Annals of Parasitology 2022, 68(2), 317–321
doi: 10.17420/ap6802.437

Original paper

Association of interleukin-6 and interleukin-1-β levels in patients with toxoplasmosis

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ABSTRACT. Toxoplasmosis is an important disease, which is caused by obligate intracellular parasite *Toxoplasma gondii*. Maternal infection with toxoplasmosis during pregnancy is frequently stimulates the immune response that may cause the fetus to be lost or deformed. A case control study were conducted in Al-Nassiriya province, southern Iraq to measure the interleukin-6 and interleukin-1-β levels. One hundred and twenty seven recurrent aborted women suspected with toxoplasmosis attending to Bent Al-Huda Maternity and Child Hospital for the period from March 2020 to March 2021, and compared to (20) healthy women as a control group. The ages ranged between 16–42 years, 5 ml of blood samples were collected for serum detection positivity of latex agglutination test, Enzyme Linked immune sorbent assay (interleukin-6 and interleukin-1-β). The distribution analysis showed that the highest toxoplasmosis among aborted women compared to healthy control group, the infection rate were (39.37%). Higher percentages of toxoplasmosis (82.35%) was found within the age group (15–25) years. In addition, the results showed a high positive of toxoplasmosis in 43 cases out of 50 cases by latex agglutination test. Also, the result indicated increasing levels of IL-6 and IL-1-β in patients' sera (24.69, 14.21) respectively, compared to healthy women.

Keywords: *Toxoplasma gondii*, interleukin-6, interleukin-1-β, toxoplasmosis

Introduction

Toxoplasmosis is one of the most common parasitic diseases where approximately one-third of the world's population is affected [1]. It is capable of causing severe and life threatening conditions specially in pregnant women and immune-compromised individuals [2]. Infections generally occur by the consumption of undercooked meat that contains tissue cysts or by water and food contaminated with oocysts present in cat faeces [3]. In addition, the infection can be transmitted directly from the mother to the fetus during the transition of the active phase tachyzoite across the placenta, rarely through blood transfusions or organs transplantation [4]. Congenital infection is one of the most important sequels of toxoplasmosis in pregnant women [5]. Congenital transmission of *Toxoplasma gondii* predominantly occurs at the first time during pregnancy [6].

The severity of congenital toxoplasmosis is highest in the first and second trimesters of pregnancy, which usually results in abortion or stillbirth [7]. Cytokines are essential for the normal development of pregnancy, any imbalance in the amount or location of expression can influence trophoblastic and endometrial reactions leading to pregnancy complications [8].

Therefore, this study was designed to analyze the association of IL-6 and IL-1β with toxoplasmosis that affects regulation of the immune response.

Materials and Methods

Patients and controls

The present study were conducted on 127 aborted women who suffer from recurrent abortion and 20 healthy women as a control group that pregnancy with normal delivery without any history of abortion. Samples were collected from patients suspected infection by toxoplasmosis and a control

Table 1. Distribution of toxoplasmosis according to age group

Age group	No. of sample	No. of positive sample	Infection rate (%)
16–25	17	14	82.35 %
26–35	33	19	57.58 %
>36	77	17	22.08 %
Total	127	50	39. 37 %

Chi-square=0.041, D.F=3, P=0.462, $P \leq 0.05$

group who attended Bent Al-Huda Maternity and Child Hospital in Nassiriya Province from March 2020 to March 2021.They were (16–42) years old age.

Blood samples

Five ml of venous blood were drowned from vein of each suspected patient and control groups by using disposable syringes, this blood were collected in a sterile serum tube and left 30 minutes at room temperature to separate the serum, which was collected into Eppendorf tube by micropipette and stored at –20°C until for serological tests.

Serological tests

Latex agglutination test (*Code No. 1201002*)

The Toxo-latex is a slide agglutination test for the qualitative and semi-quantitative detection of anti-*Toxoplasma* antibodies. Latex particles coated with soluble *Toxoplasma gondii* antigen are agglutinated when mix with samples containing antibodies anti-*Toxoplasma* [9].

Enzyme Linked Immune Sorbent Assay

Interleukin-6 (*Cat. No. E-CL-H0101*)

This IL-6 kit uses the Sandwich-IL-6 principle. The micro-IL-6 plate provided in this kit has been pre-coated with an antibody specific to Human IL-6. Standards or samples are added to the micro CLIA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-6 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro-plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-6 biotinylated detection antibody and Avidin-HRP conjugate will appear fluorescence. The relative light unit (RLU) value is measured by the chemiluminescence immunoassay analyzer. The RLU value is positively associated with the concentration of Human IL-6.

Interleukin-1-β (*Cat. No. RD194559200R*)

The Human IL-1β solid-phase sandwich ELISA is designed to measure the amount of the target bound between a matched antibody pair. A target-specific antibody has been pre-coated in the wells of the supplied microplate. Samples, standards, or controls are then added into these wells and bind to the immobilized (capture) antibody. The sandwich is formed by the addition of the second (detector) antibody, a substrate solution is added that reacts with the enzyme-antibody-target complex to produce measurable signal. The intensity of this signal is directly proportional to the concentration of target present in the original specimen.

Statistical analysis

Statistical analysis were carried out using SPSS statistical package (version 20). Analysis of variance (ANOVA) of the data was used to detect overall difference in groups mean. Data are presented as the mean ± standard error. Differences among group means were assessed using least significance difference (LSD). Proportions were compared by Chi-square. $P \leq 0.05$ was considered statistically significant.

