

# Proinflammatory cytokines and oxidative stress are key participant in recurrent spontaneous abortion

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(Submitted: 02 February 2017 – Revised version received: 15 April 2017 – Accepted: 28 May 2017 – Published online: 21 June 2017 )

**Objective** To evaluate the association of oxidative stress/impaird antioxidant defense system and inflammation with the occurrence of recurrent spontaneous abortion (RSA).

**Methods** This study consisting of 30 pregnant women with a history of RSA (study group) and 30 healthy pregnant women (control group), their age ranging from 15 to 45 years. Blood samples were collected and tested for the measurement of antioxidants (SOD, CAT), MPO and proinflammatory cytokines. Student *t*-test was used for statistical analysis.

**Results** Serum levels of SOD and CAT were significantly lower ( $P < 0.05$ ) and serum level of MPO was significantly higher ( $P < 0.05$ ) in the study group as compared with the control group. A significant increase in TNF levels was observed ( $P > 0.05$ ) between the two studied and control group, while IL-6 levels change was non-significant.

**Conclusions** Our data demonstrate that reduction impaired antioxidant defense and elevation of MPO and TNF, and the presence of possible interrelationship between those parameters may be related at least partly to the pathogenesis of RSA.

**Keywords** recurrent spontaneous abortion, oxidative stress, antioxidant, proinflammatory cytokines, myeloperoxidase

## Introduction

Abortion is a common obstetric complication in early pregnancy, mostly presenting with vaginal bleeding. It is a situation of spontaneous loss of pregnancy before 20<sup>th</sup> weeks gestational age or before fetal weight reaches 500 g and fetus reaches the viability.<sup>1</sup> Abortion can be subdivided into: threatened abortion, inevitable abortion, incomplete abortion, missed abortion, septic abortion, complete abortion, and recurrent spontaneous abortion.<sup>2</sup>

Almost all of the abortions are spontaneous, only 1% recurrent pregnancy loss occurs because of risk factors in the parents.<sup>3</sup> Recently, many studies began investigating the role of oxidative stress in abortion pathogenesis.<sup>4</sup> Clinical and experimental evidence suggesting that oxidative stress is one of the main underlying mechanisms in the pathogenesis of disease process continuum such as spontaneous abortion, hydatidiform mole and preeclampsia.<sup>5</sup> Oxidative stress defined as an imbalance between pro-oxidant and antioxidant levels. This rate may happen with the reduction of antioxidant defense mechanisms or change of increased levels of ROS and/or reactive nitrogen species.<sup>6</sup> Consequently, it may cause disorders in normal redox levels of tissues, peroxides in all cellular compartments, including DNA, RNA, lipids and proteins, and toxic effects because of a generation of free radicals.<sup>7</sup> During the pregnancy, the oxidative stress occurs in response to the inadequate fetoplacental energy. Oxygen pressure in the intervillous space is about 20 mmHg during the first trimester, placental tissues contain a low activity of antioxidants such as catalase, SOD, GPx, Cu/Zn and Mn, because of low concentrations in syncytiotrophoblasts, the tissue becomes susceptible to oxidative stress.<sup>8</sup> Myeloperoxidase (MPO) is the a haem proinflammatory enzyme that is most abundant in granules of human inflammatory cells such as activated neutrophil and macrophage.<sup>9</sup> MPO has been found to be involved in different diseases, including atherosclerosis,<sup>10</sup> myocardial infarction,<sup>11</sup>

and transplant rejection.<sup>12</sup> Its oxidant products are a part of the inflammatory cascade initiated by these diseases. Recent studies have suggested roles for MPO in abortion. However, the role of MPO in the pathogenesis of abortion is still unclear.

The maternal immune system plays a critical role in the establishment and maintenance of pregnancy and successful birth.<sup>13</sup> This role is primarily accomplished through many hormonal changes as well as alteration in the cytokine concentrations both locally (within the uterus) and systematically.

