**ORIGINAL ARTICLE**

# Reduced total antioxidant status in postterm pregnancies

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**Abstract**

**Background:** The placenta is the major source of oxidative stress in normal human pregnancy. The placental tissue is typically functional in postterm pregnancies. We hypothesized that such pregnancies experience deteriorating oxidative balance and increasing oxidative stress. In this case-control study, our aim was to investigate the oxidative status in postterm pregnancies comparing with term by using total antioxidant status (TAS) measurement.

**Methods:** Fifty pregnant women who were in their 41st gestational week (GW) and whose labor had not yet started were selected for the study group. Fifty subjects whose spontaneous labor onset and who delivered before their 41st GW were included for control group. Venous blood samples were obtained from each participant before the onset of labor. A premixed reagent was used to obtain serum TAS measurements from the blood samples. The Mann-Whitney test was used to compare the groups.

**Results:** Age, gravity, and parity of the subjects were similar between the groups ($p > 0.05$). Body mass index (BMI) were statistically higher in postterm group ($p = 0.011$). The median (interquartile range) TAS level was lower in the pregnancies beyond 41 weeks than term pregnancies [1.69 (0.12) mM vs 1.75 (0.20) mM, ($p < 0.05$)].

**Conclusions:** A lower total antioxidant status in past days pregnancy suggests an association with decreased oxidative status compared to term. It can be speculated that pregnancies beyond 41 weeks are associated with decreased oxidative stress and this may be play a role in the etiology of the prolonged pregnancy.

**Keywords:** Oxidative stress, postterm pregnancy, total Antioxidant Status

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## Introduction

A free radical is defined as an atom or molecule with one or more unpaired electrons that is capable of an independent (usually short) existence. During normal cell metabolism, free radicals are continuously formed as intermediate products. Free radicals sometimes interact with molecular oxygen and form free oxygen radicals, which constitute the most important free radicals in biological systems[1].

The accumulation of reactive oxygen species (ROS) and free radicals in a cell affects many important compounds, such as lipids, proteins, DNA, carbohydrates, and enzymes, and can result in cell damage. Antioxidants are substances that prevent or delay the formation of ROS. In healthy humans, cells defend themselves against ROS damage through antioxidants, which prevent or counterbalance oxidation even at low concentrations. Oxidative stress occurs when an organism accumulates more ROS than can be eliminated by antioxidant defense mechanisms. This situation can be caused by weakened antioxidant defenses and/or increased ROS production. In healthy humans, the ROSs and antioxidant protection against free radical injury system are in balance[2,3].

Pregnancy is an oxidative event in itself[4]. However, in normal pregnancy there seems a balance between antioxidant and oxidant concentrations despite modest oxidative stress[5]. It has been reported that impaired oxidant/antioxidant status is involved in the etiopathogenesis of various obstetrical complications[6].

The oxidant-antioxidant balance varies as the gestational week increases and the oxidation processes are increased in pregnancy[4,7]. The increase in free radicals becomes especially profound in the late period of pregnancy, and leads to increases in the antioxidant mechanisms to compensate for the increased oxidative stress[8,9].

The reported incidence of prolonged pregnancy is 7%[10]. Although various theories have been proposed for the pathogenesis of postterm pregnancy, which is a significant obstetric problem due to the associated high risk for maternal and fetal complications, an accurate physiological mechanism has not been identified[11].

The standard internationally definition of postterm pregnancy is 42 completed weeks (294 days) or more from the first day of the last menstrual period. However, the definition of postterm pregnancy varies in the literature and in some trials prolonged pregnancy was considered to be 287 days after LMP[12-14]. Perinatal mortality and maternal complications increase as pregnancy exceeded 41 weeks[14,15]. Induction of labor appears to be an effective way of reducing perinatal morbidity and mortality associated with post-term pregnancies (>41 weeks)[14]. Therefore, in our clinical practice, we also prefer active

management beyond 41 weeks of the pregnancy.

The purpose of this study was to compare oxidative status in postterm (after 41st gestational week) and term pregnancies (between 37-40th weeks) by using total antioxidant status (TAS) measurement, and to evaluate whether the oxidative status is associated with prolonged pregnancy.

