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# Evaluation Some Antioxidants and Oxidative Stress index in Seropositive Toxoplasmosis in Pregnant Women in Ramadi city of Iraq

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**Abstract**

Toxoplasma gondii (known as T. gondii) is an agent that causes toxoplasmosis- an infection caused disease. One third of the human population is infected by Toxoplasma gondii. This study has been conducted, in the Gynecology and Obstetrics department in Al-Ramadi Teaching Hospital from November 2019 to February 2020, to analyse the effects of toxoplasmosis, in pregnant women, on some enzymatic (Superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT)), non-enzymatic (vitamin C, vitamin E, Glutathione (GSH), and Albumin) antioxidants and Oxidative stress index (Malondialdehyde (MDA)). Forty-four blood samples were collected from infected women whose age ranges were 20-45 years. Also, 22 blood samples from non-infected pregnant women as a control group were used. The results showed a significant decrease in the concentration of SOD, G-Px, CAT, vitamin C, vitamin E, GSH, and Albumin compared with control groups. However, a significant increase in the concentration of Oxidative stress index MDA in comparison with the control group. This study concluded that infection with Toxoplasma plays a role in oxidative stress induction and reduced the antioxidant – defends systems.

**Keywords:** Albumin; Antioxidants; Ascorbic Acid; Catalase; Glutathione; Superoxide Dismutase; Toxoplasmosis

## 1. Introduction

Toxoplasmosis is one of the common parasitic infections in tropical and subtropical climates. Its causative agent is Toxoplasma gondii (T. gondii). Toxoplasma gondii is a prevalent parasite, which may infect nearly every animal of warm blood and invade all nucleated cells. This has enabled this obligate intracellular parasite to infect warm-blooded animal all around the world in terrestrial and marine biomes populations [1].

Human beings are infected after birth by eating or drinking something polluted with oocysts, consuming tissue cysts of undercooked meat, or by unintentionally consuming the oocysts of the environment [2]. Toxoplasmosis causes a range of clinical symptoms in humans, although most people do not show any signs. The infection may be very hazardous and fatal in some people including fetuses, newborn babies, pregnant women and people with immunodeficiency [3]. The parasite can pass from the placenta to the fetus, causing congenital toxoplasmosis once the woman is infected in the course of pregnancy. T.gondii can also affect the central nervous system with disease and ocular which results in vision abnormalities and the failure of growth and hearing [4]. Unfortunately, no vaccine can stop the infection by this parasite. However, there are only a few numbers of effective and active medicines to cure severe T.gondii, and none is capable of removing the tissue cysts [5,6]. In addition, an imbalance between oxidative agents and antioxidant can lead to serious complications in pregnant women such as abortion, because pregnancy is characterized by low defense against prooxidizing agents [7]. The body of human owns a complicated antioxidant defense system containing the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). This system hinders the commencement of free radical chain reactions and both SOD and CAT are both necessary for intracellular survival of T. gondii. Therefore, antioxidant enzymes represent potential candidates for the prevention of toxoplasmosis [8,9]. The main objective of this study was to estimate some antioxidants levels like enzymatic antioxidants: Superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT), and non-enzymatic antioxidants: vitamin (C, E), Glutathione (GSH), Albumin and Oxidative stress index MDA, in serum of positive toxoplasmosis pregnant women. The results of the infected women were compared with the results of the control groups (uninfected pregnant women) to better understand toxoplasmosis pathogenesis.

## 2. Materials and methods

Forty-four blood samples were collected from seropositive-Toxoplasmosis women and from (22) seronegative Toxoplasmosis women. The age range was 20-45 years old and the women had been referred to Gynecology and Obstetrics Teaching Hospital in Ramadi, Anbar from November 2019 until February 2020. None of the participants of this study took a medication or supplementation upon entering the study. Serum was separated immediately and stored at – 20ºC until

biochemical analysis. Enzyme linked immunosorbent assay (ELISA) test was performed on all of the samples using immunoglobulin G (IgG) Kit (Human, German) and the final results were recorded by ELISA reader (optical absorbance, OD = 450).

