Predictive Value of the Oxidative Stress Indices in Syrian Women with Threatened Abortion

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ABSTRACT

After 10 years of increasing conflict and violence in Syria, women face significant challenges associated with lack of adequate access to maternal health services, threatening their lives along with their immediate and long-term health outcomes, among them miscarriage, which has been increasing dramatically.

Our aim was to assess whether serum total antioxidant (TAS) capacity and total oxidant status (TOS) altered during first trimester pregnancies with vaginal bleeding and abdominal pain, and to investigate the accuracy of these biomarkers in the prediction of miscarriage.

In this cross-sectional study, A group of pregnant women with vaginal bleeding and abdominal pain (n=29) served as patient group and a control group of healthy pregnancies (n=30) were included. All of the participants in the two groups were matched for age and gestational age. All pregnant women were recruited from the Khaled Kendel hospital-Sarmada, Syria, and Blood samples were collected at 6–14 weeks from all participants for measurement of oxidative stress markers. The area under curve (AUC) was used to determine the predictive value of the oxidative stress indices.

The mean TOS level was significantly (P<0.05) higher in patients whereas mean TAS level was significantly (P<0.05) decreased in patient group compared to the control group. The AUC of TOS and TAS were (0.611, 0.895, P= 0.115, 0.001) respectively. The cut-off, sensitivity, and specificity of TAS were (> 0.98, 93.5 and 70.8) respectively. TAS can be used to discriminate between TM and the control subjects (sensitivity= 0.956; P <0.0001).

Our analysis of patients presenting with threatened miscarriage in this study presents, imbalance in oxidative stress markers, we recommended supplementing of anti-oxidants Throughout pregnancy. finally, Further high-quality research in this area is warranted to confirm our results.

KEYWORDS: Threatened miscarriage, oxidative stress indices, ROC curve, Syrian pregnant

INTRODUCTION

For the preservation of integrity and function of all cells and tissues during normal pregnancy, a well-controlled balance between the generation of reactive oxygen species (ROS) and the operation of the antioxidant defense components is essential. The amount of flowing lipid peroxidation products increases physiologically over time during pregnancy[1].

There are various causes of the increase in the severity of lipid peroxidation, but the most important are increased lipoproteins circulating, placenta prooxidant and altered basal metabolism during pregnancy[2].

A well-balanced prooxidant and antioxidant processes describe physiological pregnancy[3]. The increased activity of ROS followed first and foremost by increased levels of antioxidants, cu, zn and superoxide (SOD-1), maintaining a delicate balance between the ROS-producing cellular redox balances and defensive mechanisms that detoxify them[4].The production of highly reactive oxygen metabolites exceeds the antioxidant defensive potential and induces disturbance of cells' homeostasis[5].

As oxidative stress is the product of an uneasy equilibrium of prooxidants and antioxidants, only through controlling the prooxidant and antioxidant parameters during and after the pregnancy will it be quite challenging to evaluate this degree[6]. No specific antioxidant has been found to represent the overall protective reaction since it is possible to quantify the total antioxide potential and the collective function of antioxidants in blood and biological fluids, both enzyme and non-enzyme[7].

Many studies have demonstrated that oxidative stress (OS) in pregnancy has a direct effect on systemic and placental pathophysiologic systems, contributing to disorders involving endothelial and immune impairment of placental vascularization. OS is also considered an important factor in maternal pathology such as sudden and frequent

miscarriages, preeclampsia, preterm labor, and other conditions[8][9][10].

Recurrent pregnancy loss is characterized as three or more successive spontaneous abortions before 20 weeks of pregnancy are completed. This disease affects 0.5%-3% of women during the menstrual cycle, it is estimated[11]. The cause is not known in 50%-60% of cases involving recurrent miscarriage, and this is why they are classed as idiopathic. The implications for women may be significant psychological distress and gynecological problems, in particular with multiple miscarriages. Owing to the complexity of a comprehensive understanding of their process, the topic of idiopathic persistent error focuses continuously on doctors with different specialties[12].

Placental oxidative stress may be a result of early pregnancy that may lead to complications such as repeated abortions, preeclampsia and congenital diabetes disorders. Oxidative stress is expected to be a status in recurring pregnancy loss (RPL) etiopathogensis[13].

After eight years of war and abuse in Syria, women face major problems related to the lack of proper access to reproductive health facilities and risk their life along with immediate and long-term consequences, including miscarriage. In this study, our aim was to determine the markers of OS in patients with recurrent miscarriages and comparing the level of OS with normal pregnancy, by measuring total antioxidants (TAS) and total oxidative status.

Materials and methods
This study included a total of 55 pregnant women who have been admitted to Khaled Kendel hospital, department of Obstetrics and Gynecology, Sarmada, Syria. They were concordant with participation in the study, which was confirmed by their written consent in accordance with the criteria of the Helsinki Declaration. The Research Ethics Committee of Idlep University approved the study.

