RESEARCH LETTER

Investigation of Free Radical Scavenging Enzyme Activities and Lipid Peroxidation in Human Placental Tissues With Miscarriage

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BACKGROUND: *Miscarriage (early pregnancy failure) is a pregnancy-related disease, the pathophysiology of which is still not completely understood. Lipid peroxidation and alterations in antioxidant enzyme activities may be of importance in the pathogenesis of this disorder. This study was planned to investigate the possible relation between free radical scavenging enzyme activities and lipid peroxidation levels in placenta tissues with miscarriage.*

METHODS: *Placental tissue samples were obtained from 21 patients who had miscarried and 25 normal pregnant women undergoing elective abortion as a control group. Total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities and levels of thiobarbituric acid reactive substances (TBARS), antioxidant potential (AOP), and nonenzymatic superoxide radical scavenger activity (NSSA) were measured in the placental tissues.*

RESULTS: *GSH-Px, CAT activities, and TBARS levels were found to be significantly increased, while T-SOD and NSSA values decreased in patients with early pregnancy failure when compared with women undergoing elective abortion (control group). However, there were no significant differences in AOP levels between the groups.*

CONCLUSIONS: *Our results reflect oxidative stress in placenta tissues of early pregnancy failure, as the oxidative processes seem to be counteracted by the physiologic activation of antioxidant enzymes such as CAT and GSH-Px. Moreover, a compensatory mechanism might be developed against possible oxidative damage in patients with miscarriage. (J Soc Gynecol Investig 2006;13:384–8)*

KEY WORDS: Miscarriage, antioxidant enzymes, oxidative stress, placenta.

Miscarriage is a common complication of early pregnancy, and approximately 15% of clinical pregnancies end in a miscarriage, most of them in the first trimester.[1–3] The majority of early pregnancy failure is usually treated with dilation, curettage, and several drugs and/or hormones, including prostaglandin and thromboxane analogs such as misoprostol.[4,5] Thromboxane and prostacyclin are metabolites of arachidonic acid and exert diverse biologic activities. A balance between the actions of prostacyclin and thromboxane is important during normal pregnancy. Imbalance between thromboxane and prostacycline in pregnancy is significantly correlated with the imbalance between lipid peroxides and antioxidants.[6]

Lipid peroxides are formed when lipid interacts with a radical, like oxygen. They are not only unstable and highly reactive but are also very damaging compounds.[7] For example, lipid peroxides or hydroperoxides can cause ongoing lipid peroxidation of other circulating lipids and lipoproteins and thus result in disseminated endothelial dysfunction.[8] Moreover, lipid peroxidation is an oxidative process that normally occurs at low levels in all cells and tissues.[9,10] Finally, uncontrolled lipid peroxidation may occur and impair normal endothelial cell function in a disease state.[11]

Lipid peroxidation and oxidative stress have been implicated in many pathologic, clinical, and physiologic conditions, including pregnancy and its complications.[6,12]

Biologic systems have many defense mechanisms to protect against attack from free radicals. For example, enzymatic and non-enzymatic mechanisms protect cell constituents from damage by reactive oxygen species (ROS). In this enzymatic defense mechanism, glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) enzymes play a primary role. On the other hand, living cells and plasma have many non-enzymatic free radical scavengers such as ascorbic acid, α-tocopherol (vitamins C and E) uric acid, and sulfhydryl groups.[6,13–16] In several studies, lowered, increased, or unchanged SOD, CAT, GSH-Px activities, and non-enzymatic antioxidant levels were observed in some placental tissues or

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1071-5576/06/$32.00
doi:10.1016/j.jsgi.2006.04.003

Table 1. Characteristics of Control and Missed Abortion Groups

	Control (n = 25)	Missed abortion (n = 21)	P
Pregnancy number	3.20 ± 1.20	2.86 ± 1.75	NS
No. of living children	1.77 ± 0.70	1.67 ± 0.70	NS
Birth no.	2.40 ± 1.89	1.75 ± 0.75	NS
Induced abortion	1.20 ± 0.04	1.50 ± 0.50	<.005
Abortion	1.33 ± 0.74	1.46 ± 0.58	NS
Gestational time (wks)*	6–10	6–13	—
Fetal heart tones/activity	(+)	(−)	—

NS = not significant.
* Data are presented as range.

plasma with pregnancy-related complications.[17–20] In light of these studies, there is no agreement between the results of several research groups.

The aim of the current study was to investigate the role of oxidative stress and, further, to elucidate the relationship between T-SOD, GSH-Px, and CAT enzyme activities and levels of thiobarbituric acid reactive substances (TBARS), antioxidant potential (AOP), and nonenzymatic superoxide radical scavenger activity (NSSA), parameters that were measured as indicators of antioxidant activity in placental tissues in women with miscarriage and controls.

