

Impaired serum thiol/disulphide homeostasis may be another explanation for the pathogenesis of missed abortion

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Summary

Aim: Oxidative stress is an important factor in pregnancy physiology and embryogenesis. Placental oxidative stress results from deficient trophoblast invasion found to be a cause of a spectrum of disorders including miscarriage, missed miscarriage, and early- and late-onset preeclampsia. Herein, the authors aimed to evaluate serum dynamic thiol/disulphide concentrations in patients with missed abortion and in healthy pregnant women and attempted to determine whether serum thiol/disulphide homeostasis have a role in the pathogenesis of missed abortions. **Materials and Methods:** This prospective case control study was conducted at the current clinic between January 2015 and January 2016. The demographic characteristics and laboratory parameters of all participants were retrieved prospectively from the each pregnant woman. First-trimester missed abortion was defined as sonographic evidence of an intact gestational sac, no evidence of fetal cardiac activity (after six weeks from last menstrual period), a closed cervical os, and a history of no or minimal bleeding. Patients in the control group were followed up at least until the 20th week of gestation. Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu. **Results:** A total of 90 pregnant women were included in this study. The study group consisted of 45 patients with missed abortion, whereas 45 healthy pregnant women were chosen as the control group. All of the patients in the two groups were matched for age, gestational age, and BMI. The authors found significantly increased levels of disulphide ($17.1 \pm 4.8 \mu\text{mol/L}$ vs. $11.9 \pm 4.1 \mu\text{mol/L}$) in the sera of the study group compared to the control group ($p < 0.001$). However serum levels of native thiol and total thiol were statistically insignificant between the two groups. **Conclusion:** As a conclusion, the authors found a significant increase in serum disulphide levels from the oxidative stress markers in patients with missed abortion. Impaired serum thiol/disulphide homeostasis may be another explanation for the pathogenesis of missed abortion.

Key words: Missed abortion; Oxidative stress; Antioxidants.

Introduction

Ten to 15 percent of clinically recognized pregnancies, the majority of which occurred during the first trimester, are lost. It was shown that more than half of all pregnancy losses are associated with chromosomal abnormalities [1]. Missed abortion, also known as delayed miscarriage, is defined as the cessation of embryonic or fetal development in the first trimester [2]. In some cases, this situation occurs for a certain time before the expulsion the embryo or fetus. Some substances pass to the maternal circulation after the death of embryo or fetus and pregnancy hormones begin to decrease in a short time. Thus corpus luteum which supports the pregnancy is collapse. Eventually, uterine contractions are triggered and conceptus material is expelled. This process usually begins within few days following the death of embryo or fetus. However, this expulsion process sometimes does not occur, although two weeks have passed since the embryo died. In case of missed abortion, the cervix is usually closed and there may be no or slight bleeding. An empty gestational sac or a fetal pole without cardiac

activity is seen on the sonographic examination. After the initial evaluation, the definitive treatment is medical or surgical evacuation of the uterus [3].

Oxidative stress is called as the shift of the balance between free radicals that emerge during normal metabolism or pathologically and antioxidant defense system which is a protective against them in favor of free radicals in living organisms [4]. It is a condition in which the balance between oxidants and antioxidants is impaired, resulting in the formation of more free radicals than the antioxidant defense system can tolerate, and leads to damage to important macromolecules of the cells [5]. Oxidative stress is now accused in the pathophysiology of many diseases. It is generally thought to lead to diseases due to toxic effects on carbohydrate, protein, lipid, and DNA metabolism [6]. In recent years, there have been a number of studies on the negative effects of free radicals on the human body and the preventive methods for those effects. Different oxidant or antioxidant parameters have been investigated in this regard, but no convenient result for a specific marker has been achieved for routine use.

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Abnormal placentation in the first trimester of pregnancy leads to oxidative stress, while endothelial dysfunction, which is the result of oxidative stress, plays a key role in the development of pregnancy complications such as miscarriage [7]. Morphological and immunohistochemical markers of cellular stress and damage are shown to be increased in tissues obtained from missed abortions compared with controls. This effect was found to be more prominent in early gestational weeks. There is convincing evidence that oxidative stress is one of the underlying pathogenesis of spontaneous abortion [8]. Hempstock *et al.* claimed that placental oxidative stress with resultant damage to the syncytiotrophoblast secondary to the early onset of the maternal circulation may be the final mechanism in missed abortion cases [9].