The criteria for entry into the study were pregnancy between the weeks of 11 and 12 and single tone pregnancy (pathological and cytogenetic evidence of prior pregnancies that no fetal defects or chromosome aberrations occurred) and three previous spontaneous abortions (singles). Only non-smokers were included in the study.

Multiple births, polyhydramniotics, maternal diseases (diabetes, asthma, obesity, preeclampsia, cardiac and renal failure and infections of the urinary tract), fetal disease (fetal delayed development) and local causes such as uterus and vagina anatomical malformations, cervical inadequacies, malignancies, alcohol, smoking or any drug use and other diseases impacting the stage became the requirements for exclusion.

5mlof venous blood samples were collected from the participants; the serum samples were obtained by centrifuge at 3000 RPM for 10 minutes and were eventually placed in the eppendorf tubes and stored at-20°C until analysis.

The total oxidant status (TOS) of serum was determined with the use of a new automatic colorimetric measuring system, discovered by Erel (Rel Assay Diagnostics®, Gaziantep, Turkey). Under this process, iron-o-dianisidine complex is oxidized into the ferric ions using oxidants in the sample[14]. The results are given in a liter equivalent of hydrogen peroxide (H_2O_2eqmicromol / L).

Absolute serum antioxidant status (TAS), assessed using a modern automatic colorimetrical measurement (Rel Assay Diagnostics ®, Gazyentep, Turkey). Find Erel's method. The hydroxy radical reacted with the Fenton reaction using this process to form a radical light-yellowish brow dianisyl substratum with colorless o-dianisidine. The findings were shown in Trolox as milli moles / liter equivalent (TroloxEqmmol / L) for calculation[15]. Oxidative stress index (OSI) was calculated by the ratio of TOS to TAS.

Urea, total proteins and albumin were determined by commercial assay kit (Biosystem- Spain) spectrophotometrically by a Biosystem BT 350as per manufacturer's recommendations in serum samples.

Statistical analysis:
Statistical analysis was performed using SPSS 21.0 for Windows version. Independent samples t- test analysis was applied normally distributed variables, while the Mann–Whitney U test were applied for non-normally distributed variables, and median (25 %–75 %) percentiles were presented. finally a p value <0.05 was considered as statistically significant. The area under curve (AUC) was used to determine the predictive value of the oxidative stress indices using MedCalc software.

Results:
In the present study, patient and control groups were compared and no significant difference was revealed in terms of age (30.08±5.28 vs. 28.53±6.19, p= 0.394), gestational weeks (10.28±2.44 vs. 11.26±2.49, p= 0.101), urea levels (20.08±6.67 vs. 17.50±5.32, p= 0.125), albumin levels (3.94±0.23 vs. 4.12±0.67, p= 0.198), and total proteins levels (5.75±0.52 vs. 5.81±0.73, p=0.714).

On the other hand, when we compared TAS, TOS and OSI levels of the patient and the control groups, there were significant differences between the groups. TAS levels (median (25-75 percentile)) (mmol /L) were (0.68 (0.23-1.15) vs. 1.78 (1.36-2.14), p= 0.0001), TOS levels (µmol/L) were (6.57 (5.06-8.18) vs. 4.07 (2.85-4.90), p= 0.0001) and OSI levels were (11.28 (5.16-22.09) vs. 3.51 (2.30-4.85), p= 0.0001), respectively (Table 1).

We demonstrated statistically significant decreased TAS level in pregnant women who hada history of recurrent pregnancy loss (p = 0.0001) (Fig. 1). We also observed significantly increased TOS and OSI levels in patient groups (p = 0.0001, separately) (Figs. 2, 3).

Receiver operating characteristic (ROC) curve analysis demonstrated that TAS was the best indicator for discriminating patients from controls with AUC, sensitivity, specificity, positive protective value, negative protective value (0.895, 0.93,0.70, 0.76, 0.93) respectively and cutoff (>0.98) at (P= 0.0001)Fig. 4. While the AUC of TOS was 0.61 with sensitivity and specificity (0.71, 0.54) respectively at (P =0.15) Fig. 5.

