Effect of *Toxoplasma gondi* Infestation on Lipid Peroxidation and Certain Antioxidants in Pregnant Women in Mosul City

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(Received 28/2/2006 , Accepted 24/4/2006)

ABSTRACT

In order to determine the effects of toxoplasmosis on the lipid peroxidation and the glutathione defense system against peroxides. Forty pregnant women with toxoplasmosis (acute and chronic) and twenty healthy pregnant women (control group) with age range of 18-45 years living in Mosul city were selected. Serum malondialdehyde (MDA), reduced form of glutathione (GSH) and gluthathione peroxidase activity (GSH-PX) were measured. The results showed a marked increased in MDA level (as an index of lipid peroxidation) in patients with toxoplasmosis in comparison with the control group. On the other hand, patients with toxoplasmosis had decreased GSH and increased GSH-PX level when compared with the control subjects.

تأثير الإصابة بداء المقوسات الكوندية في بيروكسدة الدهن وبعض مضادات الأكسدة للنساء الحوامل في مدينة الموصل

الملخص

لغرض معرفة تأثير الاصابة بداء المقوسات الكوندية (داء القط) في بيروكسدة الدهن ونظام الكلوتاثايون الدفاعي. تم قياس مستويات كل من المالونداليالديهايد, الكلوتاثايون المختزل, وفعالية إنزيم الكلوتاثايون بيروكسيديز في 40 إمرأة مصابة بداء المقوسات الكوندية (اصابة حادة ومزمنة) وفي 20 مرأة سليمة, تراوحت أعمارهن من 18-45 سنة ومن سكنة مدينة الموصل وذلك في أثناء فترة الحمل. وقد أظهرت النتائج وجود زيادة معنوية في مستوى المالونداليالديهايد (كدليل على بيروكسدة الدهن) في المرضى المصابين قياسا الى الأصحاء. ومن ناحية أخرى لوحظ انخفاض معنوي في تركيز الكلوتاثايون مع زيادة فعالية الكلوتاثايون بيروكسيديز في المصابات بداء المقوسات الكوندية بالمقارنة مع مجموعة السيطرة.

INTRODUCTION

Toxoplasma gondii is a protozoan that infects most aspects of warm-blooded animals, including humans. The primary sources of infection for cats are probably small birds, rodents containing encysted forms of the organism (Pedersen, 1988).

Toxoplasmas exist in three forms: (i) the tachyzoite, which rapidly destroys infected cells; (ii) bradyzoites, which slowly multiplies in tissue cysts; and (iii) the oocyst which produces sporozoites (Wilson and McAuley, 1999; Krugman et al., 1985).

The complete cycle with gametogeny and formation of oocysts occur only in members of the cat family (*Felidae*). Thus cats are the only known definitive or intermediate host for the sexual stages of this parasite and have been reported to excrete oocysts, and were considered as the main reservoirs of infection while the entire life cycle can be completed within cats (Pedersen, 1988; Wilson and McAuley, 1999; Krugman et al., 1985).

In other animal hosts including humans, only an incomplete cycle of the organism occurs, and no oocyst develops (Krugman et al., 1985). Presumably, immunity to the parasite prevents reactivation of symptomatic infection (Krugman et al., 1985). Thus, in humans infected with *Toxoplasma gondii* the disease is asymptomatic. However, under some conditions toxoplasmosis can cause serious pathology, including blindness, pneumonia, hepatitis and severe neurological disorders in patients with human immunodeficiency virus (HIV) or malignancies (Frenkel, 1988; Sonnerborg et al., 1988; Tenter et al., 2000).

Human infection may be acquired in several ways: (i) ingestion of undercooked infected meat containing toxoplasma cysts; (ii) ingestion of the oocyst from fecally contaminated hands or food; (iii) blood transfusion; (iv) transplacental transmission (Wilson and McAuley, 1999).

The toxoplasma parasite is known to cross the placenta. It is an important cause of abortion (Remington et al., 2001). This disease is now recognized pathophysiologically as an endometrial dysfunction that probably is related to the accumulation of lipid peroxides produced in the placenta (Hubel et al., 1989; Wang et al., 1992). The placenta also has opposite physiological activities, including the production of antioxidants that decompose the placental lipid peroxides (Walsh and Wang, 1995). Generally, a healthy pregnancy is maintained by the quantitative balance between such oxidants and antioxidants (Hubel et al., 1989; Wang et al., 1992; Walsh and Wang, 1995), and the disintegration of this equilibrium might lead to endometrial dysfunction.

