Toxoplasma gondii infection correlated to Interleukin 12 in wasit Women

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Abstract

Background: Toxoplasmosis is a common and serious parasitic infection caused by the ubiquitous obligatory intracellular protozoan organism, Toxoplasma gondii. Moreover, it has been reported that IL-12 is important in initiating an effective cell -mediated immunity against T. gondii tachyzoites,

Materials and Methods: Two hundred and seventeen blood specimens was collected from pregnant women with previous abortion, in addition to twenty specimens as a control from healthy women, through a skilled and professional procedure in a gel tube, then blood samples were centrifuged in which supernatant was extracted, extracted samples were preserved in refrigerator -4℃ until using later. Samples were taken from patients who were present in Al-Zahraa teaching hospital and Al-Kut teaching hospitals. Serological investigations were done to detect IgM and IgG in the sera of women using ELISA technique, in addition to detection of Interleukin 12 in aborted, pregnant and control samples.

Result: Correlation between IgM and IgG concentrations and Interleukin 12 outcomes indicated that Interleukin 12 was 4.13 pg/mL in cases of Toxoplasma IgM and 4.12 pg/mL in Toxoplasma IgG, regard CMV Interleukin 12 concentration was 3.79 pg/mL in cases of IgM and 3.67 pg/mL in IgG, about both infection cases results recorded 4.13 pg/mL and 4.43 pg/mL of 12 concentration in the cases of IgM and IgG respectively, finally control reported that 12 concentration was 4.79 pg/mL in IgM cases and 3.93 pg/mL in IgG cases, (p value= 0.047). Regarding comparison between IL-12 concentration according to abortion, results showed that Toxoplasma IL-12 concentration was 4.10 pg/mL in aborted and 4.01 pg/mL in non-aborted, CMV IL-12 concentration was 3.77b pg/mL in aborted and 3.59 pg/mL in non-aborted, both IL-12 concentration was 4.09 pg/mL in aborted and 4.03 pg/mL in non-aborted state, (p value= 0.052).

Conclusion: Interleukin 12 was recorded as an important factor during Toxoplasma gondii infection.

Keywords: *T. gondii, infection, Interleukin 12.*

1-Introduction

Apicomplexan parasites are responsible for several diseases of veterinary and medical relevance, including zoonotic diseases. Mosquito-borne hematozoan parasites of the genus Plasmodium are the causative agents of malaria, a life-threatening disease with over 200 million cases estimated in 2019 (Sahu et al. 2020). Toxoplasma gondii and Sarcocystis spp. are cyst-forming Coccidia as well; however, they do not present host specificity with regard to the intermediate host, T. gondii is exceptional in the variety of intermediate hosts it can infect, assumed to be all warm-blooded animals (Alvarez-García et al. 2014). Schematic diagram of ELISA. a Indirect ELISA system almost all used for detection of T. gondii antibodies rather than antigens involves the specific antigen coated onto the solid phase, enzyme-conjugated secondary antibody and substrate. b Sandwich ELISA system detecting T. gondii antigens involves the specific antibody coated onto the solid phase, enzyme-conjugated antibody and substrate (dos Santos et al. 2010). It has been reported that the infection with T. gondii enhances both humoral and cell-mediated immunity, the innate response to T. gondii includes the involvement of a wide range of cytokines such as interleukins, Interferon gamma (IFN-y), Tumour Necrosis Factor (TNF), Nitrogen monoxide (NO), a Reactive Oxygen species (ROS) and many other factors. In addition, IFN- γ and α are important for controlling tachyzoite replication during both acute and chronic phases of infection, while IL-10 and IL-12 are important at the initial phase of infection but less important during the chronic stage of infection (Al-Dorry, Yaseen, and Molan 2021).

2-Patients, Materials and methods

2-1 Specimens Collection

Two hundred and seventeen blood specimens was collected from pregnant women with previous abortion, in addition to twenty specimens as acontroll from healthy women, through a skilled and professional procedure in a gel tube, then blood samples were centrifuged in which supernatant was extracted, extracted samples were preserved in refrigerator -4℃ until using later. Samples were taken from patients who were present in Al-Zahraa teaching hospital and Al-Kut teaching hospitals. Serological investigations were done to detect IgM and IgG in the sera of women using ELISA technique.

2-2 Procedure of Human Interleukin 12 ELISA Kit

All reagent were brought to room temperature before using. It was reconstituted 120 ul from the original standard (80 ng/ml) with 120 ul of standard diluent to generate a 40 ng/ml standard stock solution, it was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. It was prepared duplicate standard points by serially diluting the standard stock solution, 1:2 with standard diluent to produce 20 ng/ml, 10 ng/ml, 5 ng/ml and 2.5 ng/ml solutions. Standard diluent was served as the zero standard (0 ng/ml). any remained solution was frozen at -20℃ and used within one month. It has been determined the number of standard wells. It had been added 50 ul standard to standard well. There was addition of 40 ul sample to sample well and then it was added 10 ul of anti-IL-12 antibody to sample well, then 50 ul streptavidin-HRP has been added to sample well and standard well, it was mixed well, covered the plate with a sealer and incubated 60 minutes at 37℃. The sealer and the plates were washed 5 times with wash buffer. It was soaked well with 300 ul wash buffer for 30 second to I minute for each wash. It was blotted the plate onto paper towels.

substrate solution A 50 ul was added to each well and then it was added 50 ul substrate solution B to each well. Plates were incubated covered with a new sealer for 10 minutes at 37 ℃ in the dark. It has been added 50 ul stop solution to each well, the blue color was changed to yellow immediately. The absorbance O.D. at 450nm using a mcrotiter plate reader was read. The OD value of the blank control well is set as zero. Assay should be carried out within 10 minutes after adding stop solution.