Results and Discussion

The result of present study showing that, the 50 patients from 127 aborted women who suffer from *Toxoplasma gondii* in serum samples, the infection rate among patients were (39.37%). According to age groups, a higher percentage in *Toxoplasma gondii* infection (82.35%) was found within the age group (16–25) years followed by (57.58%) within the age group (26–35) and the lowest one was (22.08%) within the age group (>36). There is a significant difference in the rate of infection between age groups (Tab. 1).

The prevalence of infection is related to several factors including nutritional habits, contact with soil, age, rural or urban settings, and frequency of

Table 2. The percentage of toxoplasmosis according to latex agglutination test

Latex agglutination test	No. of sample	Percentage	*P*-value
Positive	43	86%	
Negative	7	14%	0.0001
Total	50	100%	

contact with domestic animals and pregnancy and climatic condition such as humidity [10].

The age group (16–25) may be represents an optimum period of fertility, thus, this critical period of women's life has higher chances for activation of latent infection of *T. gondii* that can be transmitted vertically to the fetus, which was considered as one cause of abortion as mentioned by Remington et al. [11]. The second decay of age (26–35) represents a mother's stage and the prenatal detection of antibodies against *T. gondii* in pregnant women, it was critical with regard to the management of serious congenital complication including abortion [12].

The prevalence of toxoplasmosis observed in the study was in agreement with seroprevalence data from previous studies conducted by Mahdi et al. [13], ADdory [14] in Iraq, Alghamdi et al. [15] in Saudi Arabia, Coelho et al. [16] in Brazil and Shin et al. [17] in Korea.

Table 2 shows the relationship among latex agglutination test, the result revealed that, there is a significant relationship between recurrent aborted women (P=0.0001), 43 (86%) out of 50 were positive by latex test and 7 (14%) for recurrent aborted women was negative.

This can be explained by the constant exposure of women to the risk factors of *T. gondii* infection through their routine household tasks such as minced meat products, raw and unwashed vegetables and fruits, municipal drinking from polluted reservoirs, In addition to the spread of stray cats that play a key role in the distribution of infection [12,18].

In this study, the amount of IL-6 and IL-1-β in infected patients was (24.69 and 13.73), respectively, higher than those of controls group (14.21 and 7.35). It is significantly more than the healthy controls (Tab. 3).

The host's responses to infection, inflammation, and trauma are controlled by pro-inflammatory cytokines, which can make disease worse in co-disease states [19]. While its biological activities overlap on a large scale, IL-1-β is developed during early pregnancy by autotrophic cells at the interface between the fetus and the mother and is involved in trophoblastic invasion and tissue repair [20]. Whereas, IL-6 is a powerful vascular cytokine that stimulates endothelial cell proliferation.

Previous studies have identified a critical role for IL-6 in resistance to *T. gondii* [21,22], and although the mechanism by which IL-6 promotes resistance to this pathogen remains unclear, these findings are consistent with its role as a pro-inflammatory factor. Although IL-6 is constitutively produced during many types of acute and chronic inflammation, its function may change depending on context. The transmembrane protein gp130 is a signal-transducing component of the receptors used by several closely related cytokines that include IL-6. IL-6 and gp130 are essential for the development of a protective immune response that allows the host to control parasite replication. IL-6 contribute to the control of pathogen replication or limit pathology [23].

IL-1-β is a pro-inflammatory cytokine that plays a critical role in host defense and innate immunity. IL-1 given its potent inflammatory activities, the

Table 3. Comparison between infected patients and healthy control interleukins (IL-6 and IL-1-β) concentration

Groups	Mean ± Standard Error		
	No.	IL-6 (Pg/ml)	IL-1-β (Pg/ml)
Infected patients	50	24.69±1.66 ^{a}	14.21±1.39 ^{b}
Healthy control	20	14.21 ±1.33 ^{a}	7.35±1.02 ^{b}
LSD		3.19	1.87

production of IL-1-β can also trigger immune pathology and tissue damage, reinforcing the importance of IL-1-β regulation for maintaining innate immune function without excessive inflammation [24]. IL-1-β plays an important role in protective immunity *T. gondii* infection. Monocytes are critical for controlling *T. gondii* infection by induce the cleavage of pro- IL-1-β and the release of mature IL-1-β [25].

The extracellular pattern recognition receptor (PRR) sensing of *T. gondii* was insufficient to induce IL-1-β and that either intracellular sensing of the parasite or the activity of parasite effector molecules was required. GRA15 is secreted into host cells during invasion and is believed to be continuously released from intracellular parasites, contributing to increased nuclear translocation of the NF-B p65 subunit over time [26]. Human blood monocytes have constitutively active caspase-1 and can release mature IL-1-β in response to TLR signals alone without the need for a second signal to activate the inflammasome [27]. It is possible that $GRA15_{II}$ activation of IL-1-β transcription is sufficient to drive monocyte production of IL-1-β. Alternatively, $GRA15_{II}$ may mediate both the induction of IL-1-β mRNA via NF-kB and IL-1-β processing by activating the inflammasome through an uncharacterized mechanism. A third possibility is that $GRA15_{II}$ induces IL-1-β transcription and that another aspect of *T. gondii* infection serves as the "second signal" to activate the inflammasome. This second signal may be a different parasite effector protein, since it is now appreciated that a large number of parasite-secreted proteins gain access to the host cell cytosol [28] and may serve as potential ligands for inflammasome sensors. The second signal could also be provided as an indirect effect of infection, for instance through the release of "danger signals" such as ATP from dying cells. Although we have confirmed high monocyte viability at the end of each infection experiment, it is possible that ATP or other factors are released from the small percentage of dying cells. ATP translocation into cells via the P2X7 receptor (P2X7R) has been shown to activate the inflammasome, leading to IL-1-β processing [29], and several studies have indicated a role for the P2X7R in host defense against *T. gondii* [30,31].

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Received 27 January 2022
Accepted 05 April 2022