



The Effect of *Toxoplasma gondii* on Interleukin-8, Interleukin-10, Leukotriene B4 and Calcium Levels in Aborted Women

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ABSTRACT

This research was conducted during period of February 2016 to July 2017 to detect *Toxoplasma gondii* recruiting 106 female patients with repeated abortion with age between 18-43 years who attended Al-Fayeth clinical laboratory in Baghdad. The diagnosis was done by Immunochromatography and ELISA methods. Blood sample was taken from each patient as well as other 30 healthy control of same age group. The study included measurement of concentration of interleukin-8, interleukin-10, leukotriene B4 and calcium in sera of patients and control. The result indicated presence of the anti-*Toxoplasma* IgG level in 45 cases, and anti-*Toxoplasma* IgM in 76 cases out of 106 cases of women by immunochromatography methods whereas anti-*Toxoplasma* IgG in 40 cases and Anti-*Toxoplasma* IgM in 42 cases by ELISA methods. Also, the result indicated increasing levels of IL-8 and IL-10 in patients' sera and decreasing level of leukotrienes B4 and calcium compared to healthy women (control).

Keywords: *Toxoplasma gondii*, Interleukin-8, Interleukin-10, Leukotrienes B4, Calcium

Abbreviations: ELISA: Enzyme-Linked Immunosorbent Assay; IL-8: Interleukin-8; IL-10: Interleukin-10; LTB4: Leukotriene B4; IFN- γ : Interferon-gamma; CD8+ T cells or CD4+ T cells: Cytotoxic T cell; CD40: Cluster of Differentiation 40; CD40L: CD40 Ligand

INTRODUCTION

Intracellular parasites like *Toxoplasma gondii*, usually contaminate with an extensive level of infections to the eukaryotic cells. It is quite significant devious pathogenic organism in both humans as well as animals. Often this contamination comes with no symptoms, nevertheless, the two sets that threaten personally, the humanoid genes and the persons with immunosuppression, mainly to those who have weak immune system, which lead to lethal toxoplasmic encephalitis [1].

Infection of the *T. gondii* could be developed via infection that are inherited or it could be via carnivore, when the soft tissues swelling (cyst) which lead to regular infection (chronic) to the host. Similarly, it could be developed via digestion of diet contaminated by microbes (particularly parasites), which could be oocysts shape. These can be found in the infected cat faeces [2]. Subsequently, after food ingested, parasites converted to quick duplication to well-known tachyzoite that outcomes to disseminated system to most of soft tissues. Besides usual conditions, the infected system can be controlled successfully via the response of the immune hosts.

Parasites duplicate gradually and eventually form bradyzoite that remain in the cysts of the tissues of the neural hosts as well as the tissues of the muscles for the host life time [3]. The inherited contamination could lead to defects in birth which includes hydrocephalus, spontaneous abortion, chorioretinitis and intra cerebral calcification [4]. The innate immune system responds through monocytes, dendritic cells (DCs) and *T. gondii* which ends up infecting

tissues. These types of cells are involved and are responsible for the microbial resistance [5-10]. Furthermore, the common pathway of pathogen in addition to the creation of cytokine IL-12, which initiates the natural cell killer and the tumour cell (T cell) to produce cytokine IFN- γ which is considered as a main resistant intermediate to *T. gondii* which encourage a numerous intracellular procedures and mechanism that is fatal to all the parasites and prevent its duplication and replication [11-13].

The immune response is critical in extending the duration of contamination and development of resistance in the patients. Otherwise, it develops obstacles in the function of T cells with Blood cells (B cells) defect. Cytotoxic T cells, CD8+ T cells or CD4+ T cells survival are the important steps of the contamination, which eventually leads to increase in weakness of *T. gondii* [14-16].

Throughout initial steps of the contamination (infection), CD4+ T cells help CD8+ T and B cells to response, the capability of those cells is to regulate the chronic contamination which attribute and effect the cytokines production like CD40L (ligand deficiency) which also related to CD154, it could be initiated the macrophage mechanism, extra innate cells that express the CD40 on its surfaces [16-20] while the CD8+ T cells could regulate the contamination via producing inflammatory cytokine like IFN- γ by interaction between CD40 and CD40L and also via host cells that infected with perforin [16].

In this study, the level of IL-8, IL-10, LTB₄, and calcium was determined in patients with toxoplasmosis as inflammatory mediators in patients compared with healthy control group of similar age group and gender.

MATERIAL AND METHODS

Study groups

This research work was conducted from the period between February 2016 to April 2017. The patients' ages were between 18 years and 43 years old. The patients were divided into two groups, which include suspected patients. Blood samples were collected from 106 women suspected as *T. gondii* infected which was tested and confirmed (clinically suspected cases) by specialist doctors and the other group was control including healthy individuals.

Blood samples

The blood samples were taken from the participants' veins and were collected in pastoralized plastic tubes. Each sample was left for 30 minutes at room temperature in serum separator tubes in vertical position prior to centrifugation. The samples were centrifuged at 3000 rpm and kept for 5 min. The serum was collected from every sample using Eppendorf tube and was stored at -20°C. This sample was used to evaluate immunological and clinical biochemical aspects.