**2.1. Laboratory tests**

This study has conducted all the eight following laboratory tests:

1-SOD activity: The study determined this activity in erythrocyte hemolysates in accordance to Sun et.al's method [10]. In this method, the rate of nitroblue tetrazolium (NBT) reduction is provided to blue formazan by the superoxide anion which the xanthine oxidase (XOD) reaction generates. This reaction is spectrophotometrically monitored at 560 nm. One unit of SOD was regarded a 50% reduction of NBT inhibition under the assay conditions. The results were in U/g Hb.

2-Glutathione peroxidase activity: In this study, this activity in the serum was measured colorimetrically adopting Nelson and Kulkarni's method [11].

3-Catalase activity: The catalase activity was spectrophotometrically calculated at 240 nm. This is by measuring the degradation of H2O2 rate as the enzyme substrate by the use of the Aebi method [12].

4-Vitamin C: Ascorbic acid in the protein-free supernatant is oxidized by (Cu+2) ions to dehydroascorbic acid and diketoguloconic acid. These ketones react with 2, 4–DNPH in H2SO4 to form a red-orange phenyl hydrazone product, which absorbs at λ max 520 nm [13].

5-Vitamin E: Determination of vitamin E is based on oxidation and reduction reactions. This is by using the method of Emmerie – Engle Reaction that includes oxidation Tocopherol to Tocopherol quinone by Ferric chloride (FeCl3) which absorbs at λ max 520 nm [14].

6- Serum glutathione: Serum glutathione was measured by a modified procedure utilizing Ellman`s reagent and determined from a standard curve and expressed as nmol/mg protein [11].

7- Albumin Serum: The Albumin serum was determined by dye-binding method [15] using kit manufactured by bioMerieux. The measurement of albumin is based on its quantitative binding at pH 4.2 with bromocresol green (BCG) to form a blue-green complex.

8- MDA: MDA is a colour dependent method and the reaction between Thiobarbituric acidand MDA gives a chromatic compound on its absorbance in 532 Nanometer [16].

**2.2. Statistical Analysis**

Data were processed by SPSS version 23, One-way ANOVA test to find means and standard error. The significances of differences in proportions analysed by Duncan test, while Chi-square was used to compared the two groups according to the distribution of T.gondii [17].

## 3. Results

The results in table (1) revealed Superoxide dismutase (SOD) activity, Glutathione Peroxidase (GPx) activity and Catalase (CAT) activity significantly decreased (P< 0.05) in the serum of Toxoplasmosis woman, compared with control group. In the present study Vit.C, Vit.E, Glutathione and Albumin concentrations decreased significantly in the serum of the positive Toxoplasmosis woman when compared to the control group. On the other hand, the infections of the pregnant women with toxoplasmosis caused a significant increase in MDA concentration in comparison with the healthy pregnant women (see table 2).

**Table 1.** Enzymatic Antioxidants Level in Toxoplasmosis pregnant women

| Categories | Parameters (Mean± SE) | | |
|---|---|---|---|
| | SOD U/ml | GSH-px U/L | Catalase k / gHb |
| Control group **No.=22** | 143.60±1.470 **a** | 1.87 ± 0.37 **a** | 0.27 ± 0.085 **a** |
| Infected women **No.=44** | 83.64±6.052 **b** | 0.94 ± 0.21 **b** | 0.12 ± 0.17 **b** |

In the above table, different letterers refer to significant differences at (P<0.05).

