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Biomarkers of oxidative stress in pregnant women with recurrent miscarriages

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Abstract

Background: The aim of this study was to evaluate the markers of oxidative stress (OS), as well as the magnesium (Mg) concentration, pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (β -hCG) values in patients with previous recurrent miscarriages, with the purpose of comparing the level of OS with normal pregnancy.

Methods: The study was conducted as a prospective study, which included a total of 95 pregnant women divided into two groups. In the study group ($n=35$), there were women with at least three consecutive previous miscarriages. The control group ($n=60$) consisted of healthy pregnant women without previous miscarriages and complications in pregnancy. Blood samples were taken between the 11th and 14th weeks of gestation, and the values of superoxide dismutase (SOD-1), glutathione peroxidase (GSH-Px) and total antioxidant status (TAS) in the serum, as well as the Mg concentration, PAPP-A and β -hCG values were determined.

Results: The values of SOD and GSH-Px were statistically higher in the study group, while the values of TAS were statistically significantly higher in the control group. The values of the Mg concentration were statistically significantly higher in the control group, while the values of β -hCG were statistically significantly higher in the study group. When the study group was divided into subgroups according to the outcome of pregnancy, there were statistical differences in the level of GSH-Px, SOD between miscarriages and the term delivery group, as well as in the Mg concentration between miscarriages and the term delivery group and between the term and preterm delivery groups. Higher values of SOD and GSH-Px, and lower values of TAS and Mg concentration could be used as markers for the prediction of spontaneous miscarriages.

Conclusions: The results of this research indicated higher OS and exhaustion of antioxidant mechanisms in early pregnancy in a group with recurrent miscarriages. Also, lower values of serum Mg concentration could be one of the markers for the prediction of spontaneous miscarriages.

Keywords: biomarkers; oxidative stress; recurrent miscarriages.

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Introduction

A precisely regulated balance between the production of reactive oxygen species (ROS) and the activity of the components of antioxidant protection is required for the maintenance of integrity and function of all cells and tissues in normal pregnancy. The level of circulating products of lipid peroxidation physiologically increases continuously during pregnancy [1].

The reasons for the increased intensity of lipid peroxidation are numerous, but increased levels of circulating lipoproteins, prooxidant activity of the placenta and an altered basal metabolism in pregnancy are considered as the most important ones [2, 3].

Physiological pregnancy is characterized by well-balanced prooxidative and antioxidative processes. Increased ROS activity is accompanied by increased levels of antioxidants, Cu, Zn and superoxide dismutase (SOD-1) first of all, thereby maintaining the delicate balance between

the cellular redox equilibrium systems that produce ROS, and the defense mechanisms which detoxify them [4, 5]. Oxidative stress (OS) occurs if the production of highly reactive metabolites of oxygen exceeds the capacity of antioxidant defense, leading to the disruption of cellular homeostasis [6–8].

As OS is a result of disturbed balance of prooxidants and antioxidants, it is very difficult to determine its level only through monitoring of prooxidant and antioxidant parameters, both in pregnancy and beyond. It has been found that no single antioxidant may reflect the overall defense response as the total antioxidant status (TAS), which is a measure of the total antioxidant capacity, and the joint activity of antioxidants, both enzymatic and non-enzymatic, can in the blood and biological fluids [9, 10]. Numerous studies [11, 12] have shown that OS in pregnancy has a significant impact on systemic and placental pathophysiological processes, which leads to disorders of placental vascularization, causing endothelial and immune dysfunction. For this reason, OS is considered to be a significant factor in pathological conditions in pregnancy such as the occurrence of spontaneous and recurrent miscarriage, preeclampsia, preterm delivery, mole hydatidosis and others [13–16].

Recurrent miscarriage is defined as three or more consecutive spontaneous abortions before completing 20 weeks of gestation [17]. It is believed that this pathological condition affects 0.5%–3% of women in the reproductive period. In 50%–60% of cases with recurrent miscarriage, the cause is unknown, and this is a reason why they are classified as idiopathic. The consequences faced by women, particularly with repeated miscarriages, can be severe psychological trauma and gynecological complications [18, 19]. The problem of idiopathic recurrent miscarriage is the constant focus of doctors from various specialties, due to the complexity of a thorough understanding of their mechanisms [17, 20].

In our previous study [21], we investigated the parameters of OS in amniotic fluid and we concluded that the parameters of OS in amniotic fluid could be altered in certain pathological conditions. Their use as clinical biomarkers is limited due to great variations in the volume of amniotic fluid between patients, which favors the use of hemolysate or serum of pregnant women. We also investigated the evaluation of the markers of OS in the first trimester of pregnancy in patients with preeclampsia, and we concluded that a higher level of OS occurred in the first trimester of pregnancy with preeclampsia, which may indicate that the initiation and development of pathophysiological processes underlying preeclampsia start much earlier than the clinical syndrome exhibit [22].

