



Short Communication

Effect of Early Spontaneous Abortions on Reactive Oxygen Species, Antioxidants and Lipid Damage in Women

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ABSTRACT

In pregnancy, any disturbance in the balance between antioxidants and oxidants in favor of the later can cause serious problems such as abortion. So the investigation of the oxidants/antioxidants status during early stages of pregnancy is urgently needed to help us solving such health issues. In this current study, One hundred and thirteen aborted women (73 known causes group, 40 unknown group) and control group (23 normal delivery women (control positive), 10 normal unmarried women (control negative) were recruited in this study. Levels of lipid peroxidation (MDA level), catalase activity (CAT), total antioxidant capacity (TAC) and reactive oxygen species (ROS) were measured in the serum of all studied groups. MDA levels were significantly higher ($p \leq 0.05$) in aborted women with *T. gondii* compared to negative control. MDA levels were also significantly higher ($p \leq 0.05$) among aborted women with Cytomegalo virus compared to negative and positive control groups. All aborted women had significantly higher levels of TAC levels ($p \leq 0.05$) compared to the control groups. However, catalase activity and ROS levels in all the aborted women did not differ statistically from that of control. Our data indicated that the aborted women had increased the level of serum oxidative stress (MDA) and this elevation may be driving the detrimental effects induced by parasitic or viral infections.

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Authors' Contribution

ATSH designed the experiment. FHA conducted the lab work. Both authors wrote the manuscript.

Key words

Malondialdehyde, Catalase, Antioxidant, Oxidative stress, Abortion

Toxoplasma gondii is an intracellular parasite that is capable of infecting all warm-blooded animals including humans (Dubey, 2009). In most cases, it could cause abortion, immunocompromised individuals, encephalitis and death (Halonen and Weiss, 2013). Transmission in humans is usually occurred via the ingestion of undercooked or raw meat containing cysts or consumption of contaminated food or water with oocysts, or congenitally when the mother got the infection for the first time during pregnancy, where *T. gondii* easily transported to maternal blood and then affected the fetus via placenta (Tenter *et al.*, 2000). Spontaneous abortion is among the most gestational complications occurred in the responding to this parasitic infection (Lyu *et al.*, 2013). In the first trimester of pregnancies, more than 80% of spontaneous abortions could be occurred (Cunningham *et al.*, 2014) and the risk of abortion is boosted with increasing pregnancy age (Juliano *et al.*, 2008). Oxidative stress is an unavoidable aspect of aerobic life (Odo *et al.*, 2017). Reactive oxygen species (ROS) are molecules that are produced as byproducts of oxygen metabolism in all cells and including superoxide anion,

hydrogen peroxide, and hydroxyl radical (Jothery *et al.*, 2016). These molecules can also be generated during microbial infection, for example, the immune effectors such as eosinophils, neutrophils, and macrophages produce superoxide anion as a part of the oxidative explosion (Nathan and Shiloh, 2000). When the levels of ROS production are increased over the antioxidants capacity, oxidative stress (OS) occurs. Consequently, leading to damage among different biological molecules such as lipid, protein and DNA (Hung, 2007; Cencioni *et al.*, 2013). However, the effects of ROS can be mitigated by internal antioxidants and repair system (Halliwell and Gutteridge, 1995). Antioxidant defense mechanism involves both enzymatic and nonenzymatic system (Halliwell and Gutteridge, 1995). Catalase is one of the enzymatic antioxidant that can mitigate hydrogen peroxide and covert it to water (Kohen and Nyska, 2002). One of the main product derived from oxidative damage on lipids is malondialdehyde (MDA) (Niki, 2009). Yigenoglu *et al.* (2011) have studied the level of oxidative stress in pregnant women in first trimester with a history of frequent pregnancy loss and they found that the amount of oxidative stress levels are higher combined with the reduction of total antioxidants. Some other studies suggest that the reduction of antioxidants among pregnant women could be a serious risk factor of spontaneous abortion (Vural *et al.*, 2000). Therefore, measurements of the levels of oxidative

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damage and antioxidants among infected women who had abortion could expand the current understanding on the role of oxidative stress as a proximal mechanism in developing such medical problem.

Materials and methods

One hundred thirteen enrolled women (73 known causes and 40 unknown causes) were recruited in this study. Subjects that have chronic and genetic disease, infections, previous and recurrent abortions and smokers were excluded from the current study. Twenty-three enrolled women were considered as positive controls (normal delivery) and ten unmarried women were enrolled as negative controls.

