The Role of Some Cytokines and Trace Elements in Pregnant Women with Acute Toxoplasmosis

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Abstract

The present study was conducted on sixty five pregnant women that infected with acute toxoplasmosis, serum samples were tested for detection specific anti-*Toxoplasma gondii* IgM using the rapid Toxo IgG/IgM Chromatographic immune assay test cassette and ELISA**,** and fifty healthy married women (not pregnant) as was considered as control group.

The level of serum IL-6, IL-8, IL-12, TNF-α, TGF-β1, IP-10, MIP-1, MIG and ICAM were significantly increased (P <0.005) in pregnant patients infected with acute toxoplasmosis, while serum level of MCP-1(14.46 ± 1.38pg/ml) was not significantly increased when compared to controls(11.25 ± 0.24pg/ml) , these levels can be used as indicator to acute toxoplasmosis.

Trace elements concentrations in serum were measured during infection also, the results of this study revealed that serum Zn was markedly elevated (p<0.05) and serum Cu was significantly decreased (P<0.001) in Toxoplasmosis-seropositive women as compared with control.

Key words: *Toxoplasma gondii*, Cytokines, Zn, Cu

Introduction

T*oxoplasma gondii* is an obligate intracellular protozoan with unparalleled global distribution and mammalian host range. [1]. It is possible to differentiate between acute infections (initiated within Infection with the prior12 months) and chronic infections (more than one year since the initial infection) by using ELISA [2].Toxoplasmosis infection stimulates humoral immune response as antibody (Ab)production, in addition T cell-mediated immunity (CMI) which is essential for the host and control of intracellular infection [3]. The macrophage, T- lymphocytes and Natural killer (NK) cells, and cytokines are the major elements involved in immune response against *T. gondii*. Cytokines are secreted by a variety of cell types, including inflammatory cells, and play crucial functions in immune-regulation and immune responses including responses to parasite infection [4, 5].

Many studies have documented the role of cytokine in the resistance to acute and chronic infection to tachyzoite replication of *T. gondii* during pregnancy [6].IL-10, IL-12 and TNF-α play an important role in the innate immunity against *T gondii* and influence the adaptive immune response; these cytokines are important immune-modulators that act during the early stage of infection [7,8].

Trace element plays an important role in biological systems through their action as activators or inhibitors of enzymatic reaction, or by its essential role of direct antioxidant enzyme, or by influencing the permeability of cell [9].Zinc (Zn) is essential for maintaining the integrity of immune function of T-cells has been associated with deficiency of Zinc, which acts as a growth hormone [10]. The element copper (Cu) is an integral part of cytochrome [8], and also required for normal red blood cells information [11].

The present study aimed to determine the serum levels of 10 cytokines IL-6, IL-8, IL-12, TNF-α, IP-10, MCP-1, MIP-1, MIG-1, TGF-β1 and ICAM, also it is designed to investigate the effect of the infection with acute toxoplasmosis on the levels of essential trace elements Zn and Cu, such collective evaluations may aid in a better understanding of the immunological mechanisms that can lead to acute toxoplasmosis in Iraqi pregnant women.

Materials and Methods

Sixty-five pregnant women at first trimesters of pregnancy suffering from problems of miscarriage were attending to Department of Gynecology and Pediatrics in Al-Yarmouk Teaching Hospital and Imamein Kadhimein Medical City in Baghdad during the period from October 2014 to March 2015 were conducted in this study. Their age ranged from 20-41 years, in addition a group of 50 fertile healthy married women (not pregnant) were enrolled in this study as a control group.

Blood samples collection:

Five mL of venous blood were collected from each women and placed in plane tube where the serum was separated and stored at -20C until it is used.

Serological tests:

1-Rapid Toxo IgG/IgM Chromatographic Immune Assay Test Cassette:

Detection of parasite antibody ,the kit is supplied by CTK Biotech, San Diego, USA.

2-ELISA *T. gondii* –IgM:

The ELISA Toxo-IgM (From Biokit Diagnostics Company, Spain) is an IgM- capture immunoenzimatic assay for the determination of IgM antibodies against *T.gondii* in the patient's serum or plasma.