Numerous studies have also been conducted on thiols which are among the natural antioxidants in order to prevent oxidation. Thiols have been the subject of those investigations because of their antioxidant properties as well as their anti-cancer properties. Thiols are functional sulfhydryl groups composed of sulfur and hydrogen atoms attached to carbon and have a vital role in living organisms. The newly developed thiol/disulphide homeostasis possesses a widespread use potential and is likely to produce new knowledge and opinions in basic scientific research and clinical trials [10].

Herein, the authors aimed to evaluate serum dynamic thiol/disulphide concentrations in patients with missed abortion and in healthy pregnant women and attempted to determine whether serum thiol/disulphide homeostasis have a role in the pathogenesis of missed abortions.

Materials and Methods

This prospective case control study was conducted at the current clinic between January 2015 and January 2016. The institutional review board approved the study and written informed consent was obtained from all participants. The demographic characteristics and laboratory parameters of all participants were retrieved prospectively from the each pregnant woman. Women with additional diseases (immunologic/rheumatologic/thrombophilic disease such as antiphospholipid syndrome, endocrine diseases such as diabetes and thyroid disease, and infectious diseases) were excluded from the study. Pregnant women who applied to the antenatal outpatient clinics aged between 18-42 years, singleton pregnancies between 6 and 12^{6/7} weeks, non-smoker women, and who did not receive any multivitamin or antioxidant supplement other than folic acid were included in the study. Pelvic and physical examinations of all women were performed and age, obstetric, personal and family histories, and some laboratory values were recorded. Patients' weight and height were also recorded. The gestational weeks were calculated according to the last menstrual period and biometric measurements performed with an ultrasound machine equipped with a transvaginal transducer (7.5 MHz).

Fasting blood samples were obtained from the antecubital vein at the time of diagnosis in the missed abortion group and during the regular follow up in the control group. About 2 ml blood sample taken from pregnant women who were positive for fetal heart

beat and met the study criteria were collected in a gel tube without anticoagulant. After centrifugation at 4,000 rpm for ten minutes, serum samples were kept frozen at -80 °C until analysis.

First-trimester missed abortion was defined as sonographic evidence of an intact gestational sac, no evidence of fetal cardiac activity (after six weeks from last menstrual period), a closed cervical os, and a history of no or minimal bleeding. Patients in the control group were followed up at least until the 20th week of gestation. It was confirmed that the control group consisted of women without any vaginal bleeding, pelvic pain, and fetal anomaly until the 20th week of gestation. The statistical program on the website of the statistical department of the University of British Columbia was used to calculate the sample size and the power of this study (<http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html>). Sample size estimations were based on the results of a previous study, and assuming an alpha level of 0.05 and beta error of 0.8, 45 patients were needed per group.

Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu [10]. In summary, reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native ones were detected after reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half of the difference between total and native thiols provided the dynamic disulphide amount. After the determination of native thiol (SH) and disulphide (SS) amount, native thiol/disulphide ratio (SS/SH) was calculated. Laboratory staff performing the plasma thiol/disulphide homeostasis measurement analysis was blinded to the patients' clinical information and outcome, and results were not available to the treating physicians, study staff, or investigators during study period [10].

Statistical Package for the Social Sciences version 22 and Medcalc software (Version 16.8.4) were used for statistical analysis. Compliance with the normal distribution of data was analyzed considering the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Parametric methods were used to analyze the variables with normal distribution, whereas in the analysis of non-normally distributed variables non-parametric methods were used. In order to compare the two independent groups, independent samples of Student's *t*-test and Mann-Whitney U (exact) test were used. Differences between categorical data were evaluated using Pearson χ^2 test. Mean \pm standard deviation and median (minimum-maximum) for quantitative data, as well as numbers and percentages for qualitative data were computed. Data were examined in the 95% confidence level, and statistical significance was set at $p < 0.05$.

Results

A total of 90 pregnant women were included in this study. The study group consisted of 45 patients with missed abortion, whereas 45 healthy pregnant women were chosen as the control group. All of the patients in the two groups were matched for age, gestational age, and BMI. The age range of patients varied between 18-42 years in the study group and 22-41 in the control group. The mean BMI was calculated as 25.0 ± 4.7 kg/m² in the study group and 24.7 ± 3.6 kg/m² in the control group. Median gestational age was 10 (8-12^{6/7}) weeks vs. 11(6-12) weeks in the study and control groups, respectively ($p = 0.083$). No significant differences were observed between the two groups with regards to previous obstetric history with the exception of median gra-

Table 1. — Comparison of the demographic features of the subjects.

