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Ayşe Nur AKSOY[1]
Hülya AKSOY[2]
Nurinnisa ÖZTÜRK[2]
Çağlar BULUT[2]

Erythrocyte TAO and TBARS levels in patients who suffered missed miscarriage

Aim: Missed miscarriage is a pregnancy-related condition, and lipid peroxidation and alterations in antioxidant levels may be of importance in the pathogenesis of this disorder. The aim of the study was to assess whether erythrocyte total antioxidant activity (TAO) and thiobarbituric acid reactive substance (TBARS) levels altered in patients who suffered missed miscarriage.

Materials and methods: We measured levels of TAO and TBARS, as a lipid peroxidation marker, in erythrocytes of women with a diagnosed missed miscarriage (n = 36) during 7-16 weeks of pregnancy. The control group consisted of women (n = 34) with uncomplicated pregnancy similarly matched for maternal and gestational age.

Results: We found a statistically significant decrease in erythrocyte TAO values in cases with missed miscarriage when compared to healthy pregnant group ($P < 0.01$). Additionally, mean TBARS levels were significantly higher in the missed miscarriage group than in the controls ($P < 0.001$). There was a negative correlation between erythrocyte TAO and TBARS ($r = -0.60$, $P = 0.0001$ for the missed miscarriage group and $r = -0.44$, $P = 0.009$ for the control group).

Conclusion: The obtained results indicate that in erythrocytes oxidant-antioxidant defense system may be impaired in missed miscarriage.

Key words: TAO, TBARS, miscarriage

[1] Department of Obstetrics and Gynecology, Nenehatun Hospital, Erzurum - TURKEY

[2] Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum - TURKEY

Missed abortus hastalarında eritrosit TAO ve TBARS düzeylerinin ölçümü

Amaç: Missed abortus gebelikle ilişkili bir hastalık olup patogenezinde lipid peroksidasyon ve antioksidant seviyelerindeki değişimler önemli olabilir. Bu çalışmanın amacı missed abortuslu hastalarda eritrosit TAO ve TBARS düzeylerinde değişiklik olup olmadığını tesbit etmekti.

Yöntem ve gereç: Bu çalışmada 7-16 gebelik haftasında missed abortus tanısı alan kadınların (n = 36) eritrositlerinde bir lipid peroksidasyon markeri olarak bilinen tiyobarbiturik asit reaktif maddeleri (TBARS) ve total antioksidant aktivite (TAO) düzeyleri ölçüldü. Kontrol grubu gebelik haftası ve yaşı benzer olan, komplikasyonsuz gebeliğe sahip kadınlardan (n = 34) oluşturuldu.

Bulgular: Missed abortuslu hastalarda eritrosit TAO düzeyleri, sağlıklı gebe grubuna göre daha düşük bulundu ($P < 0,01$). Ayrıca, ortalama TBARS seviyeleri missed abortus grubunda kontrol grubuna göre daha yüksekti ($P < 0,001$). Korelasyon analizinde eritrosit TAO ve TBARS seviyeleri arasında negatif bir ilişki tespit edildi (missed abortus grubu için $r = -0,60$, $P = 0.0001$ ve kontrol grubu için $r = -0,44$, $P = 0,009$)

Sonuç: Missed abortusta eritrosit oksidant-antioksidant defans sistemi bozulabilmektedir.

Anahtar sözcükler: TAO, TBARS, missed abortus

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Correspondence
Ayşe Nur AKSOY
Department of Obstetrics and Gynecology,
Nenehatun Hospital
Erzurum - TURKEY

draysenuraksoy@hotmail.com

Introduction

Early pregnancy failure is a common pregnancy complication with 15% to 20% of clinically recognized pregnancies ending in miscarriage (1,2). Missed miscarriage is a condition in which a dead immature embryo or fetus is not expelled from the uterus for 2 weeks or more. The uterus diminishes in size and

symptoms of pregnancy abate, maternal infection and blood clotting disorders may follow. The fetus and placenta may become necrotic, less commonly the fetus becomes calcified and the rest of the products of conception are resorbed.

Although oxygen is essential for sustaining life in cells, it undergoes extensive metabolism that can result in the production of toxic derivates. These molecular species derived from oxygen metabolism are designed as reactive oxygen species (ROS). Increased ROS levels may cause damage to cell functions. To prevent ROS-induced damage, cells have evolved an antioxidant system to prevent free radical damage. When balance is perturbed by an excess ROS production, a state of oxidative stress ensues leading to cell damage and cell dysfunction. Lipid peroxides are formed when lipid interacts with a radical, like oxygen.

The etiology of miscarriage is associated with embryonic and maternal factors (3). While approximately 50% of early miscarriage are linked to chromosomal abnormalities, maternal diseases, such as diabetes, immune disorders, as well as external factors, such as radiation exposure may be responsible for this condition. However, miscarriage is still not completely understood. There are many reports about antioxidant defenses in miscarriage (4-6) and it has been demonstrated that increased placental oxidative stress might be a factor in the pathogenesis of early pregnancy failure (7).