Calculation of results:

The mean of duplicate absorbance of the low positive calibrator was calculated which represents the cut-off value: Cut-off = mean of low positive absorbance. The absorbance of

each sample was divided by the cut-off value, and then the results were compared with the following ratios to differentiate between positive, negative and equivocal samples**.**
Positive sample: Ratio absorbance/cut-off > 1.0
Negative sample: Ratio absorbance/cut-off < 0.9
Equivocal sample: Ratio absorbance/ cut-off ≥ 0.9 ≤ 1.0 sample was retested with a fresh new sample after 2-4 weeks.
Through a result of serological tests that mentioned above, it was able to diagnose 65 cases of acute toxoplasmosis infection of pregnant women (with high titer IgM antibody) and 50 healthy married women (not pregnant) to measure serum level of cytokines and chemokines.

3-Serum Level of Cytokines and Chemokines:

Sera of pregnant women that have a positive IgM antibody against *T. gondii*, and control group were enrolled to measure levels of cytokines IL-6, IL-8, IL-12, TNF- α and chemokine MCP-1, IP-10, MIP-1, ICM, MIG-1 and growth factor TGF-β1 by using ELISA test. All kits were provided from Pepro Tech; USA. The manufacturer's protocols were followed for each kit and recombinant reference cytokine and chemokine samples were served as positive control for calibration.

4-Serum level of Zn and Cu:

Serum diluted (0.1 ml) to total volume of 1ml using 6%n-butanol solution and analyzed for Zn and Cu levels using atomic absorption spectrophotometer (Shimadzu AA-646) using Zn and Cu hollow cathode lamps at wavelengths 213nm and 324.75nm respectively.

Statistical analysis:

Statistical analysis was computer assisted using Statistical Package for Social Science (SPSS) version 13.0 was applied and least significant difference (LSD) between groups.

Results

Table (1) shows that serum sample from the study population were successfully analyzed for IgM by ELISA. The median values of IgM –*Toxoplasma* antibodies titer was statistically significant .It is higher in the group of pregnant women with positive diagnosis by ELISA (1.98±0.850), compared to those with negative diagnosis of toxoplasmosis (0.41±0.315) There were statistically significant differences between the two groups (P<0.001).The highest rate (27.0%) of Toxo-IgM was found in pregnant women from the age group 26-31 years old.

The results of cytokines and chemokines levels show in table (2) there was a significant increase in cytokines IL-6, IL-8, IL-12, NF- α, IP-10, MIP-1, ICM, MIG-1 and TGF-β1 in pregnant women that they were positive to *T. gondii* IgM serology which significant increase (P < 0.05), while the mean of MCP-1 serum concentration (14.46±1.38pg\ml) was significantly decreased (P <0.05) in patients in comparison to control group(11.25±0.24pg/ml) (Table 2).

In table (3) Zn and Cu levels analysis in sera of positive women and in control, the mean levels of serum Zn were significantly lower in patient group(p≤0.05) than in the healthy controls whereas serum Cu concentrations were significantly (p≤0.001) higher in patient group when compared with control..

Discussion

The results of the present study showed that median value of IgM- *Toxoplasma* antibodies in women with toxoplasmosis was 1.98±0.850, this result reflects that infected women had acute toxoplasmosis .ELISA technique for detection of IgM and IgG of *T. gondii* must be used within *Toxoplasma* serologic profile that included different

agglutination and ELISA ,which are routinely used to assess whether infection was acquired during pregnancy[6].

The serum level of IL-6 was higher than its level in control group which is considered as an indicator for acute inflammation during infection and the results were compatible with that recorded by AL-Damoshi *et al*., [7]. This cytokine may occur early in infection before the immunological shift from Th2 into Th1 that might be responsible for the several cases of abortion [12].