Variables	Missed abortion (n=45)	Control group (n=45)	p value
Age (years) x± SD	29.1 ± 6.6	27.9 ± 4.6	0.327
BMI (kg/m ²) x± SD	25.0 ± 4.7	24.7 ± 3.6	0.763
Gravida median (min-max)	3 (1-6)	2 (1-6)	0.043
Parity median (min-max)	1 (0-4)	1 (0-4)	0.222
No. of live children median (min-max)	1 (0-4)	1 (0-4)	0.284
No. of Abortion median (min-max)	0 (0-3)	0 (0-2)	0.989
D&C median (min-max)	0 (0-3)	0 (0-2)	0.012
Gestational age (weeks) median (min-max)	10 (8-14)	11 (6-12)	0.083
Sonographic age (weeks) median (min-max)	7 (6-12)	11 (6-12)	< 0.001
Bleeding n (%)	11 (24.4)	0	< 0.001

Table 2. — Dynamic thiol/disulphide homeostasis parameters of the patients.

Variables	Missed abortion (n=45)	Control group (n=45)	p value
Native thiol (μmol/l) x± SD	386.2 ± 34.5	400.4 ± 48.6	0.112
median (min-max)	385.3 (264.9-466.9)	399.9 (293.4-493.2)	
Total thiol (μmol/l) x± SD	420.2 ± 35.9	423.1 ± 50.7	0.755
median (min-max)	423.6 (292.0-502.2)	429.7 (310.5-506.8)	
Disulphide (μmol/l) x± SD	17.1 ± 4.8	11.9 ± 4.1	< 0.001
median (min-max)	18.0 (6.0-27.9)	11.4 (3.6-22.8)	
Disulphide/Native thiolx100 x± SD	4.4 ± 1.3	3.0 ± 1.1	< 0.001
median (min-max)	4.7 (1.6-7.5)	2.9 (1.0-7.3)	
Disulphide/Total thiolx100 x± SD	4.1 ± 1.1	2.8 ± 1.0	< 0.001
median (min-max)	4.4 (1.6-6.5)	2.7 (1.6-6.5)	
Native thiol/Total thiolx100 x± SD	91.9 ± 2.2	94.7 ± 2.7	< 0.001
median (min-max)	91.4 (87.0-96.9)	94.7 (87.2-106.9)	

P < 0.05 was considered statistically significant.

vidity (Table 1).

The authors found significantly increased levels of disulphide (17.1 ± 4.8 μmol/l vs. 11.9 ± 4.1 μmol/l), disulphide/native thiolx100 (4.4 ± 1.3 vs. 3.0 ± 1.1), disulphide/total thiolx100 (4.4 ± 1.1 vs. 2.8 ± 1.0), and native thiol/total thiolx100 (91.9 ± 2.2 vs. 94.7 ± 2.7) in the sera of the study group compared to the control group (*p* < 0.001). However serum levels of native thiol and total thiol were statistically insignificant between the two groups (386.2 ± 34.5 μmol/l vs. 400.4 ± 48.6 μmol/l, and 420.2 ± 35.9 μmol/l vs. 423.1 ± 50.7 μmol/l, respectively) (Table 2).

Discussion

In the current study, the authors compared the serum thiol/disulphide levels of missed abortion patients and healthy pregnant women for the assessment of whether thiol/disulphide homeostasis is involved in the etiology of missed abortion. They found that serum disulphide levels which have oxidant properties were statistically significantly higher in the missed abortion patients when compared to controls. There was no significant difference between the two groups in terms of serum levels of native thiol and total thiol with antioxidant activity. This is the first study which evaluates serum thiol/disulphide homeostasis in patients with missed abortion in the literature.

Vaginal bleeding seen in the first trimester is a common complication of pregnancy and it may be an early marker for placental dysfunction. Oxidative stress is an important factor in pregnancy physiology and embryogenesis. Endothelial dysfunction occurring during abnormal placentation is thought to be caused by oxidative stress, and this plays a key role in the emergence of pregnancy complications such as abortion [7]. Placental oxidative stress resulting from deficient trophoblast invasion may cause a spectrum of disorders including miscarriage, missed miscarriage, and early- and late-onset preeclampsia [11].

Dalle *et al.* [12] showed that lipid peroxidation increased in the placenta during pregnancy, howbeit in response to this oxidative stress, antioxidant defense mechanism also increased in healthy pregnancies. Gubaljevic *et al.* [13] analysed serum levels of an oxidative stress marker in pregnancy. They found that oxidative stress increased in healthy pregnancies compared to non-pregnant women. When they compared this marker between the first and the second trimester, a significant increase was found in the second trimester pregnant women compared to the first trimester pregnant women. Hempstock *et al.* [9] investigated placental oxidative stress in early pregnancy loss. Placental specimens were evaluated immunohistochemically for oxidative stress and morphologically for tissue damage in their study. The authors concluded that patients with missed

abortion had significantly increased oxidative stress and tissue damage, in the accordance with the current results.

