Decreased oxidative stress may contribute to the disease process in placenta accreta

Efser ÖZTAŞ1, Sibel ÖZLER1,*, Merve ERGİN2, Özcan EREL1, Başak GÜMÜŞ GÜLER4, Ali Turhan ÇAĞLAR1, Aykan YÜCEL1, Dilek UYGUR3, Nuri DANİŞMAN1

1Department of Perinatology, Zekai Tahir Burak Women’s Health Training and Research Hospital, Ankara, Turkey
225 Aralık State Hospital, Gaziantep, Turkey
3Department of Clinical Biochemistry, Faculty of Medicine, Yıldırım Beyazit University, Ankara, Turkey
4Department of Obstetrics and Gynecology, Liv Hospital, Ankara, Turkey

* Correspondence: sibelozler@gmail.com

1. Introduction
Placenta accreta is defined as complete or partial adhesion of the placenta to the myometrium and its incidence has increased up to 3 per 1000 deliveries and seems to be parallel with the increasing rate of cesarean delivery (1). Placenta accreta is a catastrophic clinical condition that threatens survival, due to intrapartum and postpartum bleeding and warrants a multidisciplinary approach. The rates of maternal mortality and morbidity with placenta accreta have been reported to be as high as 7% and 60%, respectively (2). While increasing cesarean sections seem to be responsible for the increased incidence and deficiency of decidualization, increased angiogenesis, and overinvasiveness of the trophoblasts are implicated in the pathophysiology of placenta accreta, the molecular basis of this condition remains unclear (1,3). Placental angiogenesis and trophoblastic invasion are balanced during pregnancy. On the other hand, deterioration of the balance between angiogenic and antiangiogenic factors and also an imbalance between proinvasive and antiinvasive factors might cause abnormal uteroplacental vascularization and subsequently abnormal placental invasion (4).

Previous studies have widely studied the impaired growth of placental vasculature in pregnancy-associated pathologies that lead to placental insufficiency, including preeclampsia, intrauterine growth restriction, intrauterine fetal demise, and miscarriage (5–8).

Background/aim: The main aim of this study was to investigate serum total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and arylesterase levels in pregnant women with placenta accreta and to compare those with age-matched healthy pregnant women.

Materials and methods: A total of 27 pregnant women who had clinically and pathologically proven placenta accreta and 30 age- and BMI-matched healthy pregnant women were enrolled in this case control study. Maternal serum TOS, TAS, OSI, and arylesterase levels were evaluated using logistic regression analysis to determine if there was an association with abnormal placental invasion or not.

Results: Decreased OSI (OR= 0.999, 95%CI: 0.998–1.000, P = 0.035) and increased arylesterase levels (OR= 0.981, 95%CI: 0.970–0.993, P = 0.001) were significantly associated with the presence of placenta accreta. Maternal serum TOS, TAS, OSI, and arylesterase levels were not predictive for adverse perinatal outcomes (P > 0.05).

Conclusions: Decreased OSI and increased arylesterase levels are significantly associated with placenta accreta and may contribute to the abnormal invasion process.

Key words: Arylesterase, oxidative stress index, total antioxidant status, total oxidant status, placenta accreta

Received: 25.05.2016 ● Accepted/Published Online: 08.04.2017 ● Final Version: 23.08.2017
preeclampsia (12,13). As increased oxidative stress is already shown to contribute in limited angiogenesis, we suggest that increased angiogenesis in placenta accreta may also be associated with an imbalance between oxidant and antioxidant mechanisms.

In the current study we aimed to investigate maternal serum TOS, TAS, OSI, and arylesterase, and antioxidant enzyme levels in pregnant women with placenta accreta, and to determine whether any changes in their levels are useful in predicting adverse pregnancy outcomes in placenta accreta.