MATERIALS AND METHODS

Human placental tissues were obtained from 21 patients who had miscarried and 25 normal pregnant women undergoing elective abortion as the control group at the Gazi University Medical Faculty, Department of Obstetrics and Gynecology. The ages of the patients ranged from 20 to 37 years (mean ± SD 26.46 ± 4.94 years) and those of the controls from 19 to 36 years (mean ± SD 27.10 ± 4.81 years). None of the participants was medicated, and all were free from hepatic, renal, cardiovascular, endocrine, and any metabolic disorder. Some characteristics of the subjects are summarized as mean ± SD in Table 1.

Placental tissues were collected immediately after surgery operation and washed with cold NaCl solution (0.154 M) to discard blood contamination and kept in a deep freeze at −70C until analysis. The placental tissues were prepared for the analyses by first homogenizing (B. Braun Melsungen Model, Melsungen, Germany) at 1000 U for 3 minutes. After centrifugation at 10,000 × g for 60 minutes, the upper clear layer was removed. The protein amount and TBARS levels were measured as described by Lowry et al[21] and Van Ye et al,[22] respectively, in this fraction. Part of the homogenate was extracted in ethanol/chloroform mixture (5/3 vol/vol) to discard the lipid fraction, which caused interferences in the activity measurements of T-SOD, GSH-Px, and CAT and levels of AOP and NSSA. After centrifugation at 10,000 × g for 60 minutes, the upper clear layer was removed and used for the analyses.

In the upper clear layer, T-SOD and CAT enzyme activities were measured.[23,24] The SOD activity method is based on the measurement of absorbance increase at 560 nm due to the reduction of NBT to $NBTH_2$. One unit of SOD activity has been defined as the enzyme protein amount causing 50% inhibition in $NBTH_2$ reduction rate and results were expressed in U/mg protein. The GSH-Px activity method is based on the measurement of absorbance decrease at 340 nm due to consumption of NADPH,[25] and that of CAT is based on the measurement of absorbance decrease due to H_2O_2 consumption at 240 nm. The GSH-Px and CAT activities were given in mIU/mg protein and IU/mg protein, respectively. The antioxidant potential (AOP) assay was performed using the method of Durak et al,[26] which is mainly based on the determination of TBARS levels before and after exposure to superoxide radicals produced by xanthine/xanthine oxidase system. The NSSA assay was performed using the method of Durak et al.[27] In the NSSA assay, proteins including SOD are first precipitated using trichloroacedic acid solution 20% (wt/vol) and then NSSA assay is performed in the upper clear solution without protein as with the SOD activity measurement.

The study protocol was approved by the Gazi University, Medical Faculty Ethics Committee for Medical Research. All procedures were performed at 4C throughout the experiment.

Data were analyzed by Mann-Whitney U test using SPSS software package (SPSS Inc, Chicago, IL), and P values less than .05 were judged as significant.

RESULTS

Mean ±SD values of T-SOD, GSH-Px, and CAT enzyme activities and levels of TBARS, AOP, and NSSA in the human placenta tissues of women with miscarriage and the control group are presented in Figure 1.

T-SOD activity values were ranging from 11.15 to 16.85 U/mg protein (median 14.47; mean ± SD 14.23 ± 1.60) in the miscarriage group and from 10.48 to 17.36 U/mg protein (median 16.26; mean ± SD 15.38 ± 2.52) for the control group. The activity of GSH-Px ranged from 4.20 to 17.32 mIU/mg protein (median 11.14; mean ± SD 11.71 ± 3.61) in patients with miscarriage, while those of the control group ranged from 7.12 to 11.24 mIU/mg protein (median 7.50; mean ± SD 8.20 ± 1.64). CAT activities were in the range of 56.31 to 117.12 IU/mg protein (median 90.66; mean ± SD 87.62 ± 8.19) for the miscarriage group and 40.50 to 50.68 IU/mg protein (median 43.36; mean ± SD 44.47 ± 3.79) for the control group. TBARS levels ranged from 0.360 to 0.870 nmol/mg protein (median 0.682; mean ± SD 0.657 ± 0.136)