A large number of women are at risk of hypomagnesemia due to insufficient food intake. In developed countries, as many as 23% of women consume less than two-thirds of the recommended daily intake of magnesium (320 mg), while in developing countries, such as India, it is estimated that approximately 45% of pregnant women have low levels of serum magnesium (<0.75 mmol/L) [23].

Some recent studies have recommended prophylactic oral magnesium supplementation as an inexpensive way of decreasing the risk of preterm labor among all pregnant women or those at higher risk [24, 25]. Serum magnesium level can be used as a predictive tool for preterm labor. Serum magnesium evaluation must be carried out in pregnant women in order that high-risk preterm labor can be predicted and prevented. Measurement of magnesium may also help in the cases of preterm labor to select patients who benefit from MgSO_4 as a tocolytic agent [26]. A pregnancy loss that occurs after a positive human chorionic gonadotropin (β -hCG) or a raised serum β -hCG, but before ultrasound or histological verification, is defined as a biochemical loss occurring before 6 weeks of gestation, whereas clinical miscarriage is the loss of positive ultrasound examination or histological evidence for intrauterine pregnancy [27].

Progesterone and β -hCG concentration together were raised for potential of miscarriage predictor as they are referred to as reliable single markers [28].

A suboptimal rise in serum β -hCG (lower than 66% with 2 days interval in serial measurement) was previously considered as having a good prognostic accuracy. However, β -hCG should be carefully interpreted. It is advisable to combine it with other tests [29].

β -hCG is a glycoprotein produced by trophoblasts and is detectable in maternal serum within a few days after implantation. The prognostic significance of serum β -hCG measurement is based on its natural variability level during the course of pregnancy, with a high slope of increase during the first 8 weeks of pregnancy and then a slow decline until labor. β -hCG is beneficial to predict complications in pregnancy including pregnancy loss or ectopic pregnancy [30, 31].

Inhibin A and β -hCG are two substances produced by the growing trophoblast and the lower level of these two hormones was found to be associated with miscarriage [32].

However, supplementation with β -hCG had no potential advantage to overcome threatened miscarriage [33].

Three studies with 236 women analyzed pregnancy-associated plasma protein A (PAPP-A) to predict miscarriage [34–36]. PAPP-A had a poor and wide sensitivity that ranged from 25 to 64% but a high specificity ranging from

88 to 94%. Westergaard et al. were the first to evaluate PAPP-A in the prediction of pregnancy outcome in women presenting with a threatened miscarriage. They concluded that PAPP-A measurement might be useful in differentiating between pregnancies that will have a normal outcome and those that will not [37].

Aim of the study

The first aim of this study was to evaluate the markers of OS in patients with previous recurrent miscarriages, with the purpose of comparing the level of OS with normal pregnancy. The second aim of this study was to evaluate the values of magnesium (Mg), PAPP-A and β -hCG in patients with previous recurrent miscarriages, with the purpose of comparing the level of examined markers with normal pregnancy.

Materials and methods

The study was conducted at the Department of Obstetrics and Gynecology, Clinical Center of Vojvodina in Novi Sad as a prospective in a 4-year period. This study included a total of 95 pregnant women who were concordant with participation in the study, which was confirmed by their written consent in accordance with the criteria of the Helsinki Declaration. The protocol was approved by the Ethics Committee of the Medical Faculty in the Novi Sad Clinical Center of Vojvodina (Novi Sad).

The criteria for inclusion in the study were pregnancy age between 11 and 14 weeks, singleton pregnancy and the existence of three previous spontaneous abortions (with pathophysiological and cytogenetic confirmation of previous pregnancies that there were no fetal abnormalities or chromosomal aberrations). The study included only non-smokers.

The criteria for exclusion were: multiple pregnancies, polyhydramnios, maternal diseases (diabetes, hypertension, obesity, preeclampsia, kidney and heart disease, and urinary tract infections), fetal diseases (delayed development of the fetus), and local factors such as anatomical malformations of the uterus and vagina, cervical insufficiency, malignancies and any disease that can affect the levels of the biochemical markers in the serum, such as autoimmune diseases and hormonal imbalance.

From the originally formed database of patients, for the purpose of this study, 95 patients were isolated and these data were analyzed.

The study involved two groups of pregnant women: a study group ($n=35$) with at least three previous recurrent miscarriages and a control group ($n=60$), which consisted of healthy women without miscarriage and complications in previous pregnancies. After anamnesis and clinical examination, blood samples were taken from the patients – whole blood and serum – in which certain basic hematological and biochemical parameters were determined including the markers of OS such as SOD-1, TAS and glutathione peroxidase (GSH-Px), as well as Mg concentration, PAPP-A and β -hCG values.

The complete blood count (CBC) and C-reactive protein (CRP) were determined using an automated hematology analyzer ABX Micros CRP 200 (HoribaABX Diagnostics, Oberursel, Germany).