Free radical species are constantly formed as a byproduct of aerobic respiration and other metabolic reactions. Most of the oxygen (O_2) used during the oxidation of dietary organic molecules is converted into water, but a significant amount (1–2%) is diverted into highly reactive oxygen species (ROS), mainly the superoxide, hydroxyl, peroxyl, and hydroperoxyl anions (Burton et al., 2003). These metabolites are highly reactive, and consequently antioxidant defenses have evolved to detoxify them. Superoxide dismutase is present in all aerobic cells. It converts superoxide to hydrogen peroxide, which in turn is reduced to water by the antioxidant enzymes catalase and glutathione peroxidase. In addition to the antioxidant enzymes, other molecules called non-enzymatic antioxidants, such as thiols, ceruloplasmin, transferrin, glutathione and dietary vitamins, play a crucial role in tissue defense against free radicals (Burton et al., 2003). A complex homeostatic balance is thus achieved, and at physiological levels, free radicals regulate a wide variety

of cell functions through their influence on the thiol-disulfide redox state (Burton et al., 2003). If generation of free radicals exceeds the cellular defenses, however, indiscriminate damage can occur to proteins, lipids, and DNA, resulting in oxidative stress.

Although direct evidence for the involvement of ROS in the pathogenesis of toxoplasma infection is rather sparse, some data in support of this hypothesis are available in the literature. One point that has emerged clearly is the fact that Preeclampsia is associated with a quantitative imbalance between lipid peroxide and an antioxidant produced in the placenta (Takanashi et al., 2000).

Cellular mechanisms for defense against the potentially damaging effects of reactive oxygen species produced during Toxoplasma infestation rely heavily upon glutathione (GSH), which also participates in many other functions essential to normal cell physiology (Meister, 1989; Nelson and Kulkarni, 1990). The presence of GSH is necessary, but not sufficient, for effective functioning of GSH-dependent antioxidant defense mechanisms, which require the contributions of enzymes that include glutathione peroxidases (GPX). We also determined the extent of thiobarbituric acid reactive substances (TBARS) as the amount of malonaldehyde (MDA) in serum, in order to verify the possible correlation between the cellular enzymatic antioxidant capacity and the degree of membrane lipid peroxidation.

Thus, the aim of this study was to evaluate the status and the interrelationships of the antioxidant enzymatic activity, glutathione level, TBARS levels, and toxoplasma infestation due to *T. gondii* in blood samples from pregnant women.

SUBJECTS AND METHODS

Experimental Design

Forty pregnant women with toxoplasmosis and twenty healthy pregnant women (had no history of infection with toxoplasmosis were selected as a control group) with age ranging of 18-45 years living in urban and suburban area of Mosul city were participitated in this study. The patients with toxoplasmosis included 20 pregnant with acute case of infection and 20 pregnant with chronic case of toxoplasmosis.

Sample Collection and Analysis

Blood samples were collected from the non-treated patients during the first three months of gestation. A general information about each patient were recorded, included age, living area, main job, number of children, contact with cats, history of infection with toxoplasma and any teratogenic effects of the previous children. Serum was separated immediately and divided into two parts. The first part was stored at – 20ºC until assayed for glutathione peroxidase activity, glutathione and malondialdehyde. The second part of serum was used immediately for detection of antibody.

Glutathione peroxidase activity in serum was assayed colorimetrically, a method used by Nelson and Kulkarni, (1990). Serum glutathione was measured by a modified procedure utilizing Ellman`s reagent (Godin et al., 1988), while the level of malondialdehyde was carried out using the modified method of (Gilbert et al., 1984).

Detection of Toxoplasma Antibody:

Toxoplasmosis usually is diagnosed on the basis of antibody detection. This was confirmed by direct latex agglutination screening test using a kit produced by Biokit. SA

Company, Spain. A titre of 1:40 and above were considered positive (Desmonts and Thulliez, 1985). Two-Mercaptoethanol (2ME) was then used to differentiate between acute and chronic infection. This chemical compound acts to destroy the immunoglobulin M (IgM) through reducing the disulfide bonds connecting the five arms of this immunoglobulin. This can be done by mixing an equal volume (0.2ml.) of 2ME (0.2 M), (prepared previously by diluting stock 2ME in phosphate buffer solution (P.B.S.) at pH 7.2) and a diagnosed patient's serum, then were allowed for incubation at 37ºC. After one hour incubation, the mixed sample was tested again using a direct latex agglutination test. This method belongs the modified latex agglutination test (MAT) (Abdulla, 2001; الخفاف، 2001).