Blood samples (5 ML) were collected from all enrolled women in appropriate sterile tubes by arm venous puncture. Serum was separated from whole blood by centrifugation at 2000 R.P.M for 5 minutes, then collected serum was kept at -20°C until being used.

Serum anti-toxoplasma (IgG and IgM) and anti-cytomegalic (IgG and IgM) antibodies were identified by enzyme linked immunosorbent assay (ELSA) using a commercial kit (Company Calbiotech, USA).

Total ROS in serum was determined using a method developed by Erel (2005). TAC were measured by using CUPRAC (cupric reducing antioxidant capacity) spectrophotometric method (Apke *et al.*, 2008). Serum lipid peroxidation was estimated by thiobarbituric acid reactive substances (TBARS) (Buege and Aust, 1978). Catalase activity was measured according to the method mentioned in Hadwan and Abed (2016).

The data presented here were tested for normality (Shapiro-Wilk) and the correction was performed for those non-normal distributed data. Statistical Analysis and histograms were performed utilizing Sigma-plot System (version 12.5) software. One-way ANOVA and Turkey post-tests were performed to explore the significant differences among studied groups. Mean±standard deviation were applied in the current study. Values of $p < 0.05$ were considered statistically significant.

The protocol of current study was conducted according Iraqi federal and local ethical committee policies, where all principles such as respect to persons, beneficence, risk and justice were accounted. Control and subjects were informed about the aim and details of the study.

Results and discussion

Figure 1 shows the levels of ROS, Total antioxidant capacity, catalase activity and MDA in different groups. The ROS levels were generally reduced non-significantly in all aborted women groups compared to what we have seen in the control groups.

Post-hoc analysis showed significant (P value= 0.001)

variations in serum total antioxidants and catalase activity between unknown aborted women and controls (negative

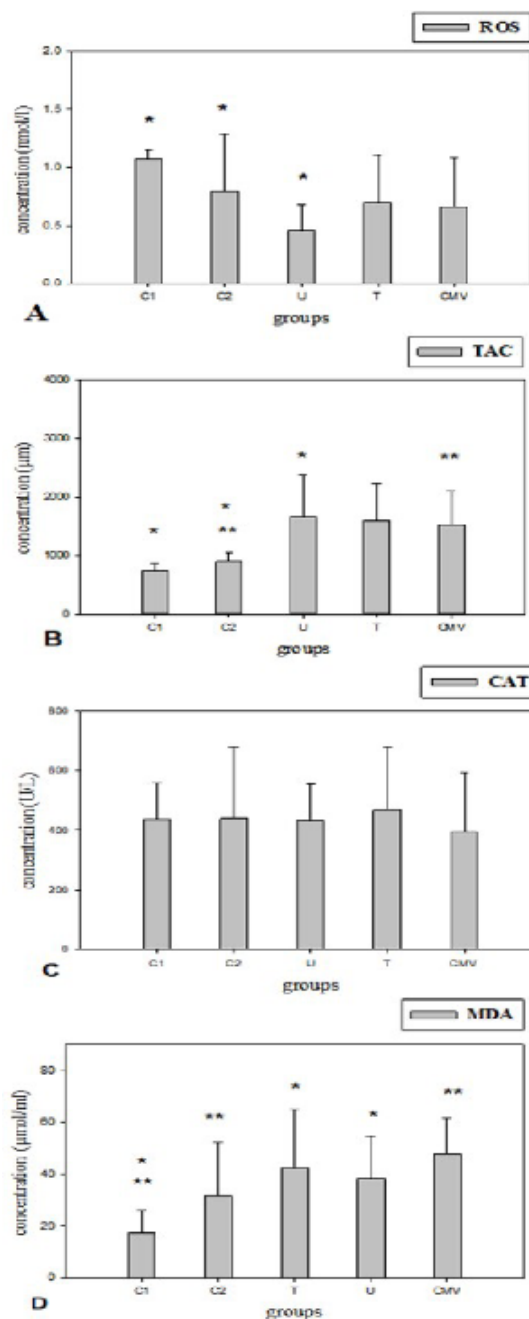


Fig. 1. Concentration of reactive oxygen species (nmol/l) (A), total antioxidant capacity(µM) (B), catalase activity (U/L) (C), and MDA (µmol/ml) (D) in aborted women with unknown abortion, *T. gondii* and *Cytomegalovirus* comparing with controls. Where C1, negative control; C2, positive control; U, unknown abortion; T, *T. gondii*, CMV, Cytomegal virus. Different stars indicate significant differences between groups, post-hoc Tukey tests, $p < 0.05$.

and positive). In addition, same direction was also true for the levels of serum total antioxidants (TAC) between Cytomegalic group and control positive groups. The levels of enzymatic antioxidant activity (Catalase) were, however, not significantly (P value < 0.05) changed among all groups.

