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Short Communication

Antioxidants: Their Role in Pregnancy and Miscarriage

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ABSTRACT

The aim of this study was to examine the role of antioxidants within the normal menstrual cycle, in healthy pregnancy, and in women suffering first-trimester miscarriage. The antioxidants chosen comprised of two from peripheral blood–plasma thiol and ceruloplasmin—and two extracellular parameters—superoxide dismustase (SOD) and red cell lysate thiol. We found that antioxidant levels varied little throughout the menstrual cycle. Pregnancies that went successfully to term were associated with increased levels of ceruloplasmin and SOD early in the first trimester. These changes were thought to offer the cell protection from the damage caused by the increased oxidative stress associated with pregnancy. First-trimester miscarriage was associated with significantly reduced levels of SOD. A subgroup of patients who miscarried in their first pregnancy, but whose second pregnancies were successful, had higher levels of plasma thiol and significantly reduced levels of red cell lysate thiol in the on-going pregnancy compared to levels at the time of miscarriage. Miscarriage and pregnancy appear to be associated with increased oxidative stress. In a successful pregnancy, however, changes occurred within the peripheral blood that offered protection from oxidant attack. Antiox. Redox Signal. 2, 623–628.

INTRODUCTION

THE BODY HAS A MULTILAYERED ANTIOXIDANT SYSTEM to cope with excessive reactive oxygen species (ROS) production. However, if the balance between ROS production and the protective mechanism is shifted in favor of the prooxidants, then excessive ROS can be damaging.

ROS have been shown to play a role in pregnancy. Placental studies have shown ROS and catalase are present in significantly greater amounts in early rather than late pregnancy, suggesting that these antioxidants may protect the fetus from ROS in the maternal/fetal circulation (Bogess, 1998; Yoshioka *et al.*, 1990). Other placental studies have shown the superoxide radical and its scavenging system play an important role in endothelial function but are not involved in parturition (Sugino, 1996; Telfer, 1997). Increased levels of serum malondialdehyde, a measure of lipid peroxide activity, have been found to be increased in women undergoing spontaneous abortion (Sane *et al.*, 1991). This was supported by the work of Nicotra *et al.*, who found increased levels of lipid peroxides in placental material taken from patients suffering spontaneous abortion compared to normal pregnancy (Nicotra *et al.*, 1994). More recently, glutathione (GSH), which is essential to the detoxification of reactive free

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radicals and lipid hydroperoxides, has been shown to have a role to play in maintaining pregnancy (Peterson *et al.*, 1998).

We now report the presence of altered levels of antioxidants in the peripheral blood of first-trimester pregnant women admitted suffering miscarriage compared to healthy pregnant women.

SUBJECTS AND METHODS

Patients

Ethical permission was obtained from all patients prior to inclusion in the study. The subjects studied fell into five groups: Group 1 comprised 60 healthy nonpregnant women (mean age 29.4 ± 4.5 years). Of these 12 were sampled in the follicular phase of their menstrual cycle, 18 in the luteal phase, 18 were ovulating, and 12 were menstruating. Group 2 comprised 28 first trimester (7.2 ± 1.5 weeks gestation) pregnant women (mean age 27.2 ± 5.5 years). All of these pregnancies continued to term. Group 3 comprised 22 women in the first trimester (7.5 ± 1.9 weeks gestation) of pregnancy (mean age 25.5 ± 4.8 years). All had been admitted suffering miscarriage and all samples were taken prior to the women going to theatre for evacuation of the retained products of conception. Group 4 comprised 19 women (mean age 24.7 ± 5.7 years) whose first pregnancy had continued to term but who had been admitted in the first trimester (7.5 ± 1.5 weeks gestation) of their second pregnancy suffering miscarriage. Again all samples were taken prior to the women going to theatre for evacuation of the retained products of conception. Group 5 comprised 9 women from Group 3 who were now in the first trimester (6.6 ± 1.8 weeks gestation) of a second pregnancy, which went successfully to term.