The GSH-Px activity was determined by a modified method of Paglia and Valentine with cumene hydroperoxide using RanSel (Randox, Ireland) tests (21). The activity of SOD-1 was measured by the xanthine oxidase (XOD) method using RanSOD tests (Randox, Ireland). TAS was determined by monitoring the inhibition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)+colors using the TAS BIOREX (BIOREX Diagnostic Limited, Antrim, UK) kit. Mg was measured by standard biochemical methods using the clinical chemistry analyzer ADVIA 1800 (Siemens Healthcare GmbH, Erlangen, Germany). PAPP-A and free β -hCG values were determined by immune fluorimetric method using a Kryptor Brahms analyzer (Brahms GmbH [Thermo Scientific], Germany).

Data were analyzed using the statistical package IBM SPSS Statistics, version 23 and MedCalc for PC (MedCalc Software, Belgium). p -Values less than 0.05 were considered statistically significant. The results are presented either on graphs or in tables.

Results

The demographic and clinical characteristics of the patients in the study and control groups are shown in Table 1. There were significant differences between the groups with respect to the number of miscarriages ($p<0.05$), gestational week of pregnancy outcome ($p<0.05$), erythrocyte (Er) concentration ($p<0.05$), GSX-Px ($p<0.05$), SOD ($p<0.05$), TAS ($p<0.05$), Mg concentration ($p<0.05$), β -hCG ($p<0.05$) and outcome of pregnancy ($p<0.05$). No differences were found between the study and control groups with respect to the age of patients ($p>0.05$), body mass index (BMI) ($p>0.05$), body weight and length of the child ($p>0.05$), Apgar score ($p>0.05$), leukocyte

Table 1: T test – demographic and clinical characteristics of pregnant women.

	Group	n	$\bar{X} \pm SD$	SE	Test results	p-Value	s/ns
Age	Control group	60	27.80 ± 4.994	0.645	t = -1.414	0.162	ns
	Study group	35	29.29 ± 4.908	0.830			
BMI	Control group	60	23.15 ± 3.08	0.398	t = -0.57	0.955	ns
	Study group	35	23.18 ± 2.87	0.486			
Number of miscarriages	Control group	60	0.23 ± 0.533	0.069	t = -19.720	0.000	s
	Study group	35	3.49 ± 0.887	0.150			
Gestational week of pregnancy outcome	Control group	60	38.88 ± 1.427	0.184	t = 2.754	0.009	s
	Study group	35	32.54 ± 13.576	2.295			
Body weight of the child	Control group	60	3353.17 ± 453.231	58.512	t = 1.263	0.212	ns
	Study group	35	3211.43 ± 566.834	95.812			
Body length of the child	Control group	60	49.88 ± 2.315	0.299	t = 1.091	0.278	ns
	Study group	35	49.43 ± 1.720	0.291			
Apgar score	Control group	60	9.49 ± 0.622	0.080	t = 1.420	0.163	ns
	Study group	35	9.08 ± 1.655	0.280			
Er conc.	Control group	60	4.31 ± 3.29	4.25	t = 2.229	0.029	s
	Study group	35	4.14 ± 3.73	6.32			
Leu conc.	Control group	60	9.29 ± 2.22	2.87	t = 0.130	0.897	ns
	Study group	35	9.23 ± 2.17	3.68			
Plt conc.	Control group	60	248.82 ± 65.88	85.06	t = 0.160	0.873	ns
	Study group	35	246.85 ± 52.68	89.04			
Hgb conc.	Control group	60	121.08 ± 9.61	1.24	t = 0.835	0.406	ns
	Study group	35	119.48 ± 8.60	1.45			
Hct conc.	Control group	60	0.36087 ± 0.029752	0.003841	t = 1.008	0.317	ns
	Study group	35	0.35483 ± 0.027208	0.004599			
CRP conc.	Control group	60	3.55333 ± 4.333530	0.559456	t = 0.511	0.611	ns
	Study group	35	3.14000 ± 3.455618	0.584106			
GSH-Px	Control group	60	534.14000 ± 151.731577	19.588462	t = -6.137	0.000	s
	Study group	35	732.28571 ± 151.847935	25.666986			
SOD	Control group	60	29.58667 ± 13.348059	1.723227	t = -5.869	0.000	s
	Study group	35	52.22286 ± 20.414702	3.450714			
TAS	Control group	60	1.99667 ± 1.208495	0.156016	t = 2.804	0.007	s
	Study group	35	1.24543 ± 1.288340	0.217769			
Mg conc.	Control group	60	0.89583 ± 0.096941	0.012515	t = 5.463	0.000	s
	Study group	35	0.73429 ± 0.158509	0.026793			
PAPP-A	Control group	60	1.36068 ± 0.771191	0.099560	t = -0.039	0.969	ns
	Study group	35	1.36649 ± 0.663651	0.112178			
β-hCG	Control group	60	1.12687 ± 0.580405	0.074930	t = -2.062	0.045	s
	Study group	35	1.59526 ± 1.268417	0.214402			
Dwelling place	Control group	60	/	/	$\chi^2 = 0.620$	0.284	ns
	Study group	35	/	/			
Problems in the current pregnancy	Control group	60	/	/	$\chi^2 = 3.117$	0.210	ns
	Study group	35	/	/			
Outcome of pregnancy	Control group	60	/	/	$\chi^2 = 12.926$	0.005	s
	Study group	35	/	/			
Sex of the child	Control group	60	/	/	$\chi^2 = 0.002$	0.568	ns
	Study group	35	/	/			