**Material and methods**

This case-control study was conducted in an Educational and Research Hospital and was approved by the Local Ethics Committee. Informed consent was obtained from all subjects.

Pregnant women, with regular uncomplicated antenatal care with a single fetus with cephalic presentation and intact amniotic membranes, and who completed their 37th gestational week, were enrolled in the study.

Pregnant women with membrane rupture, placental pathology, maternal or fetal complications, such as gestational diabetes, preeclampsia, intrauterine growth restriction, maternal chronic disease such as thyroid function disorders, chronic hypertension, diabetes, systemic diseases etc., fetal anomaly or fetal distress findings, were excluded from the study. Also, pregnant women who had previous cesarean section and smokers, because of the effect of smoking on oxidative stress, were not included in the study. The last menstrual date (if known) or ultrasonographic measurements from the first trimester were used to determine the gestational age.

From the pregnant women, who met the criteria mentioned above, peripheral venous blood samples (5 mL each) were collected weekly from the 38th gestational week until the day of delivery. The samples were protected from light and centrifuged for 10 min at 3000 / min. The resulting serum was kept at -80 °C.

Fifty pregnant women, who completed 41th weeks and had not delivered yet, were consecutively accepted as the study group.

It was decided to enroll the study and control subjects with the ratio 1:1. Fifty consecutive subjects, who delivered before their 40 weeks and 6 days of gestation, were included as control group. Thirty-nine (78%) cases were delivered via vaginal route, eleven cases (22%) were delivered with cesarean section with the indications of cephalopelvic disproportion (n = 5) and fetal distress (n = 6).

In 14 pregnant women the labor has begun spontaneously and labor induction was applied to the remained cases (n = 36, 72%) in the study group. The cesarean delivery rate was 46% (n = 23) in the study group.

From the last blood samples of all subjects, obtained before labor onset, TAS levels were measured and compared between the groups.

The general antioxidant status was measured using premixed TAS-manual kit (Randox Laboratories, UK). The TAS measurements (in mM) were made on a Hitachi 911 autoanalyzer following the manufacturer's instructions. The principle underlying TAS measurement was based on the interaction of the 2,2'-azinobis (ABTS) radical cation with the ferrylmyoglobin radical species (generated by the activation of metmyoglobin with $H_2O_2$) to create $ABTS^+$. Antioxidant compounds suppress the absorbance of the $ABTS^+$ radical cation to a degree and on a time-scale dependent on the antioxidant capacity. Decolorization was used as the assay endpoint[16]. The TAS values of the study (postterm) and control (term) groups were compared.

Statistical analysis was performed using SPSS software (version 11.5, IBM SPSS Inc, Chicago, IL, USA). Data normality was tested via the Shapiro Wilk normality test before performing statistical analyses. The comparison of the values distributed normally ($p < 0.05$) was performed using t-test and the nonparametric Mann-Whitney test was used for comparisons of the data did not distributed normally ($p > 0.05$) between groups. The results are given as the median (interquartile range [IQR]) and mean ± standard deviation (SD). A p-value < 0.05 was accepted as statistically significant.

**Results**

The age, gravity, and parity of the patients were similar between the groups. The mean gestational age when the maternal blood sample was taken was 41.2 ±0.2 weeks in the postterm group and 38.1 ±0.6 weeks in the control group ($p < 0.001$).(Table 1) Body mass index (BMI) was statistically higher in postterm group ($p = 0.011$).

The minimum and maximum TAS values were 1.32 and 1.76 mM, respectively, in the postterm group and 1.31 and 1.87 mM, respectively, in the term group. The TAS value was significantly lower in the postterm group than term group [median (IQR) values 1.69 (0.12) mM vs 1.75 (0.20) mM, ($Z = 2.761$, $p < 0.01$)] (Table 1, Figure 1).

