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Evaluation of gender-related differences in response to oxidative stress in *toxoplasma gondii* positive serum

Mahvash Jafari[1] PhD, Maryam Salehi[2] MS, Shahnaz Shirbazou*[3] PhD, Laila Abasian[3] MS
Fatemeh Talebi-Meymand[3] MS

[1]*Department of Biochemistry, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
[2]*Neuroscience Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
[3]*Department of Parasitology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.*

ABSTRACT

Purpose: *Toxoplasma gondii (T. gondii)* is the causative agent of toxoplasmosis. It infects up to one third of the human population. The aim of this study was to evaluate the effects of *T. gondii* infection on induction of oxidative stress in serum of infected men and women.

Materials and Methods: This case-control study was carried out on 150 individuals who had referred to our center in Tehran. Serum was obtained from venous blood samples. Immunoglobulin G (IgG) anti-*toxoplasma* antibody enzyme linked immunosorbent assay (ELISA) test was performed on all of the samples. Those who were IgG positive were regarded as the case group (52 women and 23 men) and the others as the control group (43 women and 32 men). The data were analyzed by INSTAT software using ANOVA followed by Tukey.

Results: Serum superoxide dismutase activity in men of the case group was significantly higher than in the control group (7.81 ± 0.38 vs. 6.69 ± 0.17, P = .045). Catalase activity in men of the case group was significantly higher than the control group (8.64 ± 0.55 vs. 6.23 ± 0.38, P = .006). Glutathione S-transferase activity and malondialdehyde level in women of the case group were significantly higher than the control group (5.98 ± 0.24 vs. 4.73 ± 0.28, P = .037 and 2.3 ± 0.09 vs. 1.9 ± 0.09, P = .032, respectively). Catalase activity and glutathione level in women of the case group were lower than the control group (6.0 ± 0.45 vs. 7.63 ± 0.48, P = .043 and 0.62 ± 0.05 vs. 0.89 ± 0.05, P = .007, respectively).

Conclusion: *T. gondii* infection induces oxidative stress in women's serum because of the decreased catalase activity, glutathione depletion and increasing lipid peroxidation. The increased antioxidant enzyme activities in infected men were because of the adaptive response to the generated free radicals. Women were found to be more sensitive to the effects of *toxoplasma* infection on oxidative stress induction compared to men.

Keywords: *toxoplasma gondii*; antioxidant enzymes; lipid peroxidation; human; serum.

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INTRODUCTION

Toxoplasmosis is one of the common parasitic infections in tropical and subtropical climates. Its causative agent is *Toxoplasma gondii (T. gondii)*. It exists in a chronic asymptomatic form in 500 million to 1 billion people.[1,2] In Iran, at least 30% of the population in most regions are infected with it.[3] Toxoplasmosis occurs in cases of congenital infection and in immune-compromised individuals, particularly malignant patients under chemotherapy, organ transplant recipients and people with AIDS.[4-6] Toxoplasmosis can cause serious pathologies including hepatitis, pneumonia, blindness and severe neurological disorders.[7]

T. gondii is an obligated intracellular protozoan

parasite, which infects humans and most warm-blooded animals, depending on hygiene standards, eating habits, profession and living place (urban or rural).[2,6,8] *Toxoplasma* has a complex life cycle consisting of a sexual cycle in its feline definitive hosts and an asexual cycle in its intermediate hosts. Intermediate hosts like humans can be infected by eating raw or uncooked meat of infected animals, ingestion of oocysts shed in cat feces or from mother to fetus.[4,6,9,10] Acute infections in pregnant women cause severe congenital toxoplasmosis, in which the symptoms could be mental retardation, cerebral calcifications, hydrocephaly, chorioretinitis, microcephaly, convulsions or even death of the fetus.[8,11] Chronic *T. gondii* infection can lead to cryptogenic epilepsy, headaches, and schizophrenia.[8]

T. gondii infection is associated with activation of T helper 1 (Th1) cells secreting cytokines. Cytokine-activated macrophages release a great number of reactive oxygen species (ROS), which are responsible for parasite killing in macrophages.[4,5,12-14] Antioxidant defense system including glutathione, superoxide dismutase (SOD), catalase and glutathione S-transferase (GST) form a network protecting cells against ROS.[15,16] Several studies have shown that decrease of antioxidant enzyme activities in *T. gondii*-infected patients are associated with a depletion of glutathione and an increase of lipid peroxidation, all of which can lead to oxidative stress and finally cell death.[7,17-21] Previous studies have also stated the role of cytosolic antioxidant enzymes in *T. gondii* in defense against oxidative injury.[22-24]