$\bar{X}$, mean value; β-hCG, beta human chorionic gonadotropin; BMI, body mass index; conc., concentration; CRP, C-reactive protein; Er, erythrocytes; GSH-Px, glutathione peroxidase; Hct, hematocrit; Hgb, hemoglobin; Leu, leukocytes; Mg conc., magnesium concentration; ns, non-significant; PAPP-A, pregnancy-associated plasma protein A; plt, platelets; s, significant; SD, standard deviation; SE, standard error; SOD, superoxide dismutase; TAS, total antioxidant status.

(Leu) concentration ($p > 0.05$), platelet concentration ($p > 0.05$), hemoglobin (Hgb) concentration ($p > 0.05$), hematocrit (Hct) concentration ($p > 0.05$), CRP concentration ($p > 0.05$), PAPP-A ($p > 0.05$), dwelling place ($p > 0.05$),

problems in the current pregnancy ($p > 0.05$) and sex of the child ($p > 0.05$) (Table 1).

The values of the examined parameters in pregnant women with recurrent miscarriages and in

Figure 1: Distribution of the mean value of the number of previous miscarriages by the group of patients. In the control study there was no previous miscarriages.

Figure 4: Distribution of the mean value of GSH-Px by the group of patients. GSH-Px, glutathione peroxidase.

Figure 2: Distribution of the mean value of GW by the group of patients. GW, gestational week of pregnancy outcome.

Figure 5: Distribution of the mean value of SOD by the group of patients. SOD, superoxide dismutase.

Figure 3: Distribution of the mean value of Er conc. by the group of patients. Er conc., erythrocyte concentration.

Figure 6: Distribution of the mean value of TAS by the group of patients. TAS, total antioxidant status.

Figure 7: Distribution of the mean value of Mg concentration by the group of patients.
Mg, magnesium concentration (mmol/L).

Figure 8: Distribution of the mean value of β -hCG by the group of patients.
 β -hCG, β -subunit of human chronic gonadotropin (CorMom).

the control group in the first trimester are displayed in Figures 1–8.

The number of previous miscarriages of women in the control and the study groups are displayed in Figure 1. The mean value (mean $\pm$ standard deviation [$\bar{X} \pm SD$]) for pregnant women in the study group was 3.49 ± 0.887 , whereas the mean value in the control group was 0.23 ± 0.533 . Patients with recurrent miscarriages had significantly more previous miscarriages than healthy controls ($p < 0.001$).

The gestational week of pregnancy outcome for women in the control and the study groups are displayed in Figure 2. The mean value ($\bar{X} \pm SD$) for pregnant women in the study group was 32.54 ± 13.576 , whereas the mean value in the control group was 38.88 ± 1.427 . Patients with

recurrent miscarriages had significantly lower gestational weeks of pregnancy outcome compared with healthy controls ($p = 0.009$).

The Er concentration of women in the control and the study groups are displayed in Figure 3. The mean value ($\bar{X} \pm SD$) for pregnant women in the study group was 4.14 ± 3.73 , whereas the mean value in the control group was 4.31 ± 3.29 . Patients with recurrent miscarriages had significantly lower Er concentration compared with healthy controls ($p = 0.029$).

The values of SOD in the serum of women in the control and the study groups are displayed in Figure 4. The mean value ($\bar{X} \pm SD$) of SOD activity (IU/L) in the serum of pregnant women in the study group was 52.22 ± 20.41 , whereas the mean value in the control group was 29.58 ± 13.35 . Patients with recurrent miscarriages had significantly higher mean values of SOD-1 compared with healthy controls ($p < 0.001$).

The values of GSH-Px in the serum of women in the control and the study groups are displayed in Figure 5. The mean value ($\bar{X} \pm SD$) of the GSH-Px activity (IU/L) in the serum of pregnant women in the study group was 732.28 ± 151.85 , while the average value of the control group was 534.14 ± 151.73 . The results showed significantly higher mean value of GSH-Px in pregnant women with recurrent spontaneous abortions compared to healthy control group ($p < 0.001$).

The values of TAS in the serum of women in the control and the study groups are displayed in Figure 6. The mean value ($\bar{X} \pm SD$) of TAS (mEq/L) in the serum of pregnant women in the study group was 1.26 ± 1.28 , whereas the mean value in the control group was 1.99 ± 1.20 . TAS values were significantly lower in the study group compared to the values in the control group ($p < 0.001$).