The cytokine roles have been suggested to be involved in various clinical studies including pregnancy.<sup>14</sup> The most important of these are trophoblastic invasion and placental proliferation and angiogenesis.<sup>15</sup>

Tumor necrosis factor- $\alpha$  is a Th1 response cytokine produced by macrophage, T-lymphocyte, basophils and monocytes, during pregnancy. This cytokine could be encountered which render some authors to state that whenever the level of TNF- $\alpha$  increase abortion occurs.<sup>16</sup> In a recent study, Er<sup>17</sup> found that azithromycin treatment significantly decreased the abortion rate in pregnant rats treated with lipopolysaccharide. This effect was associated with decreased serum levels of TNF- $\alpha$ . IL-6 is a multi-functional Th2-type cytokine produced by immune cells, fibroblasts, endothelial cells, adipocytes, and myocytes.<sup>18</sup> Increased plasma levels of IL-6 were reported in sporadic miscarriage.<sup>19</sup> Furthermore, elevated levels of IL-6 in the placenta, amniotic cells, and deciduous have been demonstrated in pregnancies complicated by preterm premature rupture of the membranes, intrauterine infection, and prematurity.<sup>20</sup> This study aimed to investigate the overlapping roles of TNF- $\alpha$ , IL-6, MPO and oxidative stress in pathogenesis sequences of abortion. The study hypothesized that abortion might be related to an imbalanced oxidation system and expression of inflammatory cytokines. In the present study, we compared the levels of SOD, CAT, MPO, TNF and I-6 serum from women who suffered abortions.

## Materials and Methods

### Subjects

Sixty pregnant women were recruited as samples in this study. Each group comprised of 30 women. Those with spontaneous abortion in case group and those with normal pregnancy in the control group were recruited for this study from July to September 2015. The inclusion criteria applied for cases were women with normal pregnancy who experienced a threatened abortion at 8–14 weeks gestational age, live embryo with heart beat demonstrated by ultrasound measurements, and vaginal bleeding at the time of the examination. Whereas women with normal pregnancy who carried to term were included as controls. The study was approved by the ethical committee of College of Medicine/ Al-Diwaniyaha University, and consent forms were obtained from each participant.

### Blood Samples

Venous blood (5 ml) was collected from each woman at the time of recruitment. Whole blood was transferred into plain tube and left it for 30 min, then centrifuged at 3000 rpm for 10 min; serum was separated and stored at 20°C until be used.

### Biochemical Estimation

All biochemical parameters including serum catalase (CAT), superoxide dismutase (SOD), MPO activity TNF- $\alpha$  and IL-6 were estimated, in duplicate, by ELISA kit (HSTA00C, RnD Systems Europe, Ltd, Abingdon, UK). The range of measurement was 0.5–32 pg/ml, 0.156–10 pg/ml respectively.

### Statistical Analysis

All the analysis were carried out on SPSS 16.0 version (Chicago, Inc., USA). The results are presented in mean  $\pm$  SE. The paired *t*-test was used to compare discrete variables between cases and controls. The *P*-value <0.05 was considered significant.

## Results

Mean age was similar among all the studied groups: 30.4 (range 21–34) years in case Group A, 27.2 (range 22–34) years in control, (*P* = 0.34). Similarly, mean gestational age did not differ among all the studied groups: 8.5 (range 8–10) weeks in patient group on admission, and 8.2 (range 8–9.8) weeks in control group, (*P* = 0.20). There were no significant (*P* > 0.05) difference in the mean age between patients and controls. The comparison of oxidative stress parameters of both cases and control group is summarized in Fig. 1. It was observed that the mean  $\pm$  SEM values of serum level of antioxidant enzymes – SOD was 90.57  $\pm$  13.63 (Pg/ml), CAT was 157.75  $\pm$  11.18 (Pg/ml) – were observed to be significantly (*P* < 0.01) decreased among the patient group compared to control group. However, the results significantly (*P* < 0.01) increased mean  $\pm$  SEM serum MPO 439.208  $\pm$  54.16 (Pg/ml), in patients compared to control group Fig. 2.