In another study, the role of oxidative stress on vaginal bleeding in first trimester pregnancies was investigated [14]. A small group of pregnant women at less than ten weeks of gestation with vaginal bleeding and a control group of healthy pregnancies with similar characteristics were evaluated in terms of some oxidative stress markers. The study showed that the total oxidant level is high and the total antioxidant capacity is low in pregnant women presenting with vaginal bleeding during the first trimester. In the current study, serum disulphide level which is an oxidative stress marker was found to be increased. However, the authors did not find any statistically significant difference between study group and controls in terms of serum native and total thiol level which has antioxidant activities. This result has shown that oxidative stress increases in case of missed abortion, but inadequate increase in antioxidant defense mechanisms may be an important etiologic factor in missed abortion pathogenesis.

Increased oxidative stress in the body leads to lipid peroxidation. Malondialdehyde is a marker of lipid peroxidation, whereas superoxide dismutase and glutathione peroxidase are the main enzymes responsible for the detoxification of superoxide anion. In the study of Ozkaya *et al.* [15] those markers were measured in a group of pregnant women at less than eight weeks' gestation with spontaneous abortion and vaginal bleeding and were compared with healthy pregnancies. They suggested that increased lipid peroxidation and inhibition of superoxide dismutase activity might be involved in the pathogenesis of spontaneous abortion. Another study [16] compared the levels of serum prolidase activity and oxidative stress markers including total oxidant levels, antioxidant capacity, lipid hydroperoxide, and total free sulfhydryl in healthy pregnant women and women with early pregnancy loss. Similar findings were also detected in that study.

There are different methods used to measure oxidants and antioxidants such as colorimetric, fluorescent, and chemiluminescent based methods [17]. The current authors used a novel, automated, and spectrophotometric assay for thiol/disulphide homeostasis developed by Erel and Neselioglu in their patients [10]. Recently, several studies have been conducted on different groups of patients using this method. Korkmaz *et al.* [18] investigated the effects of severity of preeclampsia on thiol/disulphide homeostasis. They found a significant correlation between impairment in degree of thiol/disulphide homeostasis and severity of preeclampsia. Ozler *et al.* found that decreased SS level at 24-28 weeks of pregnancy is a risk factor for adverse perinatal outcomes [19]. Another study by the same author evaluated the relation between thiol/disulphide homeostasis and cardiovascular disease risk in overweight adolescents with polycystic ovarian syndrome (PCOS) [20]. They found that SS and total thiol levels were significantly lower

in overweight + PCOS adolescents when compared with both normal weight PCOS and control adolescents. They suggested that decreased serum total thiol levels are a contributing risk factor for future cardiovascular disease in obese PCOS girls.

The etiology of missed abortion has not yet been clarified in detailed at about half of the cases. Today, more studies are needed for clarification of etiology and for preventing the disease. There are some studies suggesting that the impairment of antioxidant/oxidant balance is one of the factors that play a role in the etiology of the abortion. Although there is an anticipated increase in oxidative stress in pregnancy, antioxidant defense mechanisms developing against it prevent the development of any complications in pregnancy. When the current authors evaluated serum thiol/disulphide homeostasis in patients with missed abortion, they found a significant increment of those patients. Exaggerated increase in oxidative stress over the antioxidant capacity may be considered as an important etiologic factor in the pathogenesis of missed abortion.

The current study is the first study in which thiol/disulphide homeostasis was used as an oxidative stress marker in missed abortion patients. It includes a larger number of patients than similar studies evaluating the role of oxidative stress in early pregnancy losses. The cross-sectional nature of the study implies that we cannot establish a cause-effect relationship. In addition, serum levels of oxidative stress markers may also not correlate with tissue levels, and it would be better to perform a molecular or immunohistochemical study. However, measurement of serum thiol/disulphide homeostasis may be a simple method in the prediction of missed abortion patients at risk and measures to reduce oxidative stress may prevent the development of the disease.

As a conclusion, the current authors found a significant increase in serum disulphide levels from the oxidative stress markers in patients with missed abortion. Impaired serum thiol/disulphide homeostasis may be another explanation for the pathogenesis of missed abortion. For this purpose, further prospective studies are needed on antioxidant treatment as a preventative measure in missed abortion patients.

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