2. Materials and methods

A case-control study was carried out between June 2013 and May 2014 in the Obstetrics and Gynecology Department of Zekai Tahir Burak Women's Health Education and Research Hospital. A total of 86 participants were included in the study. Twenty-seven patients included in the study were diagnosed with placenta accreta during cesarean section and all cases were histologically confirmed according to previously established criteria as the abnormal adherence of placenta involving all degrees to the underlying uterine wall (14). Thirty age- and body mass index (BMI)-matched uncomplicated healthy pregnant women with normal placental location were also recruited. Adverse neonatal outcomes included low birthweight (<2500 g), preterm delivery (<37 weeks), low 1- and 5-min Apgar scores (Apgar score < 7), and neonatal intensive care unit admission. Neonatal intensive care unit admission criteria in our hospital are standard for all neonates and the neonatologists did not have any information about the present study.

Patients were excluded if any of the following disorders were present: multiple pregnancy, preterm premature rupture of membranes, previous pregnancy complicated with placenta previa, any previous complicated pregnancy, cervical or uterine surgery (myomectomy, metroplasty, uterine septal resection, conization, any intervention for cervical or uterine surgery (myomectomy, metroplasty, conization, any intervention for placenta previa, any previous complicated pregnancy, rupture of membranes, previous pregnancy complicated with placenta previa, any previous complicated pregnancy, type 1 or 2 diabetes, and patients using medication.

All participants included in the study were evaluated at their initial admission. A clinical examination was performed and anthropometric measurements as well as the previous obstetric and medical history were recorded. Gestational age was calculated from the last menstrual period and verified by ultrasonography. Blood samples were obtained after overnight fasting by venipuncture and processed within 1 h of withdrawal, and all serum samples were stored at −80 °C until the day of analysis. As oxidative stress markers can be easily affected by other factors, we took care that no additional factors such as concomitant uterine contractions, bleeding, and infection were present during the sample collection. All participants provided written informed consent. The study protocol was performed according to the principles of the Declaration of Helsinki and approved by the local Ethical Committee of our hospital.

Serum TOS and TAS levels were determined spectrophotometrically using a method previously described by Erel, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H₂O₂ eqv./L) for TOS and as millimolar trolox equivalent per liter (mmol Trolox eqv./L) for TAS (15,16). The ratio of TAS to TOS represents the OSI, an indicator of the degree of oxidative stress. The OSI value is calculated according to the formula: OSI [arbitrary unit (AU)] = TOS (µmol H₂O₂ eqv./L)/TAS (mmol Trolox eqv./L) × 1002⁵. Arylesterase activities results are expressed as kU/L serum.

2.1. Statistical analysis

IBM SPSS for Windows Version 20.0 (Armonk, NY, USA: IBM Corp.) was used for statistical analysis. Continuous variables were tested for normality by the Kolmogorov–Smirnov test. Normally distributed data were presented as mean ± standard deviations. Categorical comparisons were performed using the chi-square test. For data not normally distributed, median with data range (minimum to maximum) was used. We used the independent samples t-test and Mann–Whitney U test for parametric and nonparametric groups, respectively. The optimal cut-off points of laboratory parameters determining accreta were evaluated by ROC analyses, calculating area under the curve (AUC) as giving the maximum sum of sensitivity and specificity for the significant test. Sensitivity and specificity were also calculated at the best cut-off point for each clinical measurement. To determine if the oxidative stress markers are independently associated with the presence of accreta multiple logistic regression analysis was used. Odds ratios and 95% confidence intervals for each independent variable were also calculated. A P value less than 0.05 was considered statistically significant. Multivariate logistic regression analysis was also used to determine if a relationship between adverse neonatal outcomes and serum oxidative stress markers was present or not.

3. Results

Fifty-seven pregnant women were enrolled in the present case-control study: 27 patients with placenta accreta, and 30 age- and BMI-matched healthy pregnant controls. The baseline characteristics of the placenta accreta patients and controls are given in Table 1. There were no statistically significant differences in age, BMI, weight gain in pregnancy, or the percentage of smoking between the groups. Gravida, the percentage of preterm delivery, and prior cesarean section history were significantly higher in the accreta group. Maternal serum TOS and OSI were
significantly lower, while TAS and arylesterase levels were significantly higher in the accreta group when compared with the controls (Table 1).

Serum TAS, TOS, PON, arylesterase, and OSI levels were again evaluated with ROC analysis; cut-off levels were determined and AUC values were calculated. The AUC, best cut-off values, sensitivity, and specificity for distinguishing the groups for each parameter are given in Table 2. All parameters except TAS were found to be statistically significant.