Methods

A total of four parameters were measured in this study. Two of these are intracellular parameters: red cell superoxide dismutase (SOD), which scavenges the O_2^- radical specifically, and lysate thiol (LSH), whose measurement is related to GSH concentration. GSH is one of the main general radical scavengers inside the cell. We also measured two extracellular parameters; plasma thiol (PSH) and ceruloplasmin (CP). Plasma thiol is a measure of the total plasma thiol. In diseases with an antioxidant pathology, PSH is usually reduced (Banford *et al.*, 1982). CP is one of the main oxidants in plasma. Together, these four parameters provide a measure of oxidative stress across the erythrocyte membrane. These parameters were chosen as previous studies have shown them to be good markers of antioxidant activity within the peripheral blood (Wilson *et al.*, 1989).

The methods for measuring thiol, SOD, and CP levels have been described elsewhere (Banford *et al.*, 1982, 1989). To prevent its interference in the measurement of SOD and LSH, hemoglobin was removed from lysate preparations by precipitation with chloroform: ethanol (Chen *et al.*, 1994). Briefly, red cell lysate and PSH levels were measured using the thiol disulfide interchange reactions between 5,5-dithiobis(2-nitrobenzoic acid) and biological thiols (Ellman, 1959). Plasma CP activity was measured using a modification of the method of Menden *et al.* (1977) based on the CP catalyzed oxidation of *p*-phenylenediamine to Bandrowski's base.

Statistics

As data were not normally distributed, the results are given as medians plus ranges. Statistical significance was assessed using a Mann-Whitney test.

RESULTS

The results of changes found throughout the menstrual cycle are given in Table 1. These show little change occurred in red cell oxidative stress throughout the menstrual cycle. LSH levels were significantly higher in the follicular phase than in the luteal phase.

Antioxidant levels in healthy on-going pregnancies are given in Table 2. These show that in the first trimester of an on-going pregnancy, CP and SOD levels were raised significantly compared to the nonpregnant state. In women

Table 1. Antioxidant Levels Throughout the Menstrual Cycle

Cycle stage	*PSH*	*LSH*	*Cp*	*SOD*
Follicular	414 (250–610)	492 (300–900)[a]	17.5 (8–41)	31.0 (15–97)
Luteal	440 (190–540)	364 (80–620)	17.8 (10–31)	48.5 (10–102)
Day 14	428 (215–497)	379 (100–640)	18.6 (13–26)	44.0 (16–78)
Menstruating	420 (310–670)	427 (210–550)	17.9 (11–29)	41.5 (12–89)

Results are expressed as medians plus ranges.
[a]$p < 0.04$ versus luteal phase.

whose first pregnancy ended in miscarriage (Group 3), SOD levels were significantly lower than those found in healthy pregnant women. Similar findings were made in the women who suffered a miscarriage but who already had had a previous successful pregnancy. When 9 of the women from Group 3 were sampled at the start of their next pregnancy (which ended successfully), values of PSH were significantly higher and LSH levels significantly lower than at the time of their first pregnancy (Fig. 1). Levels of CP and SOD were also raised compared to the values found at the time of their first miscarriage, but this was not statistically significant.

DISCUSSION

This study has examined the effects of miscarriage on antioxidants using peripheral blood. This is partly due to ease of sampling and partly because red cells can synthesize thiols and have only a limited ability to repair. Because thiols are effective antioxidants, their concentrations can reflect any stress that has occurred. Therefore, the red cell provides a useful model for studying the antioxidant system. ROS exist for only fractions of a second making direct measurements difficult. The parameters measured in this study are either scavengers of ROS or reflect the results of ROS activity.

Oxidative stress causes changes in cell membrane properties and function. This study has examined the role of oxidative stress in the menstrual cycle of healthy nonpregnant women, in normal pregnancy and in miscarriage. Little change occurred in antioxidant levels throughout the menstrual cycle, with the exception of a significant increase in LSH levels during the follicular phase. The reason for this is unclear but such information on the lack of cyclical variability has important implications when obtaining samples.