The values of Mg in the serum of women in the control and the study groups are displayed in Figure 7. The mean value ($\bar{X} \pm SD$) of Mg in the serum of pregnant women in the study group was 0.73 ± 0.15 , whereas the mean value in the control group was 0.89 ± 0.09 . The values of Mg were significantly lower in the study group compared to the values in the control group ($p < 0.001$).

The values of β -hCG in the serum of women in the control and the study groups are displayed in Figure 8. The mean value ($\bar{X} \pm SD$) of β -hCG in the serum of pregnant women in the study group was 1.59 ± 1.26 , whereas the mean value in the control group was 1.12 ± 0.58 . The values of β -hCG were significantly higher in the study group compared to the values in the control group ($p < 0.045$).

The mean value ($\bar{X} \pm SD$) of PAPP-A in the serum of pregnant women in the study group was 1.36 ± 0.66 ,

whereas the mean value in the control group was 1.36 ± 0.77 . The values of PAPP-A were not significantly different in the study group compared to the values in the control group ($p < 0.969$).

In Figure 9, we show the distribution of pregnancy outcome. In the control group, there were no miscarriages, and over 90% of patients finished pregnancy in term. In the study group, almost 20% of the patients had miscarriages.

Table 2 shows the OS values in the study group depending on the outcome of the current pregnancy. We divided the study group into three subgroups: I group

Figure 9: Distribution of pregnancy outcome by the group of patients. In control study was no previous miscarriages.

– with miscarriages, II group – with preterm delivery (delivery before the 37th week of gestation) and III group – with delivery after the 37th week of gestation in current pregnancy.

There were statistically significant differences in the level of GSH-Px between the subgroups with miscarriages and term delivery ($p = 0.006$). In the other subgroups, there were no statistically significant differences in the level of GSH-Px (Figure 10).

There were statistically significant differences in the level of SOD between the subgroups with miscarriages and term delivery ($p = 0.003$). In the other subgroups, there were no statistically significant differences in the level of SOD (Figure 11). There were no statistically significant differences in the level of TAS between the subgroups (Figure 12).

In Table 3, we show the level of Mg, PAPP-A and β -hCG in the study group depending on the outcome of the current pregnancy. We divided the study group into three subgroups: I group – with miscarriages, II group – with preterm delivery (delivery before the 37th week of gestation) and III group – with delivery after the 37th week of gestation in the current pregnancy.

There were statistically significant differences in the level of Mg between the subgroups with miscarriages and term delivery ($p = 0.033$); and between the subgroups with

Table 2: ANOVA – multiple comparisons (GSH-Px, SOD and TAS).

Tukey HSD							
Dependent variable	(I) Outcome	(J) Outcome	Mean difference (I–J)	SE	Sig.	95% Confidence interval	
						Lower bound	Upper bound
GSH-Px	Miscarriages	Term delivery	248.448434 ^a	79.070582	0.006	60.08388	436.81299
		Preterm delivery	202.668571	100.543558	0.114	–36.84962	442.18676
	Term delivery	Miscarriages	–248.448434 ^a	79.070582	0.006	–436.81299	–60.08388
		Preterm delivery	–45.779862	67.581968	0.777	–206.77586	115.21614
	Preterm delivery	Miscarriages	–202.668571	100.543558	0.114	–442.18676	36.84962
		Term delivery	45.779862	67.581968	0.777	–115.21614	206.77586
SOD	Miscarriages	Term delivery	28.643855 ^a	8.585740	0.003	8.19062	49.09709
		Preterm delivery	22.094286	10.917345	0.112	–3.91338	48.10195
	Term delivery	miscarriages	–28.643855 ^a	8.585740	0.003	–49.09709	–8.19062
		Preterm delivery	–6.549570	7.338269	0.646	–24.03104	10.93190
	Preterm delivery	Miscarriages	–22.094286	10.917345	0.112	–48.10195	3.91338
		Term delivery	6.549570	7.338269	0.646	–10.93190	24.03104
TAS	Miscarriages	Term delivery	–1.237639	0.572289	0.083	–2.60096	0.12569
		Preterm delivery	–0.225143	0.727704	0.949	–1.95870	1.50842
	Term delivery	Miscarriages	1.237639	0.572289	0.083	–0.12569	2.60096
		Preterm delivery	1.012496	0.489138	0.102	–0.15274	2.17774
	Preterm delivery	Miscarriages	0.225143	0.727704	0.949	–1.50842	1.95870
		Term delivery	–1.012496	0.489138	0.102	–2.17774	0.15274

GSH-Px, glutathione peroxidase; SE, standard error; SOD, superoxide dismutase; TAS, total antioxidant status. ^aThe mean difference is significant at the 0.05 level.

Figure 10: Distribution of the mean value of GSH-Px by outcome. GSH-Px, glutathione peroxidase.

Figure 11: Distribution of the mean value of SOD by outcome. SOD, superoxide dismutase.

