Evaluation of Oxidative Stress during Toxoplasmosis in Pregnant Women

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Abstract: *Toxoplasma gondii (T. gondii) is the causative agent of toxoplasmosis. It infects up to one third of the human population. This study has conducted in Tikrit Teaching Hospital from September 2013 until March 2014, to investigated the effect of infection with toxoplasmosis, in pregnant women, on some enzymatic and non enzymatic antioxidants. Seventy six blood samples were collected from infected women their ages between (16-46) years, divided into two groups (16-26) and (27-46) years, and also 25 blood samples from non-infected pregnant women as a control group and Superoxide dismutase (SOD), catalase (CAT) and Glutathione Peroxidase (G-Px), Glutathione (GSH), Ceruloplasmin (Cp), Uric acid and Albumin were determined.The results showed a significant increase in concentration of SOD and uric acid compared with control group while significant decrease in the concentration of CAT, G-Px, and GSH, while non significant differences in the concentration of albumin compared with the control group.*

Keywords: *Toxoplasmosis, pregnant women, enzymatic and non enzymatic antioxidants*

1. 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 to1 billion people.[1,2] *T. gondii* is widespread protozoan Parasite , that infects most types of warm-blooded mammals and causes opportunistic disease in humans. [3] *T. gondii* is an obligate intracellular parasite replicating inside a parasitophorous vacuole in a broad range of host cells including macrophages. Human infection may be acquired in several ways: (i) ingestion of undercooked infected meat containing *Toxoplasma* cysts; (ii) ingestion of the oocyst from focally contaminated hands or food; (iii) blood transfusion; (iv) transplacental transmission. [4,5] Toxoplasmosis can cause serious pathologies including hepatitis, pneumonia, blindness and severe neurological disorders.[6]

Toxoplasmosis may cause severe disorders in immunocompromised patients and in Pregnant women, because of high risk of transplacental transmission and the occurrence of multiple congenital lesions in the fetus. [7]

During infection, the immune effecter cells are able to kill or inhibit its intracellular growth. This antiprotozoan activity produces a number of toxic products such reactive oxygen intermediates. While, within the host cell, *T. gondii* itself produces oxidants as by products of normal metabolism. Reactive oxygen species (ROS)are potentially destructive, capable of oxidizing proteins or lipids and causing chemical modifications to nucleic acids. [8] It is well documented that, under normal physiological conditions, an estimated 1–3%of inspired oxygen is converted to superoxide radicals and H2O2.The existence and development of cells in an oxygen containing environment would not be possible without the presence of complicated defense system that include enzymatic and nonenzymatic antioxidant components.[9]

The main objective of this study was to estimate some antioxidants level like enzymatic antioxidants : Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (G-Px),and non-enzymatic antioxidants : Glutathione(GSH) , Ceurloplasmin (Cp) , Uric acid and Albumin , in serum of positive toxoplasmosis pregnant women. Results were compared to those obtained with uninfected pregnant women (control), for a better understanding of toxoplasmosis pathogenesis.

2. Materials and methods

Seventy six blood samples were collected from seropositive- Toxoplasmosis women and from (25) seronegative Toxoplasmosis women (Control group). Age range: 16-46 years old and who had been referred to Tikrit hospital in Salaheldin from September 2013 until March 2014. None of the participants of this study took 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).

SOD activity was determined in erythrocyte hemolysates according to the method described by Sun et al. [10] This method provides that the rate of nitroblue tetrazolium (NBT) reduction to blue formazan by the superoxide anion generated by the xanthine oxidase (XOD) reaction is monitored spectrophotometrically at 560 nm. One unit of SOD was considered a 50% inhibition of reduction of NBT under the condition of the assay. The results were expressed as U/g Hb.

Glutathione peroxidase activity in serum was assayed colorimetrically, a method used by Nelson and Kulkarni. [11]

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]

Catalase activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H2O2 as the substrate of the enzyme using the Aebi method. [12] 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 H2O2 degraded/min/mg of protein.

Ceruloplasmin analysis was conducted by a spectrophotometric method, which included P-phenyldiamine dichloride (PPD) use.[13]

Uric acid concentration in plasma is determined by use of uricase to allantoine and hydrogen peroxide (2H, O). [14]

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.

Statistical Analysis:

The results are expressed as mean ± SD. Our data were analyzed statistically using one-way analysis of variance. Group differences were determined using Duncan multiple range test. Statistical significance was considered at $p<0.05$.[16]

3. Results

The results in table (1) revealed a significant increase ($P< 0.05$) in serum SOD activity between toxoplasmosis women and control group, while CAT activity and GSH-px decreased significantly ($P<0.05$) in serum of Toxoplasmosis woman, compared with control group as shown in table (1) .