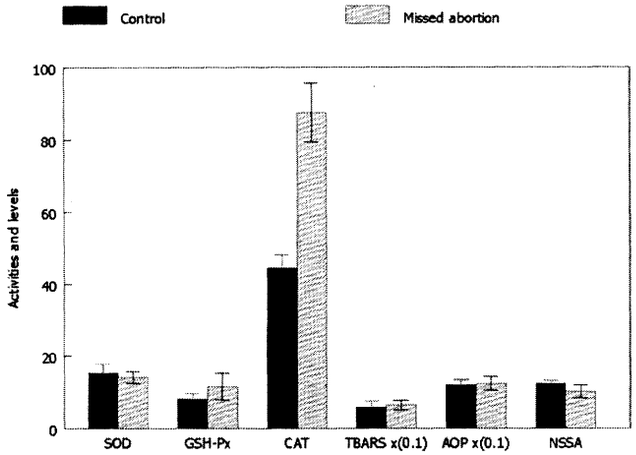

Figure 1. Mean ± SD activities of SOD, GSH-Px, and CAT and levels of TBARS, AOP, and NSSA in human placenta tissues.

and 0.335 to 0.749 nmol/mg protein (median 0.608; mean ± SD 0.593 ± 0.177) for the miscarriage and control groups. The values of AOP were in the range of 0.900 to 1.550 U/mg protein (median 1.250; mean ± SD 1.252 ± 0.191) and 1.00 to 1.44 U/mg protein (median 1.225; mean ± SD 1.218 ± 0.131) for the miscarriage and control groups. Finally, the values of NSSA ranged from 7.620 to 16.260 U/mg protein (median 10.00; mean ± SD 10.29 ± 1.90) in patients with miscarriage and in the range of 11.870 to 14.140 U/mg protein (median 12; mean ± SD 12.44 ± 0.856) in the control group. Results of intercorrelation coefficients are listed in Table 2.

As can be seen from Figure 1, GSH-Px and CAT enzyme activities were significantly higher and T-SOD activity lower in placental tissues with miscarriages than elective abortions, $P < .001$, $P < .001$, and $P < .01$, respectively. In patients with miscarriage, while placental TBARS levels were significantly higher, NSSA values were lower than those of the control group ($P < .005$ and $P < .001$), but no meaningful differences were observed between AOP levels of miscarriage and control groups.

There were strong positive correlations between T-SOD activities and NSSA levels in placental tissues in both the miscarriage and control groups, but no meaningful correlations between GSH-Px, CAT activities, and TBARS levels. However, there were moderate negative correlations between AOP values in both placental tissues from miscarriage and control tissues (Table 2).

DISCUSSION

Spontaneous abortion is one of the most serious and important complications of pregnancy, and the exact pathophysiology of the disorder is still unknown except for chromosomal aberrations. Lipid peroxides and ROS are highly reactive and very damaging compounds. Furthermore, lipid peroxides in combination with antioxidants increase to compensate their damaging and toxic effects in normal pregnancy.[28] In fact, there are many reports about antioxidant defenses in pregnancy-related disorders.[29–35] However, few studies have been performed to investigate possible relation between free radicals and abortions, especially in the human placenta. In one of these studies, Wang et al found that levels of lipid peroxides in serum are relatively stable throughout gestation, but vitamin E progressively increased.[35] In another study, Wisdom et al established a reduced plasma thiol level, and raised ceruloplasmin level and free radical activity in the normotensive pregnant group.[34] Zachara et al studied levels of selenium, glutathione, and GSH-Px activity in serum and plasma. They discovered that women with miscarriage had lowered GSH-Px activity and higher glutathione levels when compared to viable pregnancies and non-pregnant women.[31] Mover-Lev et al, who examined changes in enzymatic antioxidant activity in pregnant rats exposed to hyperoxia or hypoxia, found increased SOD and GSH-Px activity in placental tissues.[36] This study suggests that the influence of pregnancy and external oxidative stress on enzymatic antioxidant activity is not simply additive. Vural et al performed a study on antioxidant defense in recurrent abortion, and found decreased plasma ascorbic acid, α-tocopherol, total thiol groups, ceruloplasmin, uric acid, albumin, and erythrocyte glutathione levels. It was concluded that, although impaired antioxidant defense may be responsible for recurrent abortion, recurrent abortion may also result in increased oxidative stress and impaired antioxidant defense.[29] Hempstock et al reported that placental oxidative stress with resultant damage to the syncytiotrophoblast secondary to an early onset of maternal circulation may provide a mechanism for miscarriage.[37] Jenkins et al suggested that there were increased antioxidant levels in healthy pregnancy early in the first trimester, whereas first trimester miscarriage was associated with significantly reduced levels of SOD.[38] Burton and Jauniaux reported that most early pregnancy failures are associated with defective placentation, which is mainly characterized by a thinner and fragmented trophoblast shell and reduced cytotrophoblast invasion of the lumen at the tips of the spiral arteries. Therefore, this is thought to result in severe oxidative damage to the villous trophoblast, which inevitably leads to complete placenta development arrest.[39]