Lipids oxidative damage was significantly (P -value=0.001) greater in all aborted women compared to the values observed in the control groups. This difference, however, disappeared among aborted women from different groups. The correlation matrix across different measured variables within each group revealed that only lipid oxidative damage in aborted women with *T.gondii* was positively correlated with the levels of their total antioxidants capacity (Pearson correlation=0.68, $P=0.02$) (Fig. 2). However, there were no significant correlations among other variables in all studied group ($p>0.05$).

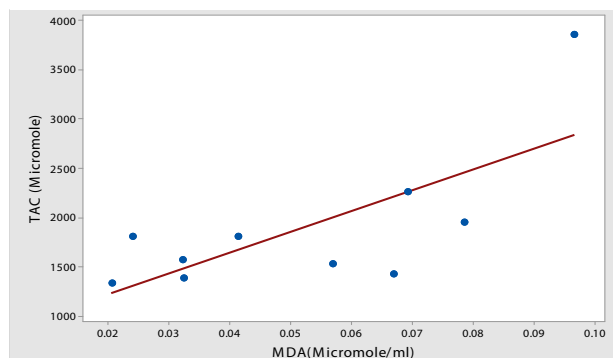


Fig. 2. The correlation between the concentration of MDA (Mean \pm SD; μ mol/ml) in aborted women with *T. gondii*, and correlation of total antioxidant capacity (TAC; μ m).

In the current study, we have investigated the oxidative status by measuring the ROS, antioxidants (catalase and total antioxidants) and lipid oxidative damage in aborted women and compared that with healthy women who had normal delivery. In agreement with the previous research (Rafeeimia *et al.*, 2014), aborted women had experienced greater levels of oxidative damage measured by serum MDA. In addition, this damage was not significantly changed among different subgroups of abortion. However, the changes observed in aborted groups compared with the controls did not seem to reflect to the changes found in the levels of ROS. Although the exact reason for such elevation of MDA is unknown, one possible mechanism is that the host's cell initiated to produce more free radicals which are important to create the toxic environment for killing the invading parasites (Dincel and Atmaca, 2016) and the elevation of these free radicals would have been more pronounced in the tissues rather than blood. This

might be a possible explanation for why we did not detect any significant change in the levels of ROS among groups. The increased levels of lipid damage was seen to be paralleled with high levels of serum total antioxidants among aborted women, suggesting that the subjects may have been under physiological stress which was expressed by greater levels of TAC. This potential explanation was confirmed by our findings in which the levels of MDA were positively correlated with levels of TAC (Pearson correlation=0.68, $P=0.02$), indicating that the women with higher lipid damage increased their antioxidant capacity as a compensating approach. It has been suggested that the levels of antioxidants are changeable according to the level of oxidative damage especially in toxoplasmosis (Bahrami *et al.*, 2016). Evaluation of total antioxidants rather than single antioxidant has been recommended as the former could reflect to all of the antioxidant capacity including enzymatic and non-enzymatic enzymes (Jothery *et al.*, 2016). Thus providing us with a comprehensive picture of the balance between oxidants and antioxidants capacity in the measured system. Previous work showed considerable evidences for the variations in the oxidative stress status (MDA and SOD) among women who had toxoplasma infections (Atmaca *et al.*, 2015). Also, experimental work on animals infected with Toxoplasma showed significant differences in endogenous antioxidant biomarkers such as CAT, SOD, MDA, GPX and GSH measured heart and liver (Türkoğlu *et al.*, 2018). In the same direction, a study done by Dincel and Atmaca (2016) on mice brain tissue indicated a significant increase in glutathione reductase and decrease in SOD activity. Since it has been demonstrated that the oxidative damage can vary among the biological molecules being measured (Jothery *et al.*, 2016). It would therefore be interesting if other markers of oxidative damage like protein and DNA damage are to be tested in the context of aborted women which could expand our understanding of the candidate mechanism underlying the abortion among pregnant women.

Conclusions

Our findings have highlighted the importance of an oxidative damage as a proximal mechanism that could drive the abortion among pregnant women. In addition, other biomarkers of oxidative damage to the biological molecules as well as different kinds of repairing markers should urgently be needed to explore more about the role of oxidative stress for aborted women. It is also suggested that the antioxidant supplementation could be an option for reducing the expected oxidative damage during pregnancy. Thus could potentially minimize the level of frequent pregnancy loss.

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Conflict of interest

Authors have declared no conflict of interest.

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