Evaluation of Erythrocyte Malondialdehyde, Glutathione Concentration and Serum Nitric Oxide Levels in Patients with *Toxoplasma gondii*

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Abstract

The aim of this study was to evaluate the biological importance of the magnitude of oxidative stress, antioxidant and the levels of nitric oxide (NO) in the female patients infected with *Toxoplasma gondii* by analyzing the levels of erythrocyte malondialdehyde (MDA) as an indicator for the oxidative stress and erythrocyte reduced glutathione (GSH) level as indicator for the antioxidant status and serum nitric oxide levels. This prospective study was conducted on fifty female patients with toxoplasmosis and thirty normal healthy females of comparable age and sex were considered as normal control. A statistically significant difference was found between patients and control group in terms of MDA, GSH and NO levels. A decrease in erythrocyte GSH levels was detected, while erythrocyte MDA and serum NO levels increased significantly as compared with normal healthy control. Consequently, the results suggest that the high infection vs control of increased erythrocyte MDA and serum NO levels probably suggest the occurrence as a mechanism of tissue change in cases of toxoplasmosis. Moreover, it is recommended that the patient levels of MDA, GSH, and NO should be evaluated in toxoplasmosis.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan in birds and mammals [1]. Toxoplasmosis is the disease that occurs when *T. gondii* invades and multiplies asexually as tachyzoites within the cytoplasm of nucleated cells [2]. When host immunity develops, multiplication of tachyzoites ceases and tissue cysts form, which remain latent, especially in the brain and muscle. Sexual reproduction of *T. gondii* occurs only in the intestinal tract of cats; the resultant oocyst passes in the feces remain infectious up to a year in soil, depending upon the temperature and moisture content [3].

The two major routes of transmission in humans are oral and congenital. Humans become infected with *T. gondii* through direct contact with oocysts in cat feces or through eating meat contaminated with the extraintestinal form of *T. gondii* [4]. The diagnosis of toxoplasmosis is most critical in four groups of patients: pregnant women who acquire infection during gestation, immunocompromised patients and patients with chorioretinitis [5]. In this research patients from the first group, were used.

Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (MDA) [6]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that causes toxic stress in cells and forms covalent protein adducts which are referred to as advanced lipoxidation end products, in analogy to advanced glycation end-products [7]. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism [8, 9].

Glutathione (GSH) is a cysteine-containing peptide found in most forms of aerobic life [10]. It is not required in the diet and is instead synthesized in cells from its constituent amino

acids [11]. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. It is one of the most important cellular antioxidants; it defends the cell against oxidative damage by undergoing reaction with free radicals and peroxidase [10].

Nitric oxide (NO) is a free radical, an uncharged molecule with an unpaired electron. NO plays multiple roles in both intracellular and extracellular signaling mechanisms [12]. This highly reactive, yet simple molecule is produced in the body by the isoenzyme nitric oxide synthase (NOS) using L-arginine as a substrate. Three iso forms of NOS have been characterized, two of them are constitutive NOS (cNOS) and the third is inducible (iNOS) by endotoxins and cytokines [13]. The aim of the present study was to evaluate the magnitude of oxidative stress by estimating the level of erythrocyte MDA levels, erythrocyte GSH concentration as indicators for the antioxidative status and levels of serum nitric oxide in patients with toxoplasmosis.

Material and methods

-Subjects: The blood samples were collected from fifty female patients, infected with *Toxoplasma gondii* and found to be positive in ELISA test. The samples were collected from Kamal Al-Samurai hospital, Al-Ilwia hospital for maternity. None of those patients was on a special diet or taking any antioxidant (vitamin E, C, etc) or treated with antioxidant drugs, no smoking or drinking habits and did not take any hormonal medication. Thirty normal healthy females of comparable age and sex were considered as normal control.

-Blood samples: blood samples (5ml) transferred into plain tubes containing (acid-citrate-dextrose) (ACD) as anticoagulant. Tubes were mixed and placed immediately in crushed ice, then assayed within (1-2) hrs. of blood collection. Blood samples were centrifuged at (5000) rpm for (10) min., then plasma and buffy coat were removed by aspiration. Erythrocytes were washed three times with phosphate buffered saline (PBS) pH= 7.4 (0.02 M phosphate; 0.123M NaCl). The packed cell volume (PCV) after the final wash was used for the assay of MDA and GSH concentration. Serum was stored at (-20˚C) and used for the determination of nitric oxide level.