**Table 2.** Non enzymatic Antioxidants Level in Toxoplasmosis pregnant women

| Categories | Parameters (Mean± SE) | | | | |
|---|---|---|---|---|---|
| | Vit.C µg/ml | Vit.E mg/L | Glutathione (µmol/L) | Albumin (g/dL) | MDA µmol/L |
| Control group **No.=22** | 6.52 ± 0.87 **a** | 16.42 ± 1.93 **a** | 1.7 ± 4.6 a | 4.67 ± 0.53 **a** | 1.37 ± 0.93 **b** |
| Infected women **No.=44** | 4.1 ± 0.98 **b** | 11.13 ± 2.3 **b** | 0.97 ± 3.2 b | 2.27 ± 0.30 **b** | 2.64 ± 1.93 **a** |

In the above table, different letterers refer to significant differences at (P<0.05).

## 4. Discussion

Superoxide dismutase is a significant physiological antioxidant defense mechanism in aerobic organism. This enzyme helps to prohibit forming the hydroxyl radical by the detoxification of hydrogen peroxide [18]. In this study, the activity of SOD of the case group, pregnant women, was lower. This suggests that the elevation of these antioxidant enzyme provides a main protection against ROS-induced tissue injury and neutrophils and macrophages release ROS as a part of the oxidative burst during T. gondii infection. ROS generation is controlled by the cellular antioxidant enzymes such as SOD which detoxifies superoxide to hydrogen peroxide (H2O2) [19]. Thus, the activity of SOD decreased in gerbils infected with Toxoplasma and might be associated with increased blood toxicity and oxidative stress [2] Others pointed there were no changes in the serum SOD activity in the infected women with T. gondii related to the increase in the severity of parasitemia and oxidative stress [21,22].

The decrease in the concentration level of GPx may be due to the fact that the cells of the inner endothelium of the placenta are formed. The fetus feeding has been subjected to a high oxidative stress during its formation, which has led to the falsification of antigen defenses. Enzymatic oxidation to scavenge the free radicals is generated as the primary function of the enzymatic antioxidant Peroxidase Glutathione to protect the mother and fetus from the oxidizing agents. These agents may cause pregnancy loss Early or perhaps due to the direct interaction of this enzyme with the hydrogen peroxide that was generated previously

in the body. It resulted in an excessive oxidation and the most damaging DNA in the universe. The enzyme is linked to protecting the cellular system and its molecules from damage and is closely related to reducing the risk of miscarriage as the aborted mother ages [23].

ROS generation is controlled by the cellular antioxidant enzymes such as catalase which convert H2O2 to H2O. A significant decrease in the catalase activity in women of the case group made H2O2, accumulate. This accumulation may be the reason to induce oxidative stress [24]. The decrease in the CAT level seems to cause abortion compared with control. This entails that there are differences between the CAT enzyme activities in cases of abortion and normal pregnancy. It is suggested that high level of free radicals and decreased antioxidants are associated with abortion. It also proposed that the end of pregnancy was due to a decrease in the CAT level that stimulates the synthesis of prostaglandins [25].

This decrease in vitamin C and vitamin E levels in the affected women may be due to the production oxidant as free radicals by the parasite T-gondii therefore vitamin C as antioxidant, prevents the body from these radicals by oxidized itself, in addition to the chemical properties of vitamin C, that allow the direct interaction with O2and OH in the aqueous phase of plasma, thus preventing damage to cells. Vitamin C plays a role in reproduction of- Tocopherol from Tocopherol Radical E, which is produced from the inhibition of lipid Peroxidation by vitamin E, therefore both vitamin C and E conserve the cell wall from damage [26,27].

Vit. C deficiency may also play a role in placental abruption. Studies have shown that low levels of Vit. C cause fetal oxidative stress in addition to a failure of placental implantation so may increase the risk of miscarriages and preeclampsia [28]. Because of the formation of free radical, oxidative stress can lead to a reduction in Vit. C level (non-enzymatic antioxidants) in toxoplasmosis patients. The increase turns over may result in the prevention of oxidative damage in the patients, suggesting an enhanced system protection against oxidant harm in toxoplasmosis disease [29].