Figure 12: Distribution of the mean value of TAS by outcome. TAS, total antioxidant status.

term and preterm delivery ($p < 0.001$). In the other subgroups, there were no statistically significant differences in the level of Mg (Figure 13).

There were no statistically significant differences in the level of PAPP-A between the subgroups. There were no statistically significant differences in the level of β -hCG between the subgroups (Figures 14 and 15).

Figures 16–19 show the interactive dot diagrams for the GSH-Px, SOD and TAS values in the study and control groups, and the receiver operating characteristic (ROC) curves for the GSH-Px, SOD and TAS values. The results indicate that the GSH-Px value cutoff level at ≥ 687.3 had a sensitivity of 83.3% and a specificity of 68.6%, and a positive likelihood ratio of 125.04 in the detection of women with recurrent miscarriages (Figure 16). The results indicate that the SOD value cutoff level at ≥ 34.8 had a sensitivity of 75% and a specificity of 80%, and a positive likelihood ratio of 113.951 in the detection of women with recurrent miscarriages (Figure 17). The results indicate that the TAS value cutoff level at ≤ 0.78 had a sensitivity of 88.3% and a specificity of 57.1%, and a positive likelihood ratio of 97.315 in the detection of women with recurrent miscarriages (Figure 18).

ROC analysis showed that the highest diagnostic accuracy was detected for SOD (area under the curve [AUC] 0.822, $p < 0.001$), GSH-Px (AUC 0.818, $p < 0.001$) and TAS (AUC 0.259, $p < 0.001$) in the detection of spontaneous miscarriages (Figure 19, Table 4).

Figures 20–23 show the interactive dot diagrams for Mg, PAPP-A and β -hCG values in the study and control groups, and the ROC curves for Mg, PAPP-A and β -hCG values. The results indicate that the Mg value cutoff level at ≤ 0.81 had a sensitivity of 80% and a specificity of 68.6%, and a positive likelihood ratio of 53.005 in the detection of women with recurrent miscarriages (Figure 20). The results indicate that the PAPP-A value cutoff level at ≤ 2.066 had a sensitivity of 93.3% and a specificity of 20%, and a positive likelihood ratio of 116.723 in the detection of women with recurrent miscarriages (Figure 21). The results indicate that the β -hCG value cutoff level at ≥ 1.34 had a sensitivity of 93.3% and a specificity of 20%, and a positive likelihood ratio of 122.268 in the detection of women with recurrent miscarriages (Figure 22).

ROC analysis showed that the highest diagnostic accuracy was detected for Mg (AUC 0.197, $p < 0.001$). PAPP-A and β -hCG did not show statistically significant diagnostic accuracy in the detection of spontaneous miscarriages (Figure 23, Table 5).

Table 3: ANOVA – multiple comparisons (Mg, PAPP-A and β -hCG).

Tukey HSD							
Dependent variable	(I) Outcome	(J) Outcome	Mean difference (I–J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Mg	Miscarriage	Term delivery	–0.15308 ^a	0.06009	0.033	–0.2962	–0.0099
		Preterm delivery	0.07371	0.07641	0.601	–0.1083	0.2557
	Term delivery	Miscarriage	0.15308 ^a	0.06009	0.033	0.0099	0.2962
		Preterm delivery	0.22680 ^a	0.05136	0.000	0.1044	0.3492
	Preterm delivery	Miscarriage	–0.07371	0.07641	0.601	–0.2557	0.1083
		Term delivery	–0.22680 ^a	0.05136	0.000	–0.3492	–0.1044
PAPP-A	Miscarriage	Term delivery	–0.57398	0.33374	0.203	–1.3690	0.2211
		Preterm delivery	–0.71043	0.42438	0.221	–1.7214	0.3005
	Term delivery	Miscarriage	0.57398	0.33374	0.203	–0.2211	1.3690
		Preterm delivery	–0.13645	0.28525	0.882	–0.8160	0.5431
	Preterm delivery	Miscarriage	0.71043	0.42438	0.221	–0.3005	1.7214
		Term delivery	0.13645	0.28525	0.882	–0.5431	0.8160
β -hCG	Miscarriage	Term delivery	0.60628	0.42081	0.324	–0.3962	1.6087
		Preterm delivery	0.93734	0.53508	0.192	–0.3373	2.2120
	Term delivery	Miscarriage	–0.60628	0.42081	0.324	–1.6087	0.3962
		Preterm delivery	0.33106	0.35966	0.629	–0.5257	1.1879
	Preterm delivery	Miscarriage	–0.93734	0.53508	0.192	–2.2120	0.3373
		Term delivery	–0.33106	0.35966	0.629	–1.1879	0.5257

β -hCG, beta human chorionic gonadotropin; Mg conc., magnesium concentration; PAPP-A, pregnancy-associated plasma protein A; SE, standard error. ^aThe mean difference is significant at the 0.05 level.

Figure 13: Distribution of the mean value of Mg concentration by outcome.
Mg, magnesium concentration (mmol/L).