Erythrocytes are susceptible to oxidative damage because of their continuous exposure to oxygen and their high concentrations of polyunsaturated fatty acids and heme iron. Oxidative stress and loss of antioxidant defenses have been implicated in many pathologic, clinical, and physiologic conditions, including pregnancy and its complications (6-10). Oxidative stress could be evaluated by some indices, such as thiobarbituric acid reactive substances (TBARS). Total antioxidant activity (TAO) has been used as a tool to assess redox status (11). The level of TAO is composed of both enzymatic and non-enzymatic antioxidants, rather than a simple antioxidant. Thus, TAO measurement gives information about the balance between oxidants and antioxidants in the biological system. Measurement of TAO in erythrocytes can also be used for evaluating oxidative stress in patients who suffered miscarriage.

The aim of the study was to assess whether erythrocyte TAO and TBARS levels altered in patients who suffered missed miscarriage. Increased erythrocyte TAO and TBARS levels may be implicated in the pathogenesis of missed miscarriage.

Materials and methods

Thirty-six pregnant women who suffered missed miscarriage during 7-16 weeks of pregnancy were included as study group (missed miscarriage group). Diagnosis of miscarriage was performed by ultrasonography according to the lack of fetal heart activity. The control group consisted of women (n = 34) with uncomplicated pregnancy similarly matched for maternal age and gestational age (control group). Medical and obstetrics histories of all patients were questioned. Women were recruited if they satisfied the following inclusion criteria: (i) age of women between 25 and 35 years; (ii) body mass index between 18 and 26 kg/m^2; (iii) no smoking during pregnancy; (iv) no history of diabetes, hypertension, impaired renal or hepatic function, thyroid disease, or autoimmune disorders. Women with recurrent (>2) abortions and uterine abnormalities, such as leiomyoma, were excluded from the study. The main clinical and obstetrics characteristics in the 2 groups are presented in Table 1. Written informed consent was obtained from all enrollees, according to the criteria of the Ethical Committee of Medical Faculty, Atatürk University.

Overnight fasting blood samples were collected in vacutainer tubes with K3-EDTA before curettage in the missed miscarriage group. Then, erythrocytes were washed with 0.9% NaCl solution 3 times and washed erythrocytes were hemolyzed by diluting with deionized water. Erythrocyte TBARS concentrations were measured by the method described in a previous study (12). TAO of erythrocytes was determined using a modified measurement method developed by Erel (13). Results were expressed as nmol/g protein and mmol Trolox equiv/g protein, respectively.

Statistical Analysis

Results were given as mean ± SD. According to the Kolmogorov Smirnov test, because distribution of all

Table 1. Main clinical and obstetrics characteristics.

	Missed abortion group (n = 36)	Control group (n = 34)
Age (years)	29.31 ± 4.00	27.65 ± 4.80
Body mass index (kg/m^2)	23.88 ± 1.65	23.44 ± 2.30
Gestational age (weeks)	10.72 ± 3.19	11.88 ± 2.43

analytes is normal, unpaired t test was used to assess the differences between groups. Correlation analyses were performed using the Pearson's correlation test. A P value <0.05 was accepted as significant.

Results

The mean erythrocyte TAO and TBARS values in patients who suffered missed miscarriage and controls are presented in Table 2. We found a statistically significant decrease in erythrocyte TAO values in cases with missed miscarriage when compared to healthy pregnant group (P < 0.01, Figure 1). Additionally, mean TBARS levels were significantly higher in the missed miscarriage group than in the controls (P < 0.001, Figure 2). There was a negative correlation between erythrocyte TAO and TBARS (Figures 3 and 4) (r = -0.60, P = 0.0001 for the missed miscarriage group and r = -0.44, P = 0.009 for the control group).

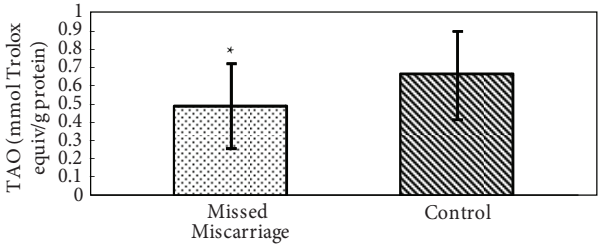

Figure 1. TAO values in missed miscarriage and control groups. * P < 0.01 compared with controls.

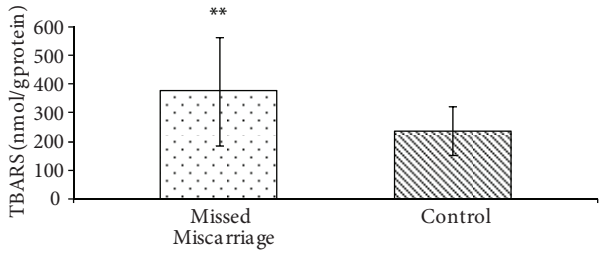

Figure 2. TBARS levels in missed miscarriage and control groups. ** P < 0.001 compared with controls.