Immunochromatographic assay

About 100 μ l of serum from each sample was added to the sample hole of the kit. The colour density is proportional to the antibody titer. The complexes (appears in colour band after 10 minutes) confirm that the test was performed correctly. This CerTest-Toxoplasma kit which qualitatively determines the *Toxoplasma* in blood samples. Pre-coating was achieved to the membrane proceeding to test band region to the monoclonal antibodies of the mouse, it was achieved against *Toxoplasma* antigens. Through test, samples were reacted with conjugated colours (anti-*Toxoplasma* of monoclonal mouse microsphere (red antibodies)), the samples were dried before that, the combination then travelled to reach membranes via the act of capillaries. While samples move via the membranes tests, tinted particle were migrated. In positive results, certain antibodies that have existed on the membranes captured these particles which lead to appearance of red tinted line that can clearly observed while the other result appears in a green tinted line (the negative results, that represent the control samples).

IgM Toxoplasma antibodies and IgG determination

To achieve the qualitative and quantitative investigation to IgG *Toxoplasma gondii* and IgM, two kinds of kits were used to detect the antibodies in infected woman serums; these kits are (*Toxoplasma* IgM, EIA, enzyme immunoassay) and *Toxoplasma* IgG EIA (enzyme immunoassay) (ACON Laboratories, Inc. San Diego, USA). *Toxoplasma* antibodies levels were assessed using ELISA technique. The level of IgM or IgG below 0.9 IU/ml was considered negative and from 0.9 to 0.99 IU/ml was equivocal limit and should be rechecked, while positive level was equal to 1.0 IU/ml or above.

Estimation of the level of IL-8, IL-10 and LTB4

The levels of IL-8, IL-10 was estimated in 42 patients who were found anti-*Toxoplasma* IgM positive by ELISA according to manual procedure of Cusabio Biotech (Germany) and leukotriene B4 was estimated by ELISA according to the manual procedure of Creative-Diagnostic Company. Calcium concentration was determined according to manufacturer's instructions of Biosystem (Spain).

Concentration of serum calcium

Serum calcium concentration was determined according to manufacturer's instructions of Biosystem (Spain).

Statistical analyses

The results were analysed using Statistical Package for the Social Sciences (SPSS) version-18 (T-test).

RESULTS AND DISCUSSION

Serological examination

The serum level of anti-*Toxoplasma* IgM present in 79 cases were 71%; also, the level of anti-*Toxoplasma* IgG present in 45 cases with a percent of 42% while the level of both present in only 12 cases with a percent of 11% out of 106 cases in immunochromatography method (Table 1).

Table 1 Distribution of anti-*Toxoplasma gondii* IgG and IgM antibodies using immunochromatography method in women with repeated abortion

Anti- <i>Toxoplasma</i> antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgG	106	45	42	66	58
IgM	106	76	71	30	29
IgM+IgG	106	12	11	94	89

The serum level of anti-*Toxoplasma* IgM present in 42 cases (39%), while in anti-*Toxoplasma* IgG present in 40 cases with 32% while the level of both present in only eight (7.5%) out of 106 cases by ELISA method (Table 2).

Table 2 Distribution of anti-*Toxoplasma gondii* IgG and IgM antibodies using ELISA method in women with repeated abortion

Anti- <i>Toxoplasma</i> antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgG	106	40	37	60	63
IgM	106	42	39	64	61
IgM+IgG	106	8	7.5	98	92.5

Interleukin-8

The serum level of IL-8 pg/ml was found to be increased significantly in patients' groups of all ages as compared with healthy females (Table 3).

Table 3 Concentration of IL-8 in patients with Toxoplasmosis as compared to healthy females of same age range

Age (years)	Group	IL-8 (pg/ml)
18-28	Positive	118.2 ± 0.4*
	Control	59.6 ± 0.8
29-39	Positive	134.6 ± 0.5*
	Control	62.5 ± 0.7
>40	Positive	133.8 ± 0.4*
	Control	87.4 ± 0.2

*p<0.05: Significant

Interleukin-10

The serum level of IL-10 pg/ml increased significantly in patient's groups of all ages in comparison with healthy control group (Table 4).

Table 4 Concentration of Interleukin-10 in patients with Toxoplasmosis as compared with healthy females of same age group

Age (years)	Group	IL-10 (pg/ml)
18-28	Positive	391.4 ± 0.2*
	Control	118.2 ± 0.4
29-39	Positive	298.2 ± 0.6*
	Control	117.1 ± 0.8
>40	Positive	365.6 ± 0.4*
	Control	126.7 ± 0.7

*p<0.05: Significant

Leukotriene B4

The serum concentration of LTB4 pg/ml decreased significantly in patients' groups of all ages in comparison with healthy control (Table 5).