Elucidating the molecular and cellular pathways activated in response to infection is crucial to understanding disease pathogenesis and to developing control strategies rationally.[25,26] Oxidative events against *T. gondii* infection are not well elucidated in animals. The response to infection and the ability to neutralize oxidant species differ between men and women.[27,28] There are few reports on the gender-dependent effects of *T. gondii* infection on induction of oxidative stress in various tissues of the host.[27] To our knowledge, this is the first study on these effects in Iran. Thus, the aim of this study was to evaluate the gender-dependent effects of *T. gondii* infection on important biomarkers of oxidative stress including malondialdehyde content as an important index of lipid peroxidation and antioxidant defense parameters such as glutathione level, activities of SOD, catalase and GST in serum of *T. gondii*-infected patients.

MATE RIALS AND METHODS

This case-control study was carried out on 150 individuals who had been referred to Baqiyatallah hospital in Tehran from January until July 2013 (age range: 20-50 years old). None of the participants of this study took medication or supplementation upon entering the study. Venous blood samples were obtained and immediately centrifuged at 1500×g for 10 minutes at 4° C. Serum was separated and stored in 0.5 mL aliquots at –70° C until biochemical analysis. Enzyme linked immunosorbent assay (ELISA) test was performed on all of the samples using immunoglobulin G (IgG) Kit (Pishtaz Teb Co., Tehran, Iran) and the final results were recorded by ELISA reader (optical absorbance, OD = 450). Using this kit, samples less than 0.9 unit/ml and more than 1.1 unit/mL were considered as negative and positive, respectively. Participants with IgG (anti-*toxoplasma* antibody) positive samples were considered as the case group (52 women and 23 men) and the rest as the control group (43 women and 32 men). This study was approved by the Ethical Committee of Baqiyatallah University of Medical Sciences.

SOD Activity Assay

The SOD activity was determined using the method described by Winterbourn, based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium by superoxide.[29] The absorbance of samples was read on a Genesys 10 UV spectrophotometer at 560 nm for 5 minutes. The amount of enzyme required to produce 50% inhibition was taken as 1 U and the results were expressed as U/mg protein.

Catalase Activity Assay

Serum catalase activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 as the substrate of the enzyme using the Aebi method.[30] A molar absorption of 43.6 Mcm^{-1} was used to determine catalase activity. Enzymatic activity was expressed as U/mg protein, one unit (U) of which was equal to 1 mole of H_2O_2 degraded/min/mg of protein.

GST Activity Assay

GST activity was assayed by monitoring the formation of the thioether product of the reaction between glutathione and 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm.[31] The enzyme activity was calculated using extinction coefficient 9.6 $mMcm^{-1}$ and expressed as µmol CDNB utilized/min/mg protein.

Determination of Glutathione Level

Glutathione level was measured using Tietz method.[32]

Glutathione in the supernatant was assayed at 412 nm by monitoring the absorbance of 5, 5'-dithiobis 2-nitrobenzoic acid for 5 minutes. Glutathione level was determined from a standard curve and expressed as nmol/mg protein.

Determination of Malondialdehyde Level

As an indicator of lipid peroxidation, malondialdehyde serum level was determined at 532 nm using 2-thiobarbituric acid according to the method of Satoh.[33] Malondialdehyde concentration was determined using 1,1,3,3-tetraethoxypropane as standard and expressed as nmol/mg protein.

Protein Level Assay

The total protein contents of the samples were measured by Bradford's method using bovine serum albumin as a standard.[34]

Statistical Analysis

All calculations were performed using INSTAT statistical software. For the sex-dependent studies, the data were statistically analyzed using ANOVA followed by Tukey post hoc multiple comparison test. *P*-values less than 0.05 were considered as statistically significant. The results were expressed as mean ± standard error of the mean (SEM), with n denoting the number of experiments performed.

RESULTS

Table 1 shows the prevalence of IgG antibodies related to *T. gondii* according to the sex of control and case groups. Overall, 75 (50%) of 150 participants were positive for anti-*T. gondii* IgG antibodies including 23 (15.33%) men and 52 (34.66%) women.

Table 2 summarizes the changes of glutathione and malondialdehyde levels in serum of control and case groups. Malondialdehyde level in women of the case group was significantly higher than in the control (2.3 ± 0.09 vs. 1.9 ± 0.09, $P = .032$), while glutathione level of the case group was lower (0.62 ± 0.05 vs. 0.89 ± 0.05, $P = .007$). In addition, malondialdehyde level was higher in women compared to men in the case group (2.3 ± 0.09 vs. 1.8 ± 0.01, $P = .028$). Serum SOD activity was significantly higher in men and women of the case group compared to the control group (7.81 ± 0.38 vs. 6.69 ± 0.17, $P = .045$ and 8.14 ± 0.23 vs. 6.76 ± 0.36, $P = .004$, respectively). (Figure 1).