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 $p<0.05$ (Steel and Torrie, 1980).

RESULTS

The effect of toxoplasma infestation on lipid peroxidation by-product, MDA is shown in Fig.1. Acute toxoplasmosis produced a highly significant increase in malondialdehyde concentration when compared with the control group. Furthermore, Chronic infection of the pregnant women with toxoplasmosis produced a significant increase in malondialdehyde concentration as compared with the control group, but did not reached a level produced by the acute one.

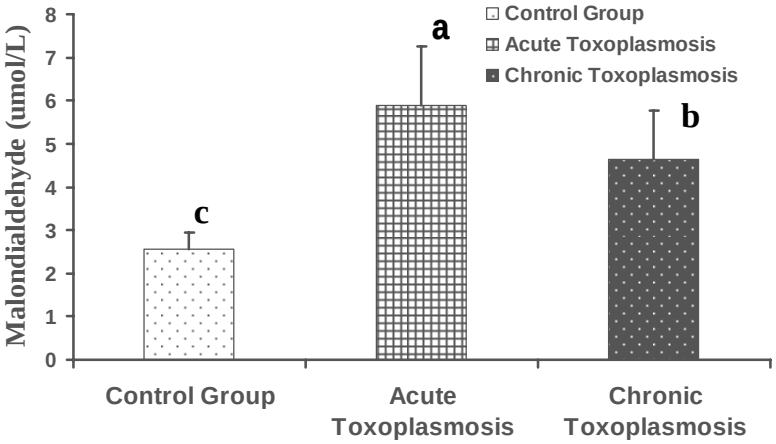

Fig.1: Effect of *Toxoplasma gondi* Infection on Serum Malandialdehyde Concentration in Pregnant Women.

On the other hand, infection of the pregnant women with chronic toxoplasmosis produced a significant decrease in glutathione content in comparison with the healthy pregnant women. Moreover, a significant decrease in glutathione content was also shown

in pregnant women infected with acute form of toxoplasma as compared with both healthy and infected groups with chronic toxoplasmosis.

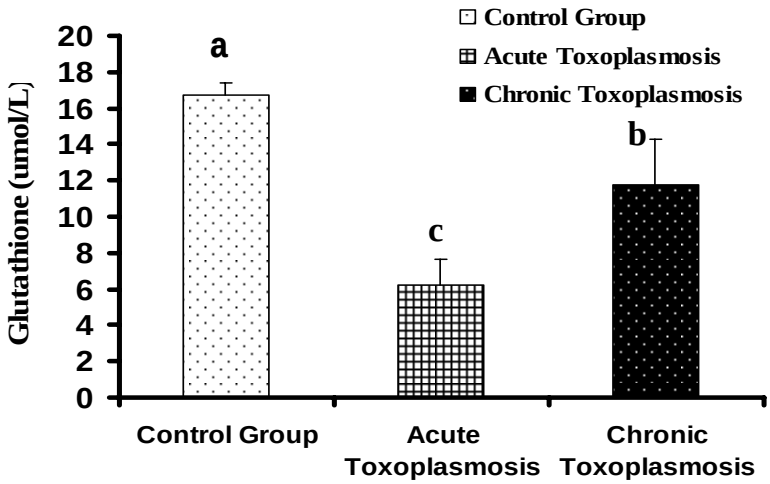

Fig.2: Effect of *Toxoplasma gondi* Infection on Serum glutathione Concentration in Pregnant Women.

Another antioxidant parameter involves glutathione peroxidase. Unlike the behavior of GSH, serum level of glutathione peroxidase showed a pronounced increase with acute toxoplasma infestation when compared with the control group. A significant increase was also noticed in pregnant women infected with chronic phase in comparison with the control group, but the increment was less significantly when compared with the acute toxoplasma group.