Figure 3 shows the serum levels of TNF- $\alpha$  and IL-6, in case group as well as in control. The mean  $\pm$  SEM TNF- $\alpha$  levels were significantly different in the patient group

(*P* < 0.05; Fig. 2). Serum values of TNF- $\alpha$  patient group was significantly higher when compared to those in control group (324.42  $\pm$  32.09 Pg/ml vs. 118.6  $\pm$  22.09 Pg/ml, *P*  $\pm$  0.02) and normal pregnancies (39.2  $\pm$  9.5 Pg/ml vs. 10.9  $\pm$  0.8 Pg/ml, *P*  $\pm$  0.01 (Fig. 3). There was no statistically significant difference in the mean  $\pm$  SEM serum IL-6 level between the patients with abortion and those with normal pregnancies (88.75  $\pm$  20.83 Pg/ml vs. 20.83  $\pm$  11.8 Pg/ml, (Fig. 4).

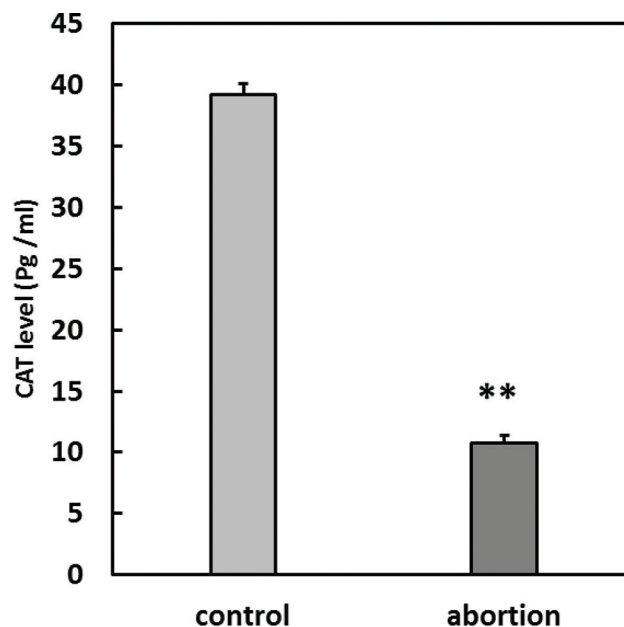


Fig. 1 Serum CAT levels in patients with RSA. Serum samples were isolated from the blood of patients with RSA. CAT levels was assessed by ELISA. Data are expressed as means  $\pm$  SEM, for 30 patients, *n* = 30 with triplicate measurements. \*\* indicates significant differences compared to the control (Student's *t*-test, *P* < 0.01).

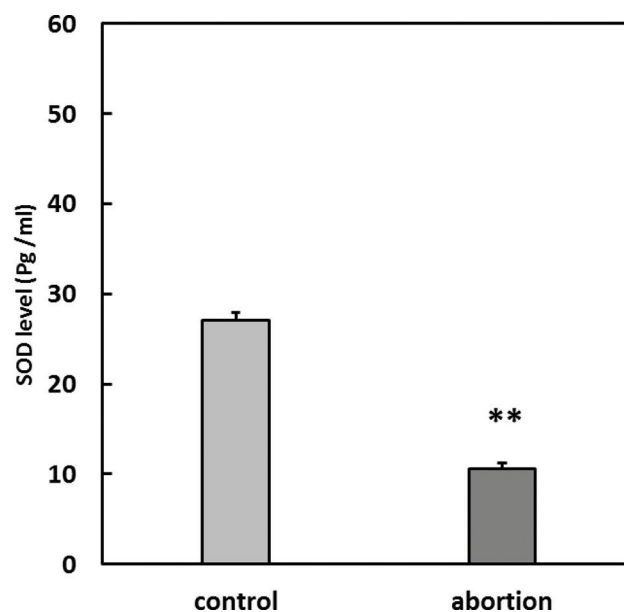


Fig. 2 Serum SOD activity in patients RSA. Serum samples were isolated from the blood of patients with RSA. SOD levels was assessed by ELISA. Data are expressed as means  $\pm$  SEM, for 30 patients, *n* = 30 with triplicate measurements. \*\* indicates significant differences compared to the control (Student's *t*-test, *P* < 0.01).