Serum catalase activity had increased in men of the case group ($P = .006$), while it had decreased in women of the case group, as compared to the control group ($P = .043$). Catalase activity in women of the case group was significantly lower than that of men ($P = .003$) (Figure 2). Moreover, serum GST activity in women of the case group was significantly higher compared to the control group (5.98 ± 0.24 vs. 4.73 ± 0.28, $P = .043$) (Figure 3).

Table 1. The prevalence of IgG antibodies specific to *toxoplasma gondii* according to the sex of the control and the case groups.

Sex	Seroprevalence		
	Negative n (%)	Positive n (%)	Total n (%)
Man	32 (21.3)	23 (15.3)	66 (36.6)
Woman	43 (28. 7)	52 (34.7)	84 (63.4)
Total	75 (50.0)	75 (50.0)	150 (100.0)

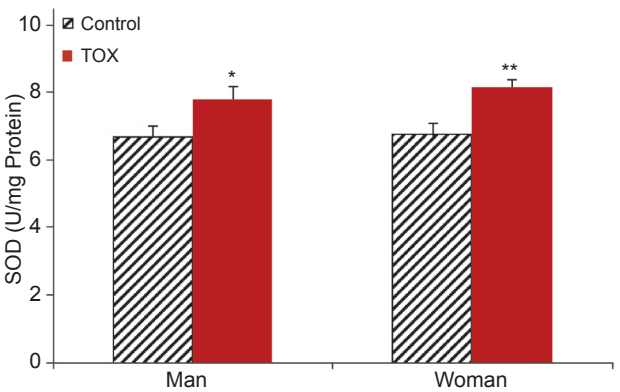

Figure 1. Effect of *toxoplasma gondii* infection on superoxide dismutase (SOD) activity in control and case groups. Values are expressed as mean ± SEM. *$P = .045$ and **$P = .004$ vs. the control group.

Table 2. Effect of *toxoplasma gondii* infection on weight, serum glutathione and malondialdehyde levels in the control and the case groups.

Parameters	Control (Mean ± SEM)		TOX (Mean ± SEM)	
	Men (n = 43)	Women (n = 32)	Men (n = 23)	Women (n = 52)
Weight (g)	36.744 ± 1.185	34.437 ± 1.382	39.565 ± 1.415	36.077 ± 0.779
GSH (nmol/mg protein)	0.838 ± 0.059	0.891 ± 0.046	0.757 ± 0.075	0.622 ± 0.053**
MDA (nmol/mg protein)	1.669 ± 0.099	1.902 ± 0.086	1.832 ± 0.107	2.305 ± 0.095*,#

Keys: GSH, glutathione; MDA, malondialdehyde; SEM, standard error of measurement.
Values are expressed as mean ± SEM. *$P = .032$ and **$P = .007$ vs. the control group; #$P = .028$ vs. infected men.

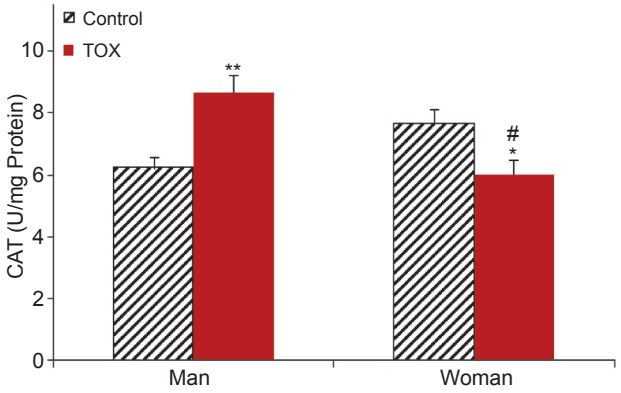

Figure 2. Effect of *toxoplasma gondii* infection on catalase (CAT) activity in control and case groups. Values are expressed as mean ± SEM. **P* = .043 and ***P* = .006 vs. the control group; #*P* = .003 vs. infected men.