Table 5 Concentration of LTB4 in patients with Toxoplasmosis as compared to healthy females of same age range

Age (years)	Group	LTB4 (pg/ml)
18-28	Positive	26.6 ± 0.4*
	Control	37.4 ± 0.8
29-39	Positive	35.5 ± 0.7*
	Control	35.6 ± 0.5
>40	Positive	5.8 ± 0.6*
	Control	36.2 ± 0.5

*p<0.05: Significant

Calcium concentration

The concentration of calcium decreased significantly in patients' groups of all ages in comparison with healthy control (Table 6).

Table 6 Concentration of calcium in patients with Toxoplasmosis in comparison with healthy control according to their interval ages

Age (years)	Group	Calcium concentration (mg/dl)
18-28	Positive	7.5 ± 0.4*
	Control	9.2 ± 0.6
29-39	Positive	7.3 ± 0.7*
	Control	9.3 ± 0.2
>40	Positive	7.8 ± 0.3*
	Control	9.6 ± 0.2

*p<0.05: Significant

DISCUSSION

The majority of acquired infection in healthy individual are benign and either asymptomatic or with vague symptoms. The ratio of positive to infection with *T. gondii* parasite that appeared in female, which amounted to 46%, is a clear indication on the capacity of the spread of the parasite *T. gondii* in different population. About one-third of the world's population is estimated to carry *Toxoplasma* infection. There are large variations in prevalence and within different countries in humans [21].

The result indicated anti-*Toxoplasma* IgG was 45 (42%) of cases while IgM was 76 (71%) cases by immunochromatography method (Table 1) and anti-*Toxoplasma* IgG was 40 (37%) while IgM 42 (39%) cases by ELISA method (Table 2). Generally, the prevalence of infection is related to several factors including nutritional habits, contact with soil, age, rural or urban settings, and frequency of contact with domestic animals and pregnancy and climatic condition such as humidity. ELISA is reported as more specific and sensitive than other tests [22]. The prevalence of anti-*Toxoplasma gondii* antibody observed in the study was in agreement with seroprevalence data from previous studies conducted in our country 66 (29.2%) from a survey carried out in Salah-Adden Government on

226 pregnant women had Toxoplasmosis. Anti-*Toxoplasma* IgG was 59 (26.1%) of cases while IgM antibody was 7 (3.1%) of cases [23].

The statistical results indicated increase of IL-8 in patients in comparison with healthy control of all ages (Table 3). This increase is due to *T. gondii* stimulate secretion of the pro-inflammatory chemokines like IL-8.

The chemokine response is dependent on invasion by live tachyzoites and subsequent host cell lysis. IL-8 is responsible for activation and recirculation of neutrophils and neutrophils can phagocyte and kill or inhibit tachyzoites of *Toxoplasma* and showed that human intestinal epithelial cells infected with *T. gondii* elicit rapid secretion of IL-8 [24].

The increasing level of IL-10 in patients in comparison with healthy control of all ages (Table 4) may be due to ability of the parasite to enhance TH2 cytokines among these was IL-10. However, IL-10 is strong enemy to macrophages capability in order to kill bacteria inside the cells microbes as well, examples are infections and *T. gondii* via numbers of pathogens, the presence of *T. gondii*, will lead to increasing in the IL-10 expression [25-28]. The decreasing level of leukotriene B4 in patients of toxoplasmosis in comparison with healthy control in all interval ages (Table 5) may be due to the parasite alter or fail to activate the 5-Lipoxygenase pathway in human monocyte.

The suppression of 5-Lipoxygenase pathway may be important for survival of the parasite as LTB4 induce surface membrane vesiculation, leakages of cytoplasmic content in to space between the inner and outer surface membrane unit and intracytoplasmic vacuolation in to *T. gondii* leading cytotoxic change occur in *T. gondii* result in intracellular killing of the pathogen in contrast to intracellular replication also, *T. gondii* induced inhibition of mononuclear phagocytes by LTB4 release could be an important anti-inflammatory mechanism used by the parasite to diminish the influx of leukocyte in the tissue site of *Toxoplasma gondii* infection and inhibit IFN- γ mediated toxoplasmodicidal activity by the selective 5-lipoxygenase suggest that 5-LO arachidonic acid metabolites play important role in mediation of cytotoxic activity [29].

Also, the result indicated decreasing level of calcium in patients group in comparison with healthy control (Table 6) the reasons may be due to the intracellular Ca²⁺ changes in *T. gondii*, especially due to calcium ionophores and extracellular calcium are involved in the invasion and intracellular replication of *T. gondii* [30].

CONCLUSION

The results indicated presence of anti-*Toxoplasma* IgG in 45 cases, anti-*Toxoplasma* IgM in 76 cases out of 106 cases of aborted women suspected infected with *Toxoplasma* by immunochromatography methods in comparison with anti-*Toxoplasma* IgG 40 cases and Anti-*Toxoplasma* IgM 42 cases by ELISA methods. Also, the results indicated significant increased levels of IL-8 and IL-10 in patients' sera and significant decrease in levels of LTB4 and calcium as compared with healthy control.

DECLARATIONS

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee and all women provided informed written informed consent prior to entering the study.

Conflict of interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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