Glutathione is the most abundant non-protein thiol source in the cell, which acts as a substrate for several enzymes, including glutathione peroxidase and GST and serves multiple functions in protecting tissues from oxidative damage keeping the intracellular environment in a reduced state.[18,24] A significant depletion of glutathione and glutathione peroxidase activity were noted in the present study in serum of women infected with T. gondii which was the result of high oxidative stress and both antioxidants over-use by the cells. Moreover, the low glutathione levels, especially in the infected pregnant women with acute phase of toxoplasmosis, represent a decreased detoxicating capacity of pregnancy [30]. The decreased in level in the serum of toxoplasmosis patients has been reported [21,31].

The present study showed decrease significant differences in albumin concentrations in seropositive Toxoplasmosis woman and this result is consistent with the previous studies. Boothroyd et al. [32] stated that toxoplasmosis can increase the concentrations of serum protein and globulin. However, it can reduce the concentrations of serum albumin and decrease albumin in the acute stages which indicates a decrease in the protein metabolism or an increase catabolism [33].

In the present study, significant differences in MDA concentrations in seropositive Toxoplasmosis woman increased and this result is consistent with Atmaca et al.,[20] who found that significantly higher erythrocytes of MDA level in gebrils caused abortion in toxoplasmosis women compared with control. Also, Al-Azzauy,[34] showed a significant increase in the MDA in the toxoplasmosis women serum compared to the control group. Cuffe [35] has shown that his level is also high in people with multiple complications of pregnancy, such as recurrent spontaneous abortions and sterility compared to the control group.

Due to T. gondii infestation, the serum MDA concentration is considerably increased. This may be caused by free radicals and oxidants generated as parasitic infectious disease. These radicals can react with DNA leading to mutation or cytotoxicity. They can also be bound to the polyunsaturated fatty acid membrane destructing membranes and cellular damage. On the other side, these biotoxic agents are more produced in acute toxoplasmosis, which can be ascribed to the activation of antioxidant [36].

The reason for the high concentration of Malondialdehyde (MDA) is due to the increased production of free radicals that lead to more lipid peroxidation and then higher oxidative damage in cellular membranes.

This includes placenta membrane damage and is due to lipid peroxidase inhibiting the synthesis of prostaglandin I2 enzyme, thus causing this enzyme to malfunction, and then the vessels shrink Hematopoietic and platelet aggregation. This is followed by the anemia of ischemia and increased cell damage. Thus, the occurrence of abnormal placentation is due to an enlarged oxidative process, as well as an elevation in the concentration of MDA. It was found that time increases with an increase in the number of times the previous miscarriages [37].

## 5. Conclusions

Antioxidants including SOD activity, Glutathione Peroxidase (GPx) activity, CAT activity, vitamin C, vitamin E, Glutathione and Albumin concentrations significantly grow higher in the serum of the toxoplasmosis aborted women than in the serum of the control group women. This is because of the free radical released with infection causing increased the oxidative stress. MDA was a marker for lipid peroxidation in all patient.

## References

1. Guo M, Dubey JP, Hill D, Buchanan RL, Gamble HR, Jones JL, Pradhan AK. Prevalence and risk factors for Toxoplasma gondii infection in meat animals and meat products destined for human consumption. J Food Prot. 2015 Feb;78(2):457-76. doi: 10.4315/0362-028X.JFP-14-328. PMID: 25710166.
2. M O, Hassan Alfaki S, Mohamed El Hussein A R. Seroprevalence of Toxoplasma gondii in Chickens (Gallus domesticus) in Sudan, Int J Infect. 2017; 4(3): e40312. doi: 10.5812/iji.40312.
3. Abdulmohaimen, N., Mezban, S. Evaluation of Apoptotic proteins (p53 and Bcl-2) expression in trophoblastic tissue of women infected with Toxoplasma gondii diagnosed by polymerase chain reaction. Egypt. Acad J biology Sci, G. Microbiology 2010; 2(2): 49-57. doi: 10.21608/eajbsg.2010.16708
4. Kaye A. Toxoplasmosis: diagnosis, treatment, and prevention in congenitally exposed infants. J Pediatr Health Care. 2011 Nov-Dec;25(6):355-64. doi: 10.1016/j.pedhc.2010.04.008. Epub 2010 Jun 12. PMID: 22018426.