**Table 1: Oxidative stress markers values of studied groups:**

| Marker	Patient group (n= 25) Median (25-75%)	Control group (n= 30) Median (25-75%)	P value
TAS (mmol /L)	0.68 (0.23-1.15)	1.78 (1.36-2.14)	0.0001
TOS (µmol/L)	6.57 (5.06-8.18)	4.07 (2.85-4.90)	0.0001
OSI	11.28 (5.16-22.09)	3.51 (2.30-4.83)	0.0001
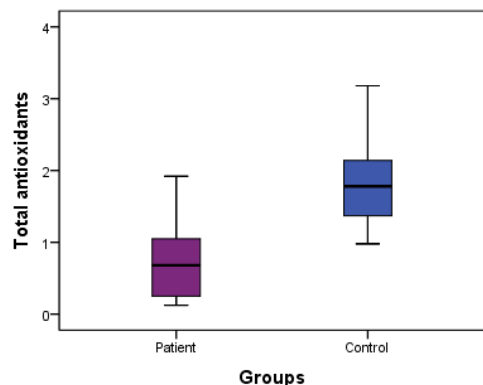
Fig.1: Box blot of TAS levels (mmol/L) in patient and control groups
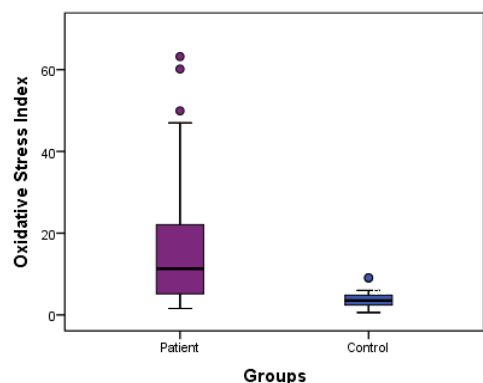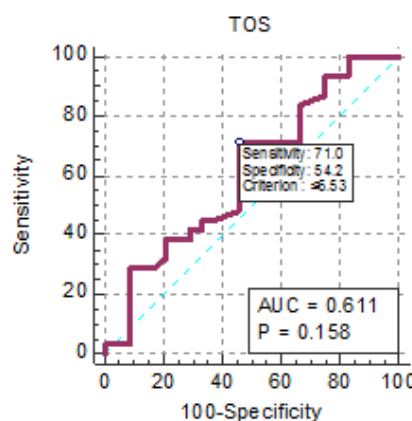

Fig.2: Box blot of TOS levels (µmol/L) in patient and control groups
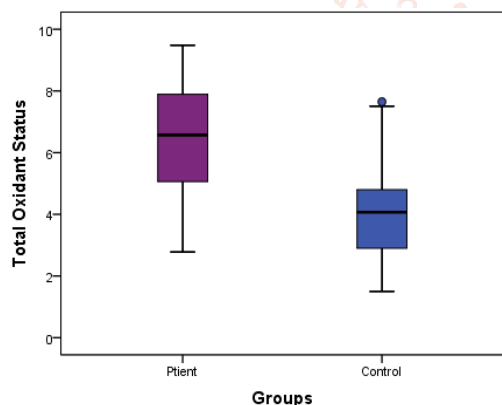
Fig. 3: Box blot of OSI levels in patient and control groups
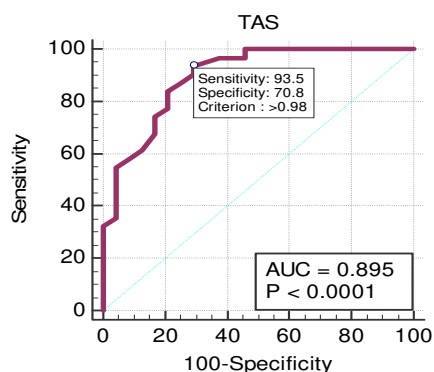
Fig.4: ROC curve for serum TAS in predicting of patients from controls

Fig. 5: ROC curve for serum TOS in predicting of patients from controls

Discussion:

Latest reports of early pregnancy loos, pre-eclampsia, and hydatidiform mole complications indicate that these are the findings of a typical pathophysiology. In first trimester the abnormal placentation leads to oxidative stress and subsequent endothelial dysfunction plays a vital role in the development of pregnancy complications, such as abortion[16][17][18].

Safronova et al. studied the generation of active oxygen formed by blood granulocytes in women with a history of habitual abortions (2–3 spontaneous abortions in the first trimester, undeveloped pregnancies). In patients with a history of loos repeated pregnancy relative to control group with normal reproductive function, they observed that development of active oxygen species in granulocytes could be increased[19].

Vural et al[20] have been tested for the evaluation of impaired antioxidant pathways by ascorbic acid, alpha tocopherol, total thiol, ceruloplasmin, uric acid, albumin and glutathione amounts. They found that defenses against damaged antioxidants could cause recurring abortions; recurring abortions could also lead to oxidative stress and degradation and poor protection of antioxidants.

Increased peroxidation in lipids and decreased in antioxidant levels contrasted with the control group in women with recurring pregnancy loos was found by Simsek et al. [21]

El-Far M, etal.[22] evaluated the amounts of Glutathione, glutathionereductase (GSH-R), glutathioneperoxidase (GSHPX), catalase (CAT), superoxide dismutase (SOD), oxide nitric (NO) and malondialdehyde (MDA), and TNF-alpha, between the recurrent pregnancy loss and control group of 40 (20 females in their first trimesters and 20 females that have been non-pregnant). They showed that antioxidant protection is compromised and oxidative reactive species increase together, which can contribute to recurring abortion because of potential oxidative damage.

In this study, levels of TOS and OSI, were significantly increased while TAS levels were significantly decreased among pregnant patients and control groups. The best predictive value was for TAS to discriminate between patients and control in cutoff (>0.98) at (P= 0.0001).

In our view, the rise in TOS and OSI levels and decreased in TAS levels may be a result of repeated pregnancy loss instead. If our use is justified, we will use this data for care or if it is the result of calculating pregnancy risk.

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