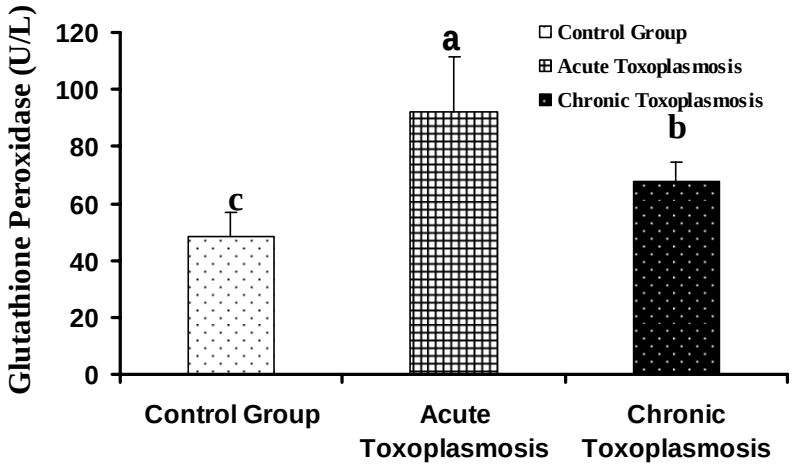

Fig.3: Effect of *Toxoplasma gondi* Infection on Serum Glutathione Peroxidase Concentration in Pregnant Women.

DISCUSSION

Free radical activity has been implicated in the pathogenesis of a variety of diseases, and many infectious diseases have been proved to be a direct and indirect sources of large numbers of these biotoxic agents (Gookin et al., 2005). Thus, the accumulation of lipid peroxides produced in the placenta in many diseases (Hubel et al., 1989; Wang et al., 1992) involves toxoplasma infestation, must be apposed or prevented through the production of antioxidants that decompose the placental lipid peroxides (Hubel et al., 1989; Wang et al., 1992; Jones et al., 2003.

Lipid peroxidation injury, as determined by the serum concentration of malondialdehyde in the present study, was significantly increased as a result of toxoplasma infestation. This may be due to these free radicals and oxidants produced as a result of parasitic infectious disease (Gookin et al., 2005). These radicals can react with DNA resulting in mutation or cytotoxicity. They also can bind to membrane lipids rich in polyunsaturated fatty acid (Fisher, 2003), producing a membrane destruction and cellular damage (Gilbert et al., 1984). On the other hand, production of these biotoxic agents was more in the acute than in chronic cases infested with toxoplasmosis, this is can be attributed to increased antioxidant intervention during chronic than during acute cases (Chade et al., 2004).

The diagnosis of *T. gondii* infection or toxoplasmosis in humans can be established by biological, serological, histological, or molecular methods or by some combination of these. Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic for a definite diagnosis. In fact, toxoplasmosis mimics several other infectious diseases. Detection of *T. gondii* antibodies (mainly immunoglobulin G [IgG] and IgM) in patients may aid diagnosis. IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 months, decline at various rates, and usually persist for life (Jenum et al., 1997). IgM antibodies may appear earlier and decline more rapidly than IgG antibodies, so the detection of IgG antibodies may be helpful for diagnosis of chronically infected patients, if IgM antibodies are negative. An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past (Wilson et al., 1997).

The production of free radicals due to toxoplasma infestation in the present study can be compared with other research of Wiznitzer et al., (1999) that human placentation associated with diseases, such as preeclampsia, hydatidiform mole, or miscarriage will be exposed easily to placental dysfunction. There is increasing evidence indicating that failure of placentation is associated with an imbalance of free radicals, which will further affect placental development and function and may subsequently have an influence on both the fetus and its mother. The mammalian embryo, and subsequently the fetus, is exposed to major fluctuations in O_2 concentration during pregnancy (Burton et al., 2003). The O_2 tension (PO_2) measured within the human placenta *in vivo* is less than 20 mm Hg at 7–10 wk gestation (Jauniaux et al., 2000). It subsequently rises to more than 50 mm Hg at 11–14 wk as the maternal intraplacental circulation becomes fully established. This inevitably leads to an increased production of ROS, and we have observed a burst of oxidative stress inside the placenta between 9 and 10 wk of gestation (Jauniaux et al., 2000).