The changes seen in pregnancy and miscarriage that are reported here occurred in the peripheral blood and may reflect changes occurring in the uterus, or, may be the result of events that have happened there. The first trimester of pregnancy was associated with increased antioxidant activity as shown by the in-

Table 2. Antioxidant Levels in Normal Pregnancy and Miscarriage

Group number	*PSH*	*LSH*	*CP*	*SOD*
1	440 (264–553)	299 (62–899)	17.7 (3–32)	39.1 (14–137)
2	434 (145–560)	415 (86–892)	22.7 (16–35)[a]	60.0 (6–90)[b]
3	412 (260–510)	408 (179–720)	19.5 (9–32)	31.6 (11–89)[c]
4	420 (237–580)	397 (103–775)	22.6 (2–42)	27.7 (9–60)[d]
5	495 (397–690)[e]	273 (110–487)[e]	21 (19–37)	55 (18–73)

Results are expressed as medians plus ranges.
[a]$p < 0.01$ versus Group 1 (nonpregnant).
[b]$p < 0.0001$.
[c]$p < 0.04$ versus Group 2 (healthy pregnant).
[d]$p < 0.01$.
[e]$p < 0.004$ versus Group 3 (miscarriage group).

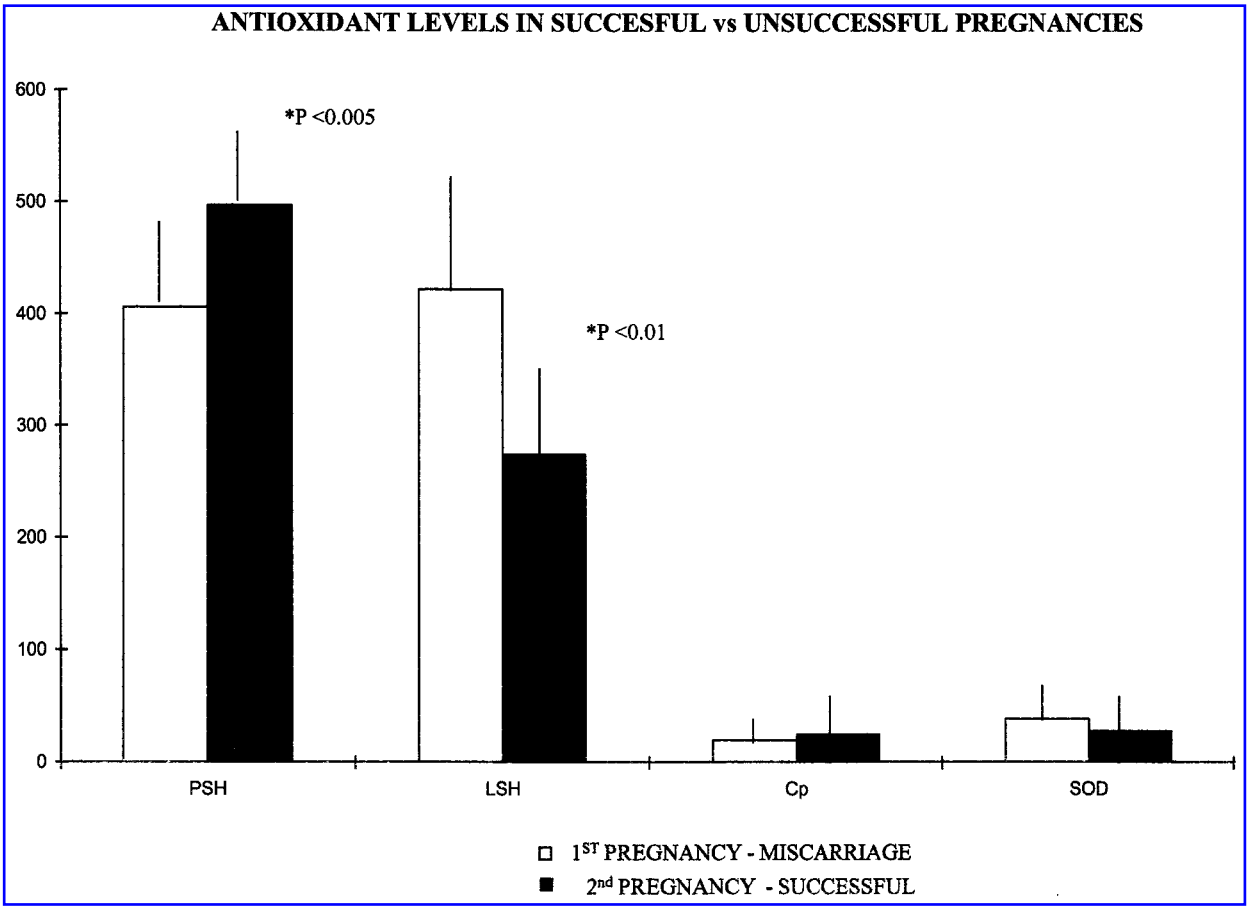

FIG. 1. Antioxidant levels in successful versus unsuccessful pregnancies.

creased levels of CP and SOD, which offer protection from ROS attack in the extracellular compartment. A previous study by Sugino *et al.* (1996) also found increased SOD activity in the endometrium in early pregnancy. These changes indicate that oxidative stress occurs in healthy pregnancy, but cellular damage is prevented by an extensive multilayered antioxidant system.

In the miscarriage group, significantly reduced levels of the O_2^- ion scavenger SOD may result in increased ROS production. Significantly reduced levels of SOD were found in Groups 3 and 4, that is those women whose first pregnancies had ended in miscarriage, and in women whose first pregnancies had been successful but whose second pregnancies had failed. This finding is important because it means that the miscarriage has been triggered by specific events, and was not influenced by a previous successful pregnancy.

Changes in antioxidant levels which influence outcome have been reported previously. Quinlan *et al.* (1997) found that patients with acute respiratory distress syndrome (ARDS) who died sustained greater levels of oxidative molecular damage and were less able to protect themselves against increased oxidative stress than those who survived. In pre-eclampsia, as a result of increased ROS activity, the intra- and extracellular antioxidant buffering levels were found to be reduced compared to normal pregnancy. This reduction in antioxidant buffering may cause many of the pathological features of the disease (Chen *et al.*, 1994).