-Chemicals: the chemicals and reagents used in this study were of annular grade unless otherwise specified and were obtained from BDH chemicals Ltd., England; Sigma, chemicals USA; Fluka A.G., Germany.

Assays

-MDA: MDA was assayed according to the method of Ohkawa *et al.* [14] with minor modification from Hirayama *et al.* [15]. The reaction to form thiobarbituric acid-reactive substances (TBA-RS) depends on the condensation of two molecules of (TBA) with one molecule of MDA to generate a reddish chromogen that absorbs light at (532) nm wave length.

-Glutathione concentration: determination of erythrocyte glutathione concentration was performed according to the method of Virgil [16] which is a modified version of that of Beulter [17]. Virtually, all of the non protein sulfhydryl groups of erythrocyte are in the form of reduced GSH. 5,5-Dithiobis (2-nitrobenzoic acid) DTNB is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at (412) nm and is directly proportional to the GSH concentration [17].

-Nitric oxide level: serum nitrite plus nitrate concentration as an index of serum NO levels were determined by the method described previously [18]. Quantification of nitrite and nitrate was based on the Griess reaction, in which chromophore with a strong absorbance at (450)nm is formed by reaction of nitrite with a mixture of naphthyl ethylenediamine and sulphanilamide. The absorbance was measured in a spectrophotometer to give the nitrite concentration. For nitrate detection, a second sample was treated with copporised cadmium in glycine buffer at pH (9.7) to reduce nitrate to nitrite, the concentration of which thus represented the total nitrite plus nitrate.

A standard curve was established with a set of serial dilutions (10^{-8} – 10^{-3}mol/l) of sodium nitrite. All samples were assayed in duplicate.

4-Hemoglobin level: Hb was determined using hemoglobin kit (Randox) procedure no. 540-UV 1996. The Hb levels were measured in the patients and control individuals to determine the MDA level and GSH concentration. Hb, erythrocyte MDA level and GSH concentration measurements of the matched sets were performed on the same day.

The results were analyzed by student's (t-test) to find out level of significance. P value ≤0.05 was considered as statistically significant.

Results

Table (1) represents the sample size (n), mean ± SD and significance of erythrocyte MDA level expressed in (n mol/g Hb) of normal healthy control and the patients of toxoplasmosis.

Erythrocyte MDA level was significantly increased in group (2) as compared with group (1) normal healthy control (P< 0.001).

Table (2) demonstrates the mean ± SD of erythrocyte GSH concentration expressed in (nmol/g Hb) of normal healthy control and the patients of toxoplasmosis. Erythrocyte GSH concentration was significantly decreased in group (2) as compared with group (1) normal healthy control (P< 0.001).

Table (3) shows the mean ± SD of serum nitric oxide level expressed in (µ mol/l) of normal healthy control and the patients of toxoplasmosis. Serum nitric oxide level was significantly increased in group (2) as compared with group (1) normal healthy control (P< 0.001).

Discussion

Toxoplasma gondii is a highly frequent obligate intracellular protozoan parasite. It is reported that about one-third of the world population is infected with T. gondii; the disease has asymptomatic progress in 90% of the patients with sound immune systems [19, 20]. It is assumed that the malondialdehyde (MDA) arising from the lipid peroxidation is an indicator of the oxidative stress in tissue and cells.

Lipid peroxidase is a derivative enzyme of feeble unsaturated fatty acid which is produced as a result of decomposition of a set of complex components [21]. In this study, the findings revealed an increase in the MDA level in the erythrocyte of *Toxoplasma gondii* patients as compared with normal healthy control (table 1). These findings are in agreement with Ulku *et al.* [22] and Yazar *et al.* [23]. The increase of MDA level in the erythrocyte of *Toxoplasma gondii* patients demonstrates the increase of lipid peroxidation. The results of the present study strongly suggest that one of the main reasons for high MDA levels in the patients infected with toxoplasmosis could be the decreased activity of the defense system protecting the tissues from free radical damage. The potentially harmful effects of reactive oxygen species are controlled by the cellular antioxidant defense system.

GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli including oxidative stress. It plays a role in preventing the transformation of hemoglobin into methemoglobin due to oxidation. Moreover, it maintains the sulfhydryl (-SH) groups in proteins in a reduced state and protects these groups against oxidation [24]. In the present study, erythrocyte GSH concentration was significantly decreased in the patients infected with toxoplasmosis as compared with healthy control (table 2). The lower GSH concentration in patient group can be explained with the oxidative stress caused by lipid peroxidation and depletion in the GSH concentration, which is an endogen antioxidant.

The results in table (3) showed increased level of serum nitric oxide in the *Toxoplasma gondii* patients. NO is the product of arginine metabolism and one of the most effective O_2-free toxins. There are also previous studies reporting an increase in the NO level in parasitic diseases [19, 22, 25]. It can be stated that the NO level increase as a defensive mechanism to protect the patient against the harmful effects of the parasite.

As a conclusion, the increase of serum NO level in the patients infected with toxoplasmosis can be associated with the stimulation of the cell mediated immune response.

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Table (1) Biostatistical calculations and student t-test of erythrocyte MDA level for normal healthy control (group 1) and the patients of toxoplasmosis (group 2).

Erythrocyte MDA level (n mol/g Hb)	Normal healthy control (group 1)	Toxoplasmosis patients (group 2)
Sample size (n)	30	50
Mean ± SD	4.43 ± 1.65	20.75 ± 2.06
Probability		< 0.001*

*normal healthy control (group 1) versus toxoplasmosis patients (group 2)

Table (2) Biostatistical calculations and student t-test of erythrocyte GSH concentration for normal healthy control (group 1) and the patients of toxoplasmosis (group 2).

Erythrocyte GSH concentration (n mol/g Hb)	Normal healthy control (group 1)	Toxoplasmosis patients (group 2)
Sample size (n)	30	50
Mean ± SD	6.95 ± 1.21	2.10 ± 0.10
Probability		< 0.001*

*normal healthy control (group 1) versus toxoplasmosis patients (group 2)

Table (3) Biostatistical calculations and student t-test of Serum nitric oxide level for normal healthy control (group 1) and the patients of toxoplasmosis (group 2).

Serum nitric oxide level (µ mol/l)	Normal healthy control (group 1)	Toxoplasmosis patients (group 2)
Sample size (n)	30	50
Mean ± SD	42.38 ± 1.51	48.47 ± 0.30
Probability		< 0.001*

*normal healthy control (group 1) versus toxoplasmosis patients (group 2)

تقدير مستوى المالون ثنائي الالدهايد وتركيز الجلوتاثايون في كريات الدم الحمر و مستوى اوكسيد النتريك في مصل الدم لدى النساء المصابات بداء المقوسات

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الخلاصة

إنَّ الهدف من هذه الدراسة هو بيان الاهمية الحيوية للزيادة الحاصلة في مستويات الاجهاد التأكسدي ومستوى اوكسيد النتريك وإنخفاض مضادات الاكسدة عند النساء المصابات بداء المقوسات من خلال تقدير مستوى المالون ثنائي الالديهايد في كريات الدم الحمر الذي يعد دليلا للاجهاد التأكسدي، وتركيز الجلوتاثايون في كريات الدم الحمر دليلا لمضادات الاكسدة ومستوى اوكسيد النتريك في مصولهن. اجريت الدراسة على 50 عينة من النساء المصابات بالمرض والمثبتة اصابتهم مختبريا، و30 عينة من نساء غير مصابات بالمرض مجموعة سيطرة سوية.

أظهرت النتائج ارتفاعاً ملحوظاً في مستوى المالون ثنائي الالديهايد في كريات الدم الحمراء للنساء المصابات بالمرض وفي مستوى اوكسيد النتريك في مصول دمائهن، كما اظهرت النتائج انخفاضاً في مستوى الجلوتاثايون في كرياتهن الحمراء مقارنة بالنساء السليمات.

ويمكن القول من خلال النتائج ان زيادة مستوى المالون ثنائي الالديهايد في كريات الدم الحمراء للمصابات ومستوى اوكسيد النتريك في مصول دم النساء المصابات اصابة شديدة بالمقارنة مع مجموعة السيطرة من المحتمل ان تكون حدثت نتيجة لتغيرالانسجة في حالة الاصابة بداء المقوسات.