5. Neville AJ, Zach SJ, Wang X, Larson JJ, Judge AK, Davis LA, Vennerstrom JL, Davis PH. Clinically Available Medicines Demonstrating Anti-Toxoplasma Activity. Antimicrob Agents Chemother. 2015 Dec;59(12):7161-9. doi: 10.1128/AAC.02009-15. Epub 2015 Sep 21. PMID: 26392504; PMCID: PMC4649158.
6. Montazeri M, Sharif M, Sarvi S, Mehrzadi S, Ahmadpour E, Daryani A. A Systematic Review of In vitro and In vivo Activities of Anti-Toxoplasma Drugs and Compounds (2006-2016). Front Microbiol. 2017 Jan 20; 8:25. doi: 10.3389/fmicb.2017.00025. PMID: 28163699; PMCID: PMC5247447.
7. Samir D, Dalal D, Noura A. Study of Oxidative Stress during Pregnancy. Glob J Pharmaceu Sci.2018: 4(5):1-4.
8. Mahadik SP, Scheffer RE. Oxidative injury and potential use of antioxidants in schizophrenia. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 1996;55(1-2):45-54.
9. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J of Med. 2018;54(4):287-93.
10. Y. Sun, L.W. Oberley, Y. Li. A simple method for clinical assay of superoxide dismutase. J. of Clin. Chem. 1988; 34: 497-500.
11. Nelson JL, Kulkarni AP. Partial purification and characterization of a peroxidase activity from human placenta. Biochem J. 1990;268(3):739-43.
12. Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105:121-6. doi: 10.1016/s0076-6879(84)05016-3. PMID: 6727660.
13. Stanley T., David T.and Howerds S. Selected method for determination of ascorbic acid in animal cells, tissues and fluids: Method in Enzymology. 1079: 62. Vitamins and Coenzymes Part D.
14. Emmerie A, Engel C. Colorimetric determination of tocopherol (vitamin E): II Adsorption experiments. Recueil des Travaux Chimiques des Pays-Bas. 1939;58(4):283-9.
15. Doumas BT, Watson WA, Biggs AG. Estimation of total protein. Clin. Chem. Acta. 1971; 31:87-96.
16. Fong KL, McCay PB, Poyer JL, Keele BB, Misra H. Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. J Biol Chem. 1973;248(22):7792-7. PMID: 4147983.
17. Abdul-Hassan NS. An investigation of Babesia spp. in goats in AL-Qadisiyah Province. AL-Qadisiyah J. of Vet. Med. Sci. 2016;15(1):77-80.
18. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev. 2012;70(5):257-65. doi: 10.1111/j.1753-4887.2012.00476. x. PMID: 22537212.
19. Corrêa G, da Silva CM, de Abreu Moreira-Souza AC, Vommaro RC, Coutinho-Silva R. Activation of the P2X7 receptor triggers the elimination of Toxoplasma gondii tachyzoites from infected macrophages. Microbes and Infect. 2010;12(6):497-04.
20. Atmaca, N., ÇinarM, G.B., KabkÇli, R., GazyaĞci, A.N., Atmaca, H.T. and Canpolat, S. Evaluation of oxidative stress, hematological and biochemical parameters during Toxoplasma gondii infection in gerbils. Ankara. Ankara Üniv Vet FakDerg, 2015; 62: 165-70.
21. AL-Khshab EM. Some antioxidants level in seropositive toxoplasmosis woman in Mosul. Tikrit J of Pure Sci. 2010;15(2):17-22.
22. A. Nurgül , Ç. Miyase,G. Bayram, K. Ruhi , N.G. Aycan,T. Hasan, and C. Sıla. Evaluation of oxidative stress, hematological and biochemical parameters during Toxoplasma gondii infection in gerbils. Ankara Üniv Vet Fak Derg. 2015; 62:165-170.