ROS are known to affect immune function, which has to adapt to tolerate pregnancy. Recent murine studies have confirmed the link between the two (Nicol *et al.*, 2000). GSH is essential for the detoxification of free radicals. Significantly reduced levels of GSH in murine antigen-presenting cells have been shown to be

associated with a T_H 2-type cytokine response, *i.e.,* favors the production of interleukin-10 (IL-10) and IL-4. Reduced levels of GSH favored a T_H 1-type response, *i.e.,* interferon-γ (IFN-γ) and IL-2 were produced (Peterson *et al.*, 1998). Murine and human studies have shown that for a pregnancy to continue a T_H2-type response must occur (Wegmann, 1998; Jenkins *et al.*, 2000). This study did not specifically measure GSH but did measure LSH, which is comprised mainly of GSH. Levels of LSH varied considerably in all patients. However, levels were found to be significantly reduced in women whose first pregnancy had failed but whose second pregnancy was successful (Group 5). This would support the recently published view that GSH favors a T_H2-type response, which is associated with an on-going pregnancy. Further indirect evidence of increased antioxidant activity in miscarriage comes from a study by Barrington *et al.* (1996), who found selenium levels were significantly reduced in patients who miscarried. Selenium scavenges hydrogen peroxide (H_2O_2), which, although not an antioxidant, can cross the cellular membrane and if not reduced to H_2O will dissociate to the hydroxyl moiety. As GSH peroxidase is a co-factor for selenium, any reduction in selenium levels as seen in the study of Barrington *et al.* (1996) will affect GSH.

In conclusion, pregnancy appears to be associated with oxidative stress, but changes in the levels of antioxidants help the body cope. When miscarriage occurs, these adaptations do not appear to occur.

ABBREVIATIONS

ARDS, Acute respiratory distress syndrome; CP, ceruloplasmin; GSH, glutathione; H_2O_2, hydrogen peroxide; IFN, interferon; IL, interleukin; LSH, lysate thiol; PSH, plasma thiol; ROS, reactive oxygen species; SOD, superoxide dismutase.

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