**Discussion**

Normal human pregnancy is considered a state of enhanced oxidative stress. All oxidative stress markers, including total antioxidant capacity (TAC), were increased

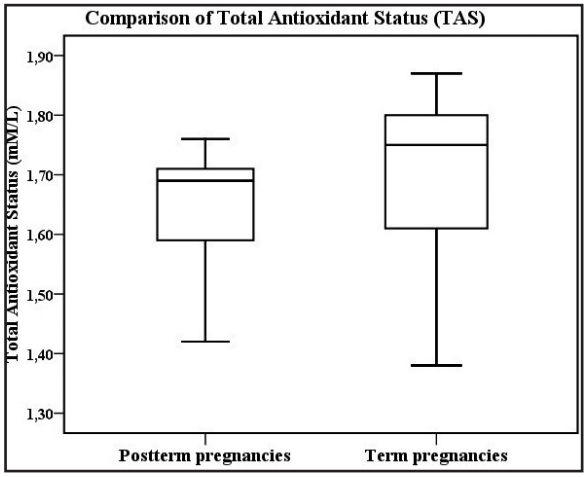


**Figure 1:** Total Antioxidant Status (TAS) levels (mM) in postterm and term pregnancies ($p = 0.006$).

**Table 1:** Patient demographics and Total Antioxidant Status (TAS) levels.

| | Postterm Pregnancies (n =50) | Term Pregnancies (n=50) | p |
|---|---|---|---|
| [a]Age (years) | 26.5 ± 5.6 | 25.2 ± 4.6 | 0.316 |
| [b]Gravidity | 2 (2.0) | 1 (1.0) | 0.068 |
| [b]Parity | 1 (1.0) | 0 (1.0) | 0.054 |
| [a,c]BMI (kg/m$^2$) | 29.7 ± 3.6 | 28.0 ±3.2 | 0.011 |
| [a,d]GW at sample collection | 41.2 ± 0.2 | 38.1 ± 0.6 | <0.001 |
| [a]GW at delivery | 41.5 ± 0.3 | 38.7 ± 0.6 | <0.001 |
| [b]Birthweight (g) | 3,243 (345) | 3,302 (317) | 0.101 |
| [b]TAS (mM) | 1.69 (0.12) | 1.75 (0.20) | 0.006 |

[a]Mean ±Standard deviation
[b]Median (Interquartile range)
[c]BMI: Body Mass Index
[d]GW: Gestational Weeks

in the third trimester[17].

Pregnancy per se is a state of oxidative stress due to the high metabolic activity of placental mitochondria that generate ROS, and also to superoxide generation from NADPH oxidase[18]. Increased oxidative stress may occur during the 3rd trimester due to the rapid development of the placenta. The placenta exhibits intense cellular activity and is the major source of pro-oxidant agents and oxidative stress in normal human pregnancy[4].

The existence of a large placenta can increase lipid peroxidation and oxidative stress due to an increase in lipid peroxide formation. Unsaturated fatty acids abundant in the placenta contribute to oxidative stress as a source of lipid peroxidation[19]. The concentration of lipoperoxides in maternal blood increases as gestation progresses. The concentration in pregnant women at 40 weeks gestation is 1.6 times higher than in nonpregnant women[20].

Although placenta contributes to increased maternal oxidative stress, it is also one of the most important sources of antioxidant enzyme-systems[20,21]. Our understanding of the role of antioxidant systems during pregnancy and fetal development is constantly evolving with research defining better the biological roles of these highly reactive species and the maintenance of optimal oxidant/antioxidant balance. The antioxidant enzyme system is upregulated during the last 15% of gestation[22]. Several antioxidant defense mechanisms, such as catalase, superoxide dismutase, beta-carotene, glutathione reductase, glutathione peroxidase, glutathione S-transferase, and glucose 6-phosphate dehydrogenase, restrict lipid peroxidation during pregnancy, especially in the placenta, and impede the oxidation progress[23,24]. The placenta is able to keep the lipid peroxidation under control in normal pregnancy[4]. Placental tissue suppresses lipoperoxide formation in the late gestational age, lowers the concentration of lipoperoxides in the cord blood. The concentration of lipoperoxides in the cord blood, however, is 70% lower than that in maternal blood[20,21].

These developmental changes provide for the transition from the relative hypoxia of intrauterine development to the oxygen-rich extrauterine environment and they protect the fetus from many kinds of oxygen radicals in the feto-maternal circulation[20-22].

Considering that the placental tissue typically is functional in postterm pregnancies, we hypothesized that such pregnancies experience deteriorating oxidative balance and increased oxidative stress, which in turn cause increased antioxidant activity.