IL-8 is a potent chemo attractant and activator of neutrophils. It is produced by wide cells, fibroblasts, neutrophils, endothelial variety of cell types, including macrophages, chondrocytes and osteoclasts, In agreement with the present results, Koumantaki *et al*., [13] found that IL-8 was significantly increased in acute with early acute inflammation or with a reactive from toxoplasmosis. The inflammatory cytokines, such as IL-8,may play a vital role in mechanism of protease induced neurogenic inflammation leading to labor or abortion by enrolling neutrophils and lymphocytes in the endometrium, whereas previous studies reported that women with spontaneous abortions had pointedly decreased plasma level of IL-8 compared to those with normal pregnancies [7,8]. In addition to IL-8, the presence of a significant increase of serum level of IL-12 is a key cytokine that drives the inflammatory response mediated by Th1 cells. Monocyte /macrophage and dendritic cells are active in IL-12 production during the first contact of *Toxoplasma* with innate immune system [14]. IL-12 is required for resistance to acute and chronic toxoplasmosis due to its essential role in stimulating production of IFN-ɣ, facilitates formation of Th1 type response and activates lymphocyte cytotoxicity. This present study showed high level IL-12 patients with toxoplasmosis in comparison with control group (Table- 2). This may be due to the fact that the infection with *T. gondii* induce strong CMI characterized by high Th1-cell response [15].

TNF-α is a Th1 response cytokine produced by macrophage, T-lymphocyte, basophils and monocytes. In toxoplasmosis, TNF-α appears to be essential for macrophage activation and inhibition of parasite replication, of this cytokine documented in resistance to the acute and chronic Toxoplasmosis, the level of this cytokine increases, abortion occurs, the current results in this regard is in consistency with Trowsdal and Betz [16].

The level of IP-10 cytokine that showed a significant increase in serum of infected group is a key mediator of the interferon response referentially attracts activated Th1 lymphocytes to sites of inflammation, and is an inhibitor of angiogenesis. The present study indicated that IP-10 is critical for survival following *T. gondii* infection and is essential for guiding antigen specific CD41 and CD81 T cells into infected organs. *T. gondii* evokes a strong humoral and cellular immune response in the infected host. The chemokine IP-10 a specific chemoat-tractant for activated T cells. These observations suggest that IP-10 may play a broader role in the localization and the function of effector T cells at sites of Th1 inflammation [17].

The chemokines, MCP-1 ,IP- 10 and MIP-1 are involved in the cellular recruitment and activation of several leukocytes such as monocytes/macrophages, polymorphonuclear cells, and lymphocyte infiltration to the sites of infection (19).The immune response against infection stimulate macrophages and lymphocytes that work on the production of MCP-1 .Low level MCP-1 (CCL2) were observed in pregnant women with acute toxoplasmosis (Table-2), because live tachyzoiest forms of *T. gondii* inhibited the synthesis of MCP-1(20),and *T. gondii* tachyzoites were found to modulate the synthesis of CCL2 in cell cultures of parturient and non-pregnant women[21].

The results of present study showed that the increase in the level of ICAM -1(Table -2), in accordance with this study Pfaff *et al*.,[22] that demonstrates ICAM-1 is the principal receptor for increased monocyte adhesion to activate primary trophoblast. IFN-ɣ plays a pivotal role in

the cell adhesion process through up regulation of ICAM-1 and in the process of congenital transmission of *T. gondii* [22].

The current study showed a significant increase in the level of MIG-1(CXCL9) in the blood serum of infected group compared to control, and this may be due to the CXCL9 is critical for the recruitment of T cells in the immune areas that breed tachyzoites to prevent chronic activate *Toxoplasma* infection [17].

The present study showed that the serum levels of Transforming factor-beta TGF-β is significantly increased in infected women. TGF-β is a cytokine which plays key roles in the regulation of immune cell functions .This cytokine leads to inhibition of B and T lymphocytes proliferation and induces homeostasis [23].TGF-β also contributes to tissue remodeling which occurs after infections and injuries, this cytokine contributes to development of Th17 and T-regulatory lymphocytes, activation and suppression of immune which play significant roles in parasite responses, against infection [24].

The results showed ,that there was significant decrease in serum Zn levels in patients ,this can be according to Kadham [25]refearing to this lowering value , probably due to the shifting of this element from the transport media (serum) to the liver which is considered as a protective mechanism against the disease.