Multivariate logistic regression analysis was then used to determine if there was a relationship between groups and the defined cut-off levels of the laboratory parameters or not. Prior cesarean delivery (OR = 0.174, 95%CI: 0.041–0.744, P = 0.018), low OSI (OR = 0.999, 95%CI: 0.998–1.000, P = 0.035), and high arylesterase levels (OR = 0.981, 95%CI: 0.970–0.993, P = 0.001) were found to be statistically significant in the determination of placenta accreta (Table 3).

In addition, we examined if there were any changes in TOS, TAS, OSI, and arylesterase levels according to the management of placenta accreta. No significant changes were observed when the accreta patients were again evaluated by dividing them into 3 subgroups according to the management procedure: hysterectomy (n = 14), compression sutures (n = 6), and compression sutures + Bakri balloon tamponade (n = 7).

Further analysis was also performed to determine whether there was a correlation between oxidative stress markers and adverse neonatal outcomes (low APGAR, low birth weight, preterm delivery, and neonatal unit admission) or not. Multivariate logistic regression analysis, used to determine the predictive value of defined cut-off levels of the laboratory parameters and adverse perinatal outcomes, failed to determine such an association (P > 0.05).

### Table 1. Baseline characteristics and laboratory parameters of the patients with placenta accreta and the controls.

<table>
<thead>
<tr>
<th></th>
<th>Placenta accreta n = 27</th>
<th>Control n = 30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.03 ± 5.28</td>
<td>30.56 ± 4.99</td>
<td>0.070</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.34 ± 4.47</td>
<td>28.07 ± 3.12</td>
<td>0.797</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>7 (25.9%)</td>
<td>3 (10.3%)</td>
<td>0.128</td>
</tr>
<tr>
<td>Prior cesarean delivery (%)</td>
<td>20 (74.1%)</td>
<td>7 (23.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAS (mmol Trolox eqv./L)</td>
<td>0.223 ± 0.077</td>
<td>0.184 ± 0.069</td>
<td>0.049</td>
</tr>
<tr>
<td>TOS (µmol H₂O₂ eqv./L)</td>
<td>3.144 ± 0.689</td>
<td>4.179 ± 1.620</td>
<td>0.003</td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>1628.43 ± 785.63</td>
<td>2559.30 ± 1353.23</td>
<td>0.003</td>
</tr>
<tr>
<td>ARES (KU/L)</td>
<td>420.49 ± 141.82</td>
<td>256.56 ± 63.95</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P value: P < 0.05 statistically significant, BMI: body mass index, TAS: total antioxidant status, TOS: total oxidant status, ARES: arylesterase, OSI: oxidative stress index, H₂O₂: hydrogen peroxide