Figure 14: Distribution of the mean value of PAPP-A by outcome.
PAPP-A, pregnancy-associated plasma protein A (CorMom).

Discussion

Controlled increase in OS physiologically encompasses all the phases of pregnancy, from the implantation of a fertilized egg through the control mechanisms that prevent the rejection of the concept generated by increased levels of the circulating markers of lipid peroxidation

accompanied by increased levels of antioxidants, which remain present throughout pregnancy [2, 38]. But, physiological pregnancy is a state of increased OS, which is certainly significantly increased in the pathological conditions in pregnancy. In the first trimester, the levels of antioxidant defense vary greatly in each individual case and depend on different factors such as BMI, age and living conditions [39].

Figure 15: Distribution of the mean value of β -hCG by outcome. β -hCG, β -subunit of human chorionic gonadotropin (CorMom).

Figure 16: Interactive dot diagram of GSH-Px levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values. GSH-Px, glutathione peroxidase; group 0, patients with recurrent miscarriages; group 1, control group; Sens, sensitivity; spec, specificity.

The results of our study indicate that a statistically significant increase in both the most important antioxidant enzymes SOD-1 and GSH-Px in the group of patients with recurrent spontaneous abortions speaks in favor of the mobilization of antioxidant defense, and it is an indicator of increased OS in this group of pregnant women. BMI, patient's age and living conditions between the study and control groups excluded the impact of these factors on the antioxidant status.

Our results are consistent with other studies [40–42] and indicate that in patients with spontaneous abortions, the entire antioxidant system is at a lower level compared to women who did not have miscarriages. This information was derived from the significantly lower levels of

Figure 17: Interactive dot diagram of SOD levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values.

SOD, superoxide dismutase; group 0, patients with recurrent miscarriages; group 1, control group; Sens, sensitivity; spec, specificity.

Figure 18: Interactive dot diagram of TAS levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values.

TAS, total antioxidant status; group 0, patients with recurrent miscarriages; group 1, control group; Sens, sensitivity; spec, specificity.

TAS in the study group. TAS is a complex parameter of antioxidant status, which, despite the enzyme activity of SOD-1 and GSH-Px, includes a number of other components, such as other enzymes (catalase), cellular antioxidants (acidum uricum and bilirubin) and non-enzymatic antioxidants (vitamins C and E, coenzyme Q) [8, 40]. In addition, pregnant women in the study group had a significantly higher incidence of miscarriages and premature births in the current pregnancy compared to the control group. In the study subgroups with poor pregnancy

Figure 19: ROC curves of the serum concentrations of GSH-Px, SOD and TAS in the first trimester of pregnancy as early markers in the prediction of recurrent miscarriages. GSH-Px, glutathione peroxidase, SOD, superoxide dismutase, TAS, total antioxidant status.

Table 4: Area under the curve (GSH-Px, SOD and TAS).

Test result variable(s)	Area	Std. error ^a	Asymptotic sig. ^b	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
GSH-Px	0.818	0.045	0.000	0.729	0.907
SOD	0.822	0.046	0.000	0.731	0.913
TAS	0.259	0.058	0.000	0.146	0.373

GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; TAS, total antioxidant status. The test result variable(s): SOD and TAS have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. ^aUnder the nonparametric assumption. ^bNull hypothesis: true area = 0.5.

outcome (miscarriages and premature birth), there was a tendency for higher values of SOD and GSH-Px in relation to the values obtained in the subgroup with normal pregnancy outcome, while TAS values were lower in the subgroups with poor pregnancy outcome. In relation to the antioxidant status of the study group, the antioxidant capacity decreased and OS increased as the duration of pregnancy was shorter, and the outcome was worse. This points to a reduced ability of antioxidant defense in the second half of pregnancy in women with previous abortions and justifies the need for frequent monitoring of these patients in the second and third trimesters of pregnancy with the initiation of dietary and antioxidant treatments [20, 40].

All the mechanisms by which the elevated levels of SOD-1 influence the delicate redox balance in the first

Figure 20: Interactive dot diagram of Mg levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values. Mg, magnesium concentration (mmol/L), group 0, patients with recurrent miscarriages, group 1, control group; Sens, sensitivity; spec, specificity.

Figure 21: Interactive dot diagram of PAPP-A levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values. PAPP-A, pregnancy-associated plasma protein A (CorMom); group 0, patients with recurrent miscarriages; group 1, control group; Sens, sensitivity; spec, specificity.

trimester of pregnancy are insufficiently clear. It is very likely that the role of SOD-1 may be strict protection but does not exclude the possibility of a simultaneous regulatory function, and it all depends on the mechanisms that lead to the induction of the synthesis of the enzyme. In their study, Frendo et al. [43] raised the claim that just an elevated level of SOD-1 in T21 cells of cytotrophoblasts has a negative impact on the differentiation of cytotrophoblasts and that it is a regulatory factor that affects

Figure 22: Interactive dot diagram of β -hCG levels in the first trimester of pregnancy in the group of patients with calculated maximum sensitivity and specificity values.