As a result, this parasite was considered as an important cause of abortion and perinatal mortality (Remington et al., 2001) during this time. In about 40 percent of the

cases in which a pregnant women has toxoplasmosis, the baby is also infected (Remington et al., 2001) producing serious health problems, including mental retardation, seizures, blindness, and death (Jones et al., 2003).

A significant decrease in serum glutathione concentration was noticed in pregnant women infected with both acute and chronic toxoplasmosis. This indicates the presence of oxidative status, which may alter a variety of cell body functions (Dolphin et al., 1989). Moreover, the low GSH levels, especially in the infected pregnant women with acute phase of toxoplasmosis, represent a decreased detoxicating capacity of pregnant uterus and may enhance the risk of toxicity for the entire organism.

The alterations of serum GSH system components in patients with toxoplasma infestation may be in part explained by the changes in the GSH-related enzyme levels. Glutathione peroxidase (GPx) activity shows an increase in patients with both acute and chronic toxoplasmosis in comparison with the controls. We believed that the increased activity of this enzyme could be due to the simultaneous induction of their synthesis in response to the increase of radical, peroxide, and electrophilic compounds. Glutathione peroxidase play a major role in detoxification of lipid peroxides and hydrogen peroxide (H_2O_2), which belongs to reactive oxygen species (Droge, 2002). These results seem to agree with those obtained by other investigators (Samarawickrema et al., 1997), who observed a marked increase in superoxide dismutase (SOD) activity as enzymatic antioxidant was detected in *Entamoeba histolytica*. Furthermore, (Keller et al., 2004) reported an increase in nitric oxide synthase activity in peripheral blood of children with malarial parasite.

In biological tissues, the superoxide anion can be converted into the nonradical species hydrogen peroxide and singlet oxygen. Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase (Fig. 4). In the glutathione peroxidase reaction glutathione is oxidized to glutathione disulfide, which can be converted back to glutathione by glutathione reductase in an NADPH-consuming process (Fig. 4) (Droge, 2002).

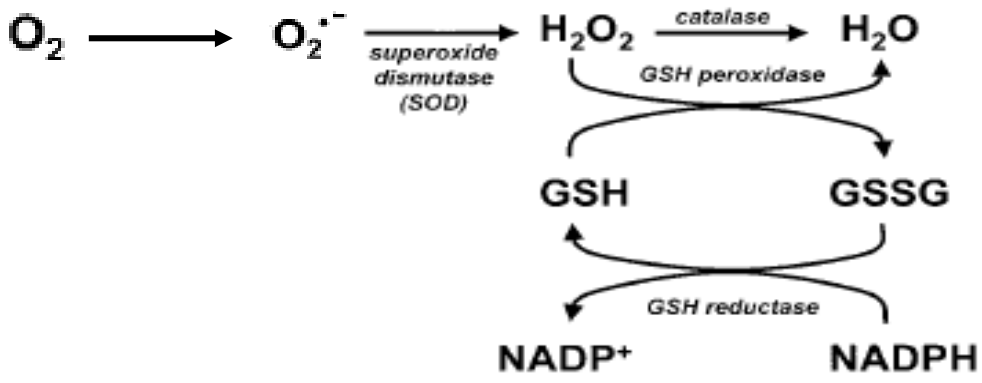

Fig.4: Pathways of reactive oxygen species (ROS) production and clearance. GSH, glutathione; GSSG, glutathione disulfide (Droge, 2002).

Decreased level of non-enzymatic antioxidant (glutathione) in the present study, has been previously reported in sheep with toxoplasmosis, (Seyrek et al., 2004). A decrease in nutrient antioxidant, zinc, which was significantly lower (68.65 ± 42.31 µg/dl) than that of healthy animals(110.58± 53.25) was reported (Seyrek et al., 2004). Furthermore, Jauniaux et al., (2004) showed limited antioxidant enzyme capacity during the first-trimester human placenta.

In the present study, blood samples were collected from the non-treated patients during the first three months of gestation. This can be based on the report by Remington et al., (2001), who said that when the mother is infected between 10 to 24 weeks gestation, the risk for severe problem in newborn is about 5 to 6 percent. When the mother is infected late in pregnancy, the chance that baby will have problems is very small. Therefore, most abortions occur in the first three months of gestation. This can be attributed to urinary 2-Hydroxyestradiol 17-Sulfate (2-OH-ES), a Potential Placental Antioxidant, that increased markedly as gestation progressed.

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