### Table 2. Cut-off levels of TAS, TOS, OSI, and arylesterase for the prediction of placenta accreta evaluated with ROC analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut off</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>AUC (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol Trolox eqv./L)</td>
<td>0.207</td>
<td></td>
<td></td>
<td>0.642 (0.494–0.790)</td>
<td>0.066</td>
</tr>
<tr>
<td>TOS (µmol H₂O₂ eqv./L)</td>
<td>3.568</td>
<td>70.37%</td>
<td>53.33%</td>
<td>0.659 (0.515–0.803)</td>
<td>0.040</td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>1623.38</td>
<td>66.67%</td>
<td>80.0%</td>
<td>0.726 (0.592–0.860)</td>
<td>0.003</td>
</tr>
<tr>
<td>ARES (KU/L)</td>
<td>338.73</td>
<td>92.59%</td>
<td>73.33%</td>
<td>0.859 (0.765–0.954)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P value: P < 0.05 statistically significant, TAS: total antioxidant status, TOS: total oxidant status, ARES: arylesterase, OSI: oxidative stress index, AUC: area under the curve, CI: confidence interval, ROC: receiver operating characteristic, H₂O₂: hydrogen peroxide
4. Discussion
In the present case-control study of oxidative stress markers in placenta accreta, increased levels of maternal serum arylesterase levels and decreased OSI were found to be associated with placenta accreta. Oxidative stress has already been suggested to contribute in the pathogenesis of placenta-related diseases of pregnancy such as preeclampsia and miscarriage (17). Various studies showed increased oxidant status in pregnancies with both preeclampsia and fetal growth retardation, and thus suggested that oxidative stress is a major contributor in preeclampsia- and fetal growth retardation-related endothelial dysfunction (18,19). Although, to the best of our knowledge, there are no studies comparing oxidative stress markers between placenta accreta and healthy pregnant women, we hypothesized that a mechanism just the opposite of preeclampsia might have contributed to the disease process in placenta accreta. In the present study, we found that oxidative stress was decreased, while antioxidant status and arylesterase, an antioxidant enzyme, were increased. Thus, it seems like there is an imbalance between oxidant and antioxidant mechanisms towards dominance in antioxidant status in placenta accreta. McMahon et al. recently demonstrated that lower levels of placental soluble fms-like tyrosine kinase-1 (sFLT-1) expression were associated with invasive placentation (20). sFLT-1 is a potent antiangiogenic growth factor and an association between increased levels and preeclampsia has been widely evaluated (21,22). Although sFLT-1 has been reported to be a predictive marker and important in the pathophysiology of preeclampsia, the mechanism of its involvement was recently demonstrated. Jiang et al. reported that in the serum of sFLT-1 treated preeclamptic mice, oxidant malonyl dialdehyde levels were higher and antioxidant superoxide dismutase levels were lower when compared with controls (23). Hence, they suggested that sFLT-1 promoted apoptosis by increasing oxidative stress. Since sFLT-1 levels were reduced in placenta accreta, it would not be inappropriate to suggest that reduced sFLT-1 resulted in decreased apoptosis of trophoblasts and increased angiogenesis, by implication of decreased oxidative stress. Our study is in agreement with the above-mentioned studies, since we found reduced oxidative stress in placenta accreta.

The antiangiogenic property of the placenta in preeclampsia and placenta accreta cases is known. Öztürk et al. observed significantly lower levels of TAS in the placenta of pregnant patients having preeclampsia when they were compared with healthy controls, and showed its significant relation with the maternal serum TAS levels (24). Moreover, Hilali et al. demonstrated significantly lower levels of TAS and increased levels of TOS and OSI in the maternal serum and cord samples of patients having mild preeclampsia (25).

Recently, Choi et al. suggested that alphafetoprotein (AFP) is a novel marker protein for the antioxidant effect of the placenta (26). In addition, it has been already proposed that unexplained elevated maternal serum AFP is associated with placenta accreta (1). These studies confirm the accuracy of our findings, since increased AFP may be responsible for the increase in antioxidant status in placenta accreta.

In conclusion, reduced OSI and increased arylesterase levels are associated with placenta accreta. Decreased oxidative stress might be either a cause of abnormal placential invasion or a consequence of increased AFP, and so this study needs to be validated with further studies investigating the causal relationship of oxidative stress in placenta accreta.

Table 3. Multivariate logistic regression analysis of several factors for the prediction of placenta accreta.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P value</td>
<td>OR (95%CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Prior cesarean delivery</td>
<td>6.667 (1.342–33.125)</td>
<td>0.020</td>
<td>0.174 (0.041–0.744)</td>
<td>0.018</td>
</tr>
<tr>
<td>Prior curettage</td>
<td>7.000 (0.501–97.751)</td>
<td>0.148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS (mmol Trolox eqv./L)</td>
<td>1.580 (0.809–3.813)</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOS (µmol H₂O₂ eqv./L)</td>
<td>0.497 (0.295–0.835)</td>
<td>0.008</td>
<td>1.620 (0.597–4.395)</td>
<td>0.343</td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>0.999 (0.998–1.000)</td>
<td>0.009</td>
<td>0.999 (0.998–1.000)</td>
<td>0.035</td>
</tr>
<tr>
<td>ARES (kU/L)</td>
<td>0.983 (0.973–0.993)</td>
<td>0.001</td>
<td>0.981 (0.970–0.993)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P value: P < 0.05 statistically significant, TAS: total antioxidant status, TOS: total oxidant status, ARES: arylesterase, OSI: oxidative stress index, OR: odds ratio, CI: confidence interval, H₂O₂: hydrogen peroxide.
References