In the present study GSG and CP activity decreased significantly in sero positive Toxoplasmosis woman in compared to the control group. On the other hand, infection of the pregnant women with toxoplasmosis produced a significant increase in uric acid concentration in comparison with the healthy pregnant women. There was no significant difference in serum albumin between control and infected women as shown in table (2).

TABLE I: Enzymatic Antioxidants Level in Toxoplasmosis pregnant women

Categories	parameters		
	SOD U/gHb	Catalase k / gHb	GSH-px umol/L
Infected women (16-26 years)	669.4 ± 249 a	0.1 ± 0.13 c	0.8 ±0.1 c
Control group	248 ± 19 b	0.1± 0.31 a	1.78 ± 0.4 b
Infected women (27-46 years)	852 ± 160 a	0.11± 0.1812 c	0.84 ± 0.118 c
Control group	235.75 ± 13 b	0.21 ± 0.075 b	0.84 ± 0.118 a

*Different letterers refer to significant differences at ($P<0.05$)

TABLE II: Non enzymatic Antioxidants Level in Toxoplasmosis pregnant women

Categories	Parameters			
	CP (mg/dl)	Glutathion (Umol/L)	Albumin (g/dL)	Uric acid(mg/ml)
Infected women(16-26 years)	41.1 ± 298.5 d	1 ± 5.1 b	1.09 ± 5.3 a	0.7 ± 7.3 b
Control group	72.9 ± 481 a	0.9 ± 9.2 a	0.408 ± 4 a	0.1 ± 6.6 c
Infected women(27-46 years)	20.9 ± 354.5 c	0.9 ± 5.3 b	5.2 ± 6.2 a	1.2 ± 7.7 a
Control group	44.7 ± 441.6 b	1.2 ± 9.3 a	0.4 ± 4.2 a	0.4 ± 6.8 c

*Different letterers refer to significant differences at (P<0.05)

4. Discussion

Superoxide dismutase is an important physiological antioxidant defense mechanism in aerobic organism. This enzyme prevents the formation of the hydroxyl radical by detoxifiying hydrogen peroxide. [17] In this study, SOD activity in pregnant women of the case group was higher, suggesting that elevation of these antioxidant enzyme provides mainly protection against ROS-induced tissue injury also neutrophils and macrophages release ROS as part of the oxidative burst during *T. gondii* infection.[18] ROS generation is controlled by the cellular antioxidant enzymes such as SOD which detoxifies superoxide to hydrogen peroxide (H2O2). [18] this result agree with[19,20] ,although others pointed there was no changes in serum SOD activity in infected women with *T. gondii*., that related to increasing the severity of parasitemia and oxidative stress.[21,22]

ROS generation is controlled by the cellular antioxidant enzymes such as catalase which converts H2O2 to H2O.[23] this result agree with[20,24]

The increased SOD activity was associated with a significant decrease in catalase activity in women of the case group leading to the accumulation of H2O2, which may be the cause of the induction of oxidative stress. [23]

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 and keeping the intracellular environment in the reduced state.[17,23] 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 pregnant [25] The decreased in level in serum of toxoplasmosis patients has been demonstrated [20,21,26]

The present study showed no significant differences in albumin ratio in seropositive Toxoplasmosis woman and this result is consistent with the of previous study.[23] But Boothroyd *et al.*[27] observed that toxoplasmosis led to an increase in serum protein and globulin concentrations and a decrease in serum albumin concentrations during the acute stage and decrease of albumin in the acute stage which indicates decrease in protein metabolism or increase catabolism. [28]

Ceruloplasmin is an acute phase protein that responses mildly to inflammation and tissue damage .[29] Some researchers pointed out that there are positive correlations between acute phase reactants and the severity of lesions as well as prognosis.[30] In the present study, CP concentration was lower in *T. gondii* seropositive pregnant women (P<0.05).It acts as an antioxidant through either prevention of decompartmentized iron acting as free radical catalysis or by directly inactivating the free radical and CP was far more effective as a peroxyl radical scavenger than SOD, deferoxamine and BSA, but slightly less effective than catalase.[31] This result agreed with other researches.[32]

In positive toxoplasmosis group, the result of uric acid was significantly increased.[33] Uric acid plays different roles in human body and plays a role as an endogenous antioxidant. Uric acid is able to react with different free radicals forming relatively stable urate radical and thus stopping radical reactions. So it is a powerful antioxidant and is a scavenger of singlet oxygen and radicals.[8,33]

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6. References

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