β -hCG, β -subunit of human chorionic gonadotropin (CorMom); group 0, patients with recurrent miscarriages; group 1, control group; Sens, sensitivity; spec, specificity.

Figure 23: ROC curves of the serum concentrations of Mg, PAPP-A and β -hCG in the first trimester of pregnancy as early markers in the prediction of recurrent miscarriages.

Mg conc., magnesium concentration; PAPP-A, pregnancy-associated plasma protein A; β -hCG, β -subunit of human chorionic gonadotropin.

the rejection of the genetically altered concept [43]. As increased OS may be one of the triggers of the induction of the synthesis of the first enzyme of antioxidant defense and as we got a lower total antioxidant capacity in our study, it is more likely that the role of SOD-1 is protective.

Our results indicate that the β -hCG value cutoff level at ≥ 1.34 had a sensitivity of 93.3% and a specificity of 20%, and a positive likelihood ratio of 122.268 in the detection

Table 5: Area under the curve (Mg, PAPP-A and β -hCG).

Test result variable(s)	Area	Std. error ^a	Asymptotic sig. ^b	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Mg conc.	0.197	0.048	0.000	0.103	0.291
PAPP-A	0.510	0.065	0.868	0.383	0.637
β -hCG	0.565	0.065	0.292	0.437	0.693

β -hCG, beta human chorionic gonadotropin; Mg conc., magnesium concentration; PAPP-A, pregnancy-associated plasma protein A. The test result variable(s): Mg conc., PAPP-A and β -hCG have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. ^aUnder the nonparametric assumption. ^bNull hypothesis: true area = 0.5.

of women with recurrent miscarriages. In the study by Lo et al. [44], the β -hCG cut-off point was 18,000 mIU/mL (sensitivity = 74.5%, specificity = 68.4%). Using logistic regression, they found that progesterone and β -hCG were useful to predict miscarriages during the first trimester of pregnancy [44]. In the study by Ajayi et al. [45], they showed that there was no statistically significant difference in the concentration of serum Mg between pregnant women with and without miscarriages [45]. In our study, there were statistically significant differences in the level of Mg between the subgroups with miscarriages and term delivery ($p = 0.033$); and between the subgroups with term and preterm delivery ($p < 0.001$). The results indicate that the Mg value cutoff level at ≤ 0.81 had a sensitivity of 80% and a specificity of 68.6%, and a positive likelihood ratio of 53.005 in the detection of women with recurrent miscarriages.

Three earlier studies with 236 women analyzed PAPP-A to predict miscarriage [34–36]. PAPP-A had a poor and wide sensitivity that ranged from 25 to 64% but a high specificity ranging from 88 to 94%. Westergaard et al. were the first to evaluate PAPP-A in the prediction of pregnancy outcome in women presenting with a threatened miscarriage. They concluded that PAPP-A measurement might be useful in differentiating pregnancies that will have a normal outcome from those that will not [37]. Our results showed that the values of PAPP-A were not significantly different in the study group compared to the values in the control group ($p < 0.969$). Also, there were no statistically significant differences in the level of PAPP-A between the subgroups. The results indicate that the PAPP-A value cutoff level at ≤ 2.066 had a sensitivity of 93.3% and a specificity of 20%, and a positive likelihood ratio of 116.723 in the detection of women with recurrent miscarriages.

Detailed recommendations for practical use in the care of pregnant women with risk of spontaneous miscarriages were not standardized. The role of OS during pregnancy and in adverse pregnancy outcomes has a basis in pathophysiology, although the role of OS and the treatment of OS during or before pregnancy remain speculative. Antioxidant defense involves several strategies, both enzymatic and non-enzymatic. In the lipid phase, tocopherols and carotenes as well as oxy-carotenoids are of interest, as are vitamin A and ubiquinol. Overall, these low molecular mass antioxidant molecules add significantly to the defense provided by the enzymes SOD-1, catalase and GSH-Pxs.

They should include supplements with some antioxidants such as vitamin C, vitamin E, Zn, Mn, melatonin, etc.

Vitamin E was found to be most effective in restoring the inherent antioxidant system. Thus vitamin E can be given as a nutritional supplement for scavenging free radicals generated in order to reduce OS.

Melatonin has the ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, and it stimulates several antioxidative enzymes which increase its efficiency as an antioxidant.

Conclusions

The results of our study indicate increased levels of OS in pregnant women with previous miscarriages, as well as the exhaustion of the antioxidant protection system that may be one of the reasons for the poor pregnancy outcome in the current study groups. Also, lower values of serum Mg concentration could be a marker for the prediction of spontaneous miscarriages. The results of this study justify the need for increased control in pregnant women in the second and third trimesters of pregnancy.

This could be just the beginning of our research of this complex topic that should be upgraded in the future with a larger number of respondents.

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