Oxidative stress may be one of the underlying mechanisms of preterm delivery[9]. Increased oxidative stress was seen in preterm mothers as well as in cord samples and is associated with prematurity[25,26].

The antioxidants may help prevent preterm birth associated with inflammation and preterm labor and lengthen the pregnancy duration[19]. This delaying action might be due to an inhibition of the synthesis of prostaglandins, which they increase the smooth muscle contractility[27]. In contrast to preterm labor, oxidative stress does not increase in postterm pregnancies. Thus, the labor mechanisms are not triggered and fetal delivery is delayed. Therefore, we alternatively hypothesized that the oxidative balance, as well as maternal TAS level do not change in postterm pregnancies.

In our study, TAS level was lower in postterm pregnancies than term pregnancies. Our result is different from the literature, which is pointed out that the antioxidant status should be increased when the pregnancy progresses[4,7]. Our result does not support our first hypothesis, also supports the second. Our result may be explained as in postterm pregnancies the contribution of the placenta in the oxidative stress status decreases, thus in the optimal milieu, the delivery mechanisms are not triggered and the pregnancy gets longer.

To test our hypotheses, we compared a single lab value of pregnant women who delivered prior to 41st weeks to those who had not begun to labor by the 41st gestational week. Radical oxidative species and antioxidant levels differ during pregnancy, making evaluation difficult[28]. We only used TAS measurement to assess the cumulative oxidative stress status, because Rice-Evans and Miller

have reported that TAS measurement reflects the cumulative antioxidant status well and TAS is more meaningful than single components of antioxidant status[29]. However, the studies examining antioxidant substances separately are dominant in the literature[30-32] and our choice may be occurred as a limitation of our study. The specific assays need to be used to confirm our results.

Although the role of oxidative stress in initiation of labor is not known, higher levels of lipid peroxidation were observed in myometrial samples obtained during term or preterm labor[33]. There is some evidence in the literature demonstrating that labor contributes to increased oxidative stress in the placenta via lipid peroxidation and the labor has an influence on maternal oxidative stress[26,3436]. In the present study, the blood samples were obtained from all subjects before the labor spontaneously begun to avoid this bias.

Obesity is one of the strongest risk factors for postterm pregnancy[37] and as expected the BMI was found higher in postterm group in the present study. At the same time, obesity elevates oxidative stress[38]. We expected according to this data TAS values should be higher in postterm group because of their higher BMI. However, in some studies, plasma (TAS) was found lower in obese persons than in non-obese persons and TAS values were 13% lower in obese than in non–obese matched controls[38-40]. Contrary to our expectation, TAS values were lower in our study and our findings were compatible with those results.

Roberts VH et al have reported that with increasing maternal BMI there is an increase in placental nitrative stress. This did not appear to be a corresponding increase in oxidative stress and they have demonstrated some evidence of a decrease in oxidative effects in these placenta samples. Potentially the formation of peroxynitrite may be consuming reactive oxygen species and reducing oxidative stress. This may be a shift in the balance between nitrative and oxidative stress, which may be a protective mechanism for the placenta[41]. Our results supported the comment of them and the reason of reduced TAS levels and indirectly oxidative stress may be that protective effect of maternal high BMI in postterm group than terms.

In conclusion, in our study, TAS value of the postterm group was significantly less than that of the term group. The low TAS can be interpreted as an indirectly sign of an altered oxidant-antioxidant balance towards decrease in postterm pregnancy compared with an uncomplicated term pregnancy. This finding may be useful for understanding the etiopathogenesis of postterm pregnancy and to speculate that the decreased oxidative stress plays a role in the etiology of the prolonged pregnancy or it is associated with postterm pregnancy. Our results may be interpreted as that when pregnancy advances and exceeds the normal duration the protective effect of the placenta becomes more dominant than the contribution to the oxidative stress. The reduction of oxidative stress can result from this situation, and at the same time this fact prevents or delays the start of labor. More information is needed on the effect and status of the oxidative stress markers in this clinical situation before drawing strong conclusions. Our hypothesis should be clarified with further experimental and clinical prospective studies with larger numbers.

**Conflict of Interest**

All authors declare that there is no conflict of interest.

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