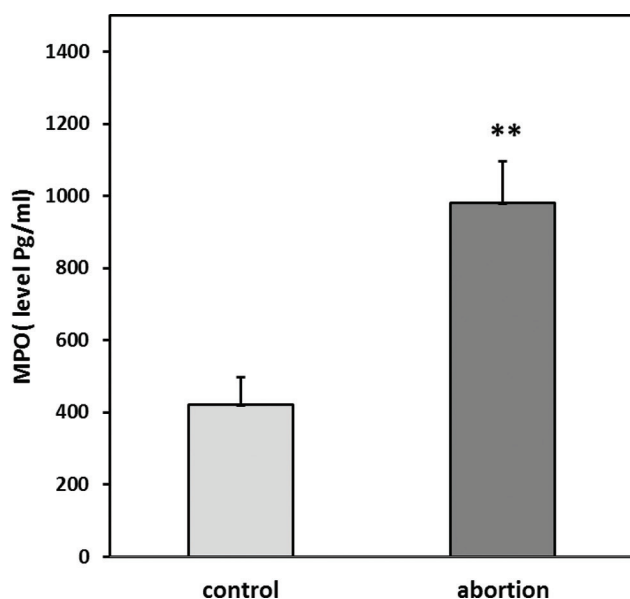


Fig. 3 Serum MPO levels in the patients with RSA. Plasma serums were isolated from blood of patients with RSA and control. MPO levels were analysed by ELISA. Data are expressed as the means  $\pm$  SEM, for 30 patients,  $n = 30$  with duplicate measurements. \*\*indicates significant differences compared to the control (Student's *t*-test,  $P < 0.01$ ).

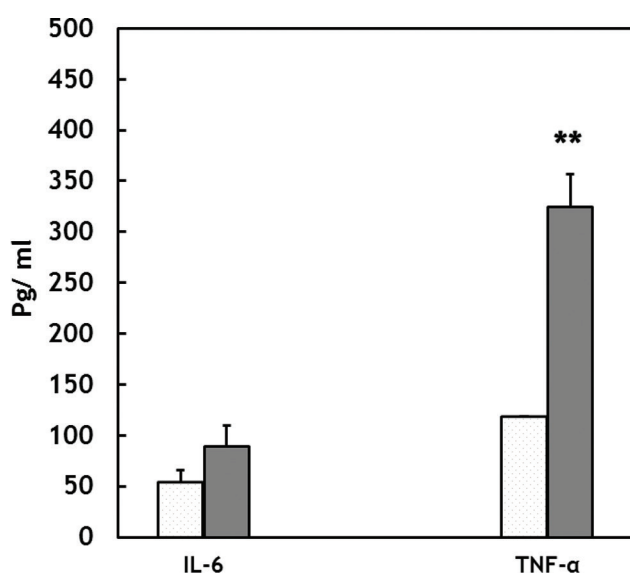


Fig. 4 Serum pro-inflammatory cytokine levels in the patients with RSA. Plasma serums were isolated from blood of patients with RSA and control. TNF- $\alpha$  and IL-6 levels were analysed by ELISA. Data are expressed as the means  $\pm$  SEM, for 30 patients,  $n = 30$  with triplicate measurements. \*\*indicates significant differences compared to the control (Student's *t*-test,  $P < 0.01$ ).

## Discussion

Oxidative stress-induced damage and increased pre-inflammatory cytokines have been hypothesized to affect on pregnancy, playing a fundamental role in spontaneous abortion and idiopathic recurrent pregnancy loss. When oxidative stress develops during pregnancy, it can impair placental development and/or enhance pregnancy loss.<sup>21</sup> In the present study, the results showed a significantly low level of SOD, CAT, in spontaneous abortion as compared to control while MPO level was found to be

increased compared to control. Similar findings were reported in recent studies. They found significantly low levels of the antioxidant enzymes GPx, SOD, and catalase in patients with idiopathic RPL, in addition to increased MPO levels.<sup>22</sup>