Zn is a metal essential for maintaining the integrity of immune system. The results showed that the specific cellular immunity is detectable in virtually all *Toxoplasma*-infected patients [10, 11]. Several investigators have found that Zn deficiency depresses antibody responses possibly owing to a loss of T- helper cell function. The increased serum level of Cu may be explained to its form of serum which is formed in response to inflammation associated with the disease [26, 27].

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Table(1): The median values of *T. gondii* IgM antibodies in pregnant women by ELISA

IgM *Toxoplasma* antibody titer	Diagnosis of Toxoplasmosis by ELISA(IU/ml)		P value
	Negative ±S.E.	Positive ±S.E.	
Range	1.20-2.9	0.17-0.8	<0.001
median	1.98 ±0.850	0.41±0.315	
No.	50	65	

Table(2): The mean level of cytokine and chemokine in sera of pregnant women with acute toxoplasmosis and control.

Cytokine	Serum Level (Mean ± S.E.; pg/ml)		LSD value
	Controls (No. = 50)	Patients (No. = 65)	
IL-6	6.38 ± 0.78	27.01 ± 3.31	7.396*
IL-8	0.611 ± 0.01	0.805 ± 0.03	0.083*
IL-12	0.56 ± 0.12	2.47 ± 0.35	5.478*
TNF-α	0.040 ± 0.002	0.056± 0.003	0.0087*
IP-10	6.68 ± 0.76	26.39 ± 2.80	6.299*
MIP-1	3.50-± 0.31	15.55 ± 1.63	3.628*
MCP-1	11.25 ± 0.24	14.46 ± 1.38	3.845N.S.
MIG-1	0.0004 ± 0.0047	0.009 ± 0.067	0.021*
TGF-β1	1.52±0.05	20.99±4.13	9.05*
1CAM	5.28 ± 0.61	13.33 ± 1.36	3.189*

N.S.: Not significant : Significant* ($P < 0.05$)

Table -3: Serum Zn and Cu levels in pregnant women with acute toxoplasmosis and control

Parameters	Mean(µM\L)±SD		P value
	Controls	Patients	
Zn	9.99±1.44	5.68±0.61	p<0.05
Cu	26.46±2.14	38.30±11.31	p<0.001

Significant ($P < 0.05$), ($p<0.001$)

دور بعض الحركيات الخلوية والعناصر النزرة لداء المقوسات الحاد في النساء الحوامل

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الخلاصة

اجريت الدراسة على 65 من النساء الحوامل المخمجات بداء المقوسات الحاد بعد الكشف عن الاضداد للمقوسة الكوندية من نوع IgM باستعمال الكروماتوكرافي الثنائي السريع (كاسيت) وفحص الادمصاص المناعي المرتبط بالانزيم (الاليزا), واشتملت ايضا على 50 من النساء المتزوجات الاصحاء (غير الحوامل) كمجموعة سيطرة. تم التحري على بعض التغيرات المناعية المصاحبة للخمج لدى هولاء النساء الحوامل المخمجة بداء المقوسات الحاد من خلال قياس مستويات بعض الحركيات الخلوية والكيميائية.اظهرت النتائج ارتفاعا معنويا($P<0.005$) بمستويات IL-6 ,IL8, IL-12 ,TNF-α,TGF-β1 ,MIP-1, IP-10 , MIG-1 وكذلكICAM بينما لم يسجل MCP- 1 مستوى معنويا (14.46 ±1.38بيكوغرام /مل) مقارنة بمجوعةالسيطرة (11.25 ± 0.24بيكوغرام/مل), وهذه يمكن أن تستعمل كمؤشرات لداء المقوسات الحاد.
اظهرت النتائج انخفاضا معنويا ($p<0.05$) في تركيز الزنك في المصل بينما لوحظ ارتفاع معنوي ($p<0.001$) في تركيز النحاس في مصل مريضات داء المقوسات الحاد مقارنة بالسيطرة غير المصابة.

الكلمات المفتاحية: مقوسات كوندي ,الحركيات الخلوية ,خارصين, النحاس