In view of the explanations cited, how can our results be assessed? In our study, the observed higher CAT and GSH-Px activities and decreased T-SOD activity are partly in agreement with the results of the previous studies in that high levels of H_2O_2 can cause an increased CAT activity, more so than an

Table 2. Intercorrelation Coefficients Between Enzyme Activities and Levels of TBARS, AOP, and NSSA in Human Placenta With Missed Abortion and Control Tissues

Control–missed abortion	*r*
T-SOD–T-SOD	0.759*
GSH-Px–GSH-Px	NC
CAT–CAT	NC
TBARS–TBARS	NC
AOP–AOP	−0.589*
NSSA–NSSA	0.686*

NC = no correlation.
* $P < .05$.

increased GSH-Px activity.[40] SOD enzyme catalyzes the dismutation of superoxide radical ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), while GSH-Px and CAT catalyze the degradation of H_2O_2 to O_2 and water. These antioxidant enzymes protect the cell constituents from damage by oxygen free radicals. ROS can also be suppressed by the endogenous antioxidant molecules such as glutathione, uric acid, albumin-bound bilirubin, sulfhydryl groups, and vitamins E and C. The antioxidant status (composed of enzymatic and non-enzymatic antioxidants) is known to be a barrier (both endogenous and exogenous) against free radical attacks in all body compartments.[6,14,16,17,41]

In the present study, placental T-SOD activity was significantly lower, but GSH-Px, CAT activities, and TBARS levels, as end products of lipid peroxidation, were significantly higher than those of the control group. However, AOP levels were not significantly elevated. Furthermore, NSSA values were significantly decreased compared with the control group. Elevations in TBARS levels suggest that there was oxidative stress in human placenta tissues with miscarriage. Decreased SOD and increased GSH-Px and CAT activities show an impaired antioxidant defense system. Hence, decreased SOD and increased GSH-Px and CAT activities might be an attempt to lower H_2O_2, a potent toxic metabolite for living cells. GSH-Px and CAT convert H_2O_2 to water and molecular oxygen. Moreover, GSH-Px operates at a lower H_2O_2 concentration, whereas CAT serves at a high H_2O_2 concentration.[42]

It has been suggested that there is a compensatory mechanism against lowered cytoplasmic defenses. AOP reflects the total capacity of the enzymatic and non-enzymatic antioxidant system.[43] Weakness of AOP indicates impairment in the total defense system. Increased TBARS levels also show that peroxidation reactions are activated due to reduced antioxidant defense capacity. These parameters have the potential to show the efficiency of using total antioxidants to reduce the malformation risk in pregnancy-related complications. In the present study, we found that oxidant reactions were enhanced in placental tissues of patients who had miscarried. When T-SOD activities are suppressed by increasing oxidant stress, $O_2^{\cdot -}$ radicals may be elevated in the placental tissues. T-SOD enzyme is the most important defense mechanism against the $O_2^{\cdot -}$ radicals producing in the cells. Increased $O_2^{\cdot -}$ radicals may be responsible for lipid peroxidation of cells membrane and increased TBARS levels. We believe that increased CAT and GSH-Px enzyme activities may protect cells against the hydroxyl radicals (OH), which are one of the most dangerous O_2-based free radicals. If H_2O_2 concentrations increase in the cells, these enzymes may easily be converted to OH and OH^- by transition metals such as Fe^{2+} and Cu^{2+}. Increased CAT and GSH-Px activities are the compensatory mechanisms to protect cells against free radical damage. Our study shows that superoxide scavenger activities of T-SOD in the placental tissues, suppressed by higher superoxide anione produced in the cells, are due to decreased NSSA levels. NSSA may reflect decreased non-enzymatic defense mechanisms that consist of vitamins E and C and GSH levels. This result is supported by increased TBARS levels. The non-enzymatic antioxidant defense system can be activated in human placenta tissues. As a result, increased oxidant load and decreased antioxidant power may lead to oxidant stress and accelerated oxidative reactions in miscarriage.

Examining correlation analysis results from Table 2, it can be seen that there are positive inter-correlations between SOD activities, NSSA levels of miscarriage, and control groups. However, the intercorrelations were negative for AOP levels and GSH-Px activities. These negative correlations might be indicators of the compensatory mechanism in the miscarriage group.

Antioxidant support might be helpful to prevent and/or eliminate oxidant stress in these patients. Nevertheless, further investigations are necessary to understand the relationship between the antioxidant defense system and miscarriage. Measurements of the level of the non-enzymatic antioxidant substances and antioxidant enzyme activities together might be of importance in the evaluation of pregnancy-related complications.

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