CAT and SOD are metalloproteins and accomplish their antioxidant function by enzymatically detoxifying the peroxides ( $-OOH$ ),  $H_2O_2$  and  $O_2$ , respectively. CAT has been suggested to provide an important pathway for  $H_2O_2$  decomposition into  $H_2O$  and  $O_2$ . Oxidative stress, of which lipid peroxidation represents a major manifestation, is involved etiologically in a variety of clinical conditions including pregnancy and miscarriage.<sup>23</sup> Oxidative stress will in turn lead to impaired placentation, which begins as blood flow disruption in the intervillous area and progresses to pregnancy termination. Abortion itself happens as the result of inadequate trophoblast invasion, and resulting in adequate supply from the spiral arteries.<sup>24</sup> The results of the present study are in accordance with previous reports suggesting that low antioxidant level increases the risk of spontaneous abortion.<sup>25</sup> Zhu et al. compare ROS, SOD and hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) levels in trophoblastic tissues between the cases with patients having missed abortion in the first trimester and cases with control group having abortion in the same trimester. As a result of this study, they suggest three factors in the pathogenesis of missed abortion. First, the ROS can cause the lipid peroxidation injury in the embryo. Secondly, increasing ROS levels can change the oxygen partial pressure in embryonic cells. Thirdly, increased generation of ROS can lead to a vicious circle of ischemia-reperfusion injury in the embryo and thus to incomplete growth. They argue that ROS, SOD and HIF-1 $\alpha$  levels could be important in missed abortion.<sup>26</sup>

The result of the present study revealed a relationship between the pregnancy outcome and MPO level. According to the recent studies, high MPO activity promotes increased generation of hypochloric acid, which actively interacts with biological amines, or reducing chloramines.<sup>27</sup> The increase in total peroxidase correlated with decrease in the antioxidant enzyme activity. Therefore, MPO seems to play a role in the pathogenesis of abortion when the antimicrobial barrier of fetal-placental system is weakened. This shows that oxidative stress biomarkers are shifted in favour of free radicals, and the damage of tissues may be induced in response to it.

The effects of pre-inflammatory on the concepts and thus on the success or failure of pregnancy are interesting and have been investigated in this study. The data reported a significant increase in TNF- $\alpha$  levels in women with spontaneous abortion compared to control. Evidence supporting this study showed that the administration of one of the Th-1 cytokines like IL-2 to normal pregnant mice causes abortion by increasing fetal resorption rates.<sup>28</sup> Therefore, the present results revealed a significant association between raised serum Th-1 cytokines (TNF- $\alpha$ ) and first trimester pregnancy loss, strengthen the relation between these agents and fetal rejection. IL-6 is, pro- as well as anti-inflammatory cytokine. Because of its properties, IL-6 has been suggested to counterbalance detrimental effects of Th1-type cytokines.<sup>29</sup> In present study, no significant differences in circulating IL-6 levels were reported for pregnant women at  $<20$  weeks of gestation with spontaneous abortion, in comparison to healthy pregnant women. These results are in agreement with the recent study by Arslan et al.,<sup>30</sup> which suggest that, an increased Th1-type immune response (in present study, increase TNF- $\alpha$ ) cannot be counterbalanced by the unaltered Th2-type immune response, thus resulting in

negative obstetric outcome. Furthermore, this result demonstrates that in spontaneous abortion, the shift in Th1/Th2 ratio is attributed only to the increase of Th1- type immune response, as it has been proposed for other types of spontaneous abortion.<sup>29</sup>

Viral infections are also potentially affect the cytokine levels of pregnant women. Therefore, one of the possible important limitations of this study is the determination of underlying infections. Both infection and inflammation have the ability to change the levels of circulating cytokine levels.

## Conclusion

Oxidative stress and ROS-induced damage in cell are brought under control by antioxidants and so no cell injury

occurs. During the pregnancy, placenta, which plays a key role in the growth and development of the fetus, an imbalance between oxidants and antioxidant level, oxidative stress occurs, and this causes injury in trophoblastic tissues. In cases where it is impossible to control such injury, pregnancy loss can occur. Oxidative stress-mediated differences in cytokine activity have been reported for normal pregnant women and those experiencing pregnancy failure. The hypothesis of Th-1 cytokines involvement in the pathogenesis of miscarriage is indicated.

## Conflict of Interest

None. ■

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