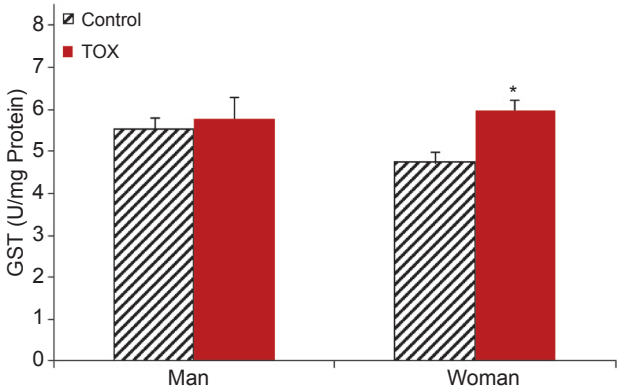

Figure 3. Effect of *toxoplasma gondii* infection on glutathione S-transferase (GST) activity in control and case groups. Values are expressed as mean ± SEM. **P* = .037 vs. the control group.

DISCUSS ION

About 30-60% of the population in different countries are infected with *T. gondii*.[35,36] Our results showed that seropositive percentual is somewhat higher in women than in men. Several studies have reported no significant difference regarding the incidence of toxoplasmosis between the two sexes.[27,37,38] However, there are those which point to higher incidence either for men[39,40] or women.[41,42]

Neutrophils and macrophages release ROS as part of the oxidative burst during *T. gondii* infection.[13,22] ROS generation is controlled by the cellular antioxidant enzymes such as SOD and catalase. SOD detoxifies superoxide to hydrogen peroxide (H_2O_2) and catalase converts H_2O_2 to H_2O.[15,43] In this study, SOD activity in men and women and catalase activity in men of the case group were higher, suggesting that elevation of these antioxidant enzymes provides mainly protection against ROS-induced tissue injury. The increased SOD activity was associated with a significant decrease in catalase activity in women of the case group leading to the accumulation of H_2O_2, which may be the cause of the induction of oxidative stress.[43] In this regard, Al-Kennany reported a significant decrease in SOD activity in placentae of ewes infected with *T. gondii*.[18] Also, Al-Khshab showed that there were no changes in serum SOD activity in infected women with *T. gondii*.[14]

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.[14,15,43] GST can remove ROS and its levels can reflect the antioxidant capacity of the body.[31] A significant depletion of glutathione and increased GST activity were noted in the present study in serum of women infected with *T. gondii* which was the result of high oxidative stress and glutathione over-use by the cells. Our finding is in agreement with the results of the previous reports in which the infection with *T. gondii* depleted glutathione in host's different tissues.[18,44-46] In addition, the decreased glutathione level in serum of toxoplasmosis patients has been demonstrated.[7,14] Evidences indicate that pre-treatment with N-acetyl cysteine as glutathione precursor can reduce *T. gondii* infection-induced oxidative stress in mice.[46]

Lipid peroxidation is the process of oxidative degeneration of polyunsaturated fatty acids membranes of tissues because of free radical generation. A common marker of lipid peroxidation is malondialdehyde, which has been frequently used as markers of oxidative stress in response to different agents such as infection.[43] In this study, malondialdehyde serum level had increased in women of the case group. The increased lipid peroxidation shows that *T. gonadii* infection-induced ROS are not totally scavenged by the antioxidant enzymes in tissues. Numerous studies have shown that malondialdehyde level in various tissues had significantly increased among *T. gondii* infected cats, ewes, chickens and mice.[18,20,44-46] Malondialdehyde level had also increased in serum of toxoplasmosis patients.[7,17,19,21,27] A study has shown that there was no change in serum malondialdehyde level in infected mice with *T. gondii*.[20] Yazar and colleagues have shown that no significant correlation could be found in malondialdehyde levels of either women and men in *T.gondii* infected and control groups.[27]

CONCLUSION

Seropositive percentual of *T. gondii* was higher for women (34.7%) than for men (15.3%). *T. gondii* infection induces the production of ROS and oxidative stress in serum of women because of the decreased catalase

activity, glutathione depletion, and increasing lipid peroxidation. Increased ROS not only kills the parasites but also damages the host cells. Women are more sensitive to the effects of *toxoplasma* infection on oxidative stress induction compared to men. Depletion of glutathione leads to oxidized glutathione production, decreasing the reduced glutathione /oxidized glutathione ratio, which may shift cells through apoptosis and necrosis.

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CONFLICT OF INTEREST

None declared.

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Corresponding Author:
Shahnaz Shirbazou, PhD
Address: Department of Parasitology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Aqdasieh St., Araj Three-way, Tehran, Iran.
Postal Code: 193956558
Tel: +98 21 22289942
Fax: +98 21 26127257
Cell Phone: +98 9121360244
E-mail: shahnazshirbazou@gmail.com

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