23. Ghneim HK, Alshebly MM. Biochemical Markers of Oxidative Stress in Saudi Women with Recurrent Miscarriage. J Korean Med Sci. 2016 Jan;31(1):98-105. doi: 10.3346/jkms.2016.31.1.98. Epub 2015 Dec 24. PMID: 26770044; PMCID: PMC4712587.
24. Jafari M, Salehi M, Zardooz H, Rostamkhani F. Response of liver antioxidant defense system to acute and chronic physical and psychological stresses in male rats. EXCLI J. 2014; 13:161.
25. Yusrawati A, Pebrina M, Herman RB. Differences in Malondialdehyde and Catalase Activity Levels Between Abortion and Normal Pregnancy. J Med Sci. 2017; 17:22-5.
26. Lee SH, Oe T, Blair IA. Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. Science. 2001;292(5524):2083-6.
27. Grodner M, Long S, DeYoung S. Health promotion through nutrition and nursing practice. Foundations and clinical applications of nutrition: A nursing approach 3rd ed. 2004; 380:385-9.
28. Juhl B, Lauszus FF, Lykkesfeldt J. Poor Vitamin C Status Late in Pregnancy Is Associated with Increased Risk of Complications in Type 1 Diabetic Women: A Cross-Sectional Study. Nutrients. 2017 Feb 23;9(3):186. doi: 10.3390/nu9030186. PMID: 28241487; PMCID: PMC5372849.
29. Al-Fartusie FS. Study of some trace elements and antioxidant vitamins in sera of Iraqi women with toxoplasmosis. Al-Mustansiriyah J of Sci. 2012;23(3):199-206.
30. Xu X, Liu T, Zhang A, Huo X, Luo Q, Chen Z, Yu L, Li Q, Liu L, Lun ZR, Shen J. Reactive oxygen species-triggered trophoblast apoptosis is initiated by endoplasmic reticulum stress via activation of caspase-12, CHOP, and the JNK pathway in Toxoplasma gondii infection in mice. Infect Immun. 2012 Jun;80(6):2121-32. doi: 10.1128/IAI.06295-11. Epub 2012 Apr 2. Retraction in: Infect Immun. 2015 Apr;83(4):1735. PMID: 22473610; PMCID: PMC3370585.
31. K Ali W, H Umar F, N Aziz B. Effect of Toxoplasma gondi Infestation on Lipid Peroxidation and Certain Antioxidants in Pregnant Women in Mosul City. Rafidain J of Sci 2006;17(11):16-25.
32. Boothroyd JC, Black M, Bonnefoy S, Hehl A, Knoll LJ, Manger ID, Ortega–Barria E, Tomavo S. Genetic and biochemical analysis of development in Toxoplasma gondii. Phil. Trans. of the R Society of London. Series B: Biological Sciences. 1997; 352(1359):1347-54.
33. Esmaeilnejad B, Tavassoli M, Asri-Rezaei S. Investigation of hematological and biochemical parameters in small ruminants naturally infected with Babesia ovis. Vet Res Forum. 2012;3(1):31-6. PMID: 25653743; PMCID: PMC4312816.
34. Al-Azzauy AA. Evaluation of Erythrocyte Malondialdehyde, Glutathione Concentration and Serum Nitric Oxide Levels in Patients with Toxoplasma gondii. Ibn Al Haitham J. for Pure and Appl. Sci. 2016; 24(1):1-6.
35. Cuffe JS, Xu ZC, Perkins AV. Biomarkers of oxidative stress in pregnancy complications. Biomark Med.

2017;11(3):295-306.
36. Ali, A. A. Toxoplasmosis: Detection of Serum Immunoglobulin by ELISA and Placenta DNA by PCR; A Comparative Study. Diyala J. For Pure Sci. 2016; 12(1): 99–07.
37. Smith C, Marks AD, Lieberman M. Oxygen toxicity and free radical injury. Mark's Basic Medical Biochemistry: A Clinical Approach. 2005:439-57.