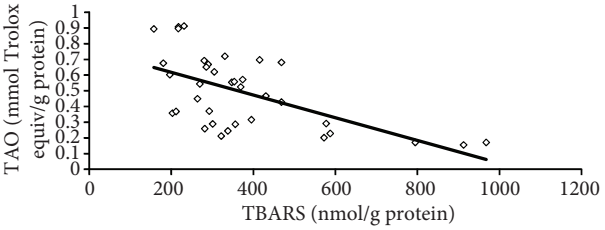

Figure 3. Correlation between TBARS and TAO in missed miscarriage group (r = -0.60, P = 0.0001).

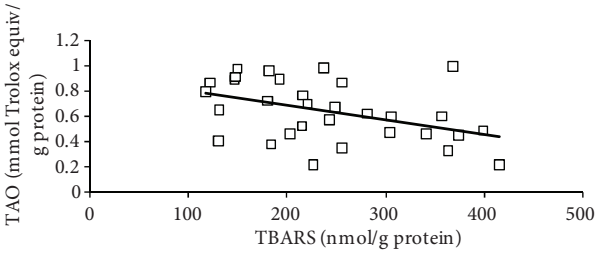

Figure 4. Correlation between TBARS and TAO in control group.

Table 2: Erythrocyte TAO values and TBARS levels.

	Missed miscarriage (n = 36)	Control (n = 34)
TAO (mmol Trolox equiv/g protein)	0.49 ± 0.23*	0.66 ± 0.24
TBARS (nmol/g protein)	376.72 ± 191. 93**	239.30 ± 86.60

Results given as mean ± SD. *P < 0.01, **P < 0.001 compared to controls.

Discussion

Missed miscarriage is associated with significant maternal mortality and morbidity, such as hemorrhage and infection. Thus, the investigation of the etiology of missed miscarriage helps preventing miscarriage. Pregnancy is a physiological state associated with enhanced oxidative stress related to high metaboli cturnover and elevated tissue oxygen requirements (14). For example it was demonstrated that there was an increase in lipid peroxidation products in normal pregnancy (15). Free radical-induced tissue damage of cell components and biomolecules (lipids, protein, and proteins) has been associated with the etiology of pregnancy-related conditions including miscarriage (16-21).

Hempstock et al.'s (22) findings indicate that morphological and immunohistochemical markers of cellular stress and damage, including expression of heat shock protein 70, and lipid peroxidation were increased in tissues obtained from missed miscarriages compared with controls. In Burton et al.'s study, it was found that syncytiotrophoblastic oxidative damage is extensive in placentas of miscarriage cases and they concluded that it may be a major contributory factor to miscarriage (23).

In a previous study, in the placenta of missed miscarriage patients, early onset of intervillous blood flow was observed when compared with normal pregnancies (24). The authors concluded that this situation might result in increased ROS and placental and peripheral oxidative damage, thus oxidative stress may contribute to miscarriage (24). In studies regarding miscarriage, various individual enzymatic and nonenzymatic antioxidant analytes were investigated in various compartments. Biri et al. (6) measured some antioxidant enzyme activities and TBARS levels in placental tissue of normal and miscarried pregnant women. In their study, glutathione peroxidase, catalase activities, and TBARS levels were found to be significantly increased, while superoxide dismutase activity was decreased in miscarried pregnant women compared with controls. In another study planned in spontaneous miscarriage patients, plasma lipid peroxidation product was found to be higher and erythrocyte superoxide dismutase activity lower than in healthy pregnancies (25). Vural et al. (26) investigated antioxidant defense in recurrent miscarriage and found decreased plasma nonenzymatic antioxidants, such as ascorbic acid and ceruloplasmin, and they suggested that recurrent abortion may result in increased oxidative stress and impaired antioxidant defense. In a study carried out by Zachara et al. (16) the levels of serum and plasma glutathione metabolism-related molecules were measured and they found lower glutathione peroxidase activity and higher glutathione levels in miscarriage cases compared to healthy pregnant and non-pregnant women.

The antioxidant activity is composed of enzymatic and nonenzymatic antioxidants and known to be a barrier against free radical attacks in all body compartments. We found decreased TAO and increased TBARS levels of erythrocytes in missed miscarriage. The decrease of TAO may indicate impairment in the total antioxidant defense system. Increased TBARS levels also show that peroxidation reactions are activated due to reduced antioxidant defense capacity in these patients. The etiology of many miscarriages remains unclear. However our findings indicate that increased levels of TBARS (as the oxidative stress marker) may be important in the pathogenesis of missed miscarriage. Further studies are necessary to investigate the effect of antioxidant therapy on missed miscarriage prevention and are needed to clarify whether oxidative stress is a cause or result of missed miscarriage.

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