2-3 Statistical Analysis

Data were entered, coded, and analyzed in SPSS (statistical package for social sciences) software program version 26. Data analysis were done using different tests. Frequency and percentages were used for the description of categorical variables. The mean and standard deviation were used to describe the continuous variables. Both Chi-square and Fisher's exact test were used for the assessment of the association between categorical variables. For the differences between means in continuous variables, the independent sample t-test, one way ANOVA test were used accordingly. Spearman correlation coefficient was used to assess the presence of correlation in non-normally distributed variables. A P-value equal to or less than 0.05 was considered significant. A bar chart was also used for the graphical presentation of the data.

3-Results

3-1 Correlation between IgM and IgG concentrations and Interleukin 12

In this study IgM and IgG concentrations were correlated the Interleukin 12 concentration, outcomes indicated that Interleukin 12 was 4.13 pg/mL in cases of Toxoplasma IgM and 4.12 pg/mL in Toxoplasma IgG, regard CMV Interleukin 12 concentration was 3.79 pg/mL in cases of IgM and 3.67 pg/mL in IgG, about both infection cases results recorded 4.13 pg/mL and 4.43 pg/mL of 12 concentration in the cases of IgM and IgG respectively, finally control reported that 12 concentration was 4.79 pg/mL in IgM cases and 3.93 pg/mL in IgG cases. The current results indicated significant differences (p value= 0.052) as shown in table (1) and (2).

Table (1): Correlation between IgM and Interleukin 12 concentration

Concentration of Interleukin-12		
Pathogens	Antibodies classes	Mean of IL-12 concentration pg/mL
Toxoplasma	IgM	4.13a
Cytomegalovirus	IgM	3.79b
Both	IgM	4.13a
Mean of Control	IgM	4.79 ab
P value	0.052	

*Similar letter means no significant difference

Each number represent mean of all tested samples

Table (2) Correlation between IgM and Interleukin 12 concentration

Concentration of Interleukin-12		
Pathogens	Antibodies classes	Mean of IL-12 concentration pg/mL
Toxoplasma	IgG	4.12a
Cytomegalovirus	IgG	3.67b

Both	IgG	4.43a
Mean of Control	IgG	3.93 a
P value	0.52	

*Similar letter means no significant difference

Each number represent mean of all tested samples

3-2 Comparison between IL-12 concentration according to abortion

Among collected specimens IL-12 concentration was tested to be correlated with abortion of patients included in this study, results showed that Toxoplasma IL-12 concentration was 4.10 pg/mL in aborted and 4.01 pg/mL in non-aborted, CMV IL-12 concentration was 3.77b pg/mL in aborted and 3.59 pg/mL in non-aborted, both IL-12 concentration was 4.09 pg/mL in aborted and 4.03 pg/mL in non-aborted state, the current results indicated significant differences (p value= 0.047) as shown in table (3).

Table (3): Comparison between IL-12 concentration according to abortion state

IL-12 concentration according to abortion		
Antibodies of infected pathogens	Concentration of IL-12 pg/mL	
	Mean in aborted	Mean in non-aborted
Toxoplasma	4.10a	4.01a
Cytomegalovirus	3.77b	3.59b
Both	4.09a	4.03a
P value	0.047	

*Similar letter means no significant difference

Each number represent mean of all tested samples

4-Discussion

Th2 lymphocytes synthesize specific cytokines (IL-4, IL-5, IL-6, IL-12, IL-13, and IL-14), which play a major role in the pathogenesis of parasitic diseases. IL-5 is the major cytokine responsible for the increase in the eosinophil population in parasitoses, whereas IL-6 stimulates production of antibodies and exerts a proinflammatory effect by stimulating the generation of acute phase proteins. IL-10 and IL-12 control the type of the immune response. The former inhibits cytokine synthesis and by blocking the production of IL-6 and TNF-α causes an advantage of the response occurring with Th2 involvement and B cell activation. However, IL-12 facilitates formation of a Th1 type response (Cooke 2012).

The present study results were compatible with Nickdel et al. (2004) proved that the early stage of T. gondii infection, coexisting with eosinophilia and increased level of IL-5, pathological changes are prominent especially for IgM antibody, which is accompanied by a simultaneous reduction in IL-12. It was noted an increase in the level of IL-5 and a decrease in the level of IL-12, as in the study of Filisetti and Candolfi (2004) revealed an IL-5 induced increase in IL-12 IgM production in toxoplasmosis. On the other hand out outcomes were disagreed with results revealed a decrease in the production of IL-12 and inhibition of Th1 response have been reported by Lang et al. (2007). A study was carried out to investigate the immune status of patients infected with toxoplasmosis. One hundred fifty samples of patients and controls which had

been tested by ELISA technique to detect anti-Toxoplasma Abs (IgG and IgM). The positive and negative toxoplasmosis samples were tested to detect the level of IL-12, IL-23 and TGF β-3. Results showed there one samples clarified that seropositive for IgM antibodies while 64 (64%) heart disease patients were seropositive for IgG antibodies and for toxoplasmosis only patients were 20 (40%) and 30 healthy as a (controls) were seronegative for IgG antibodies with significant differences ($P< 0.05$). Serum level of IL-12 was recorded an increase in a group of patient with toxoplasmosis (275.4 ±42.3 pg/ml) with highly differences of significant ($P < 0.05$) also IL-23 level was increased in a group of patient only (80.3 ±42.3 pg/ml) with no significant differences .TGF β-3 levels was highly in heart disease patient with toxoplasmosis (381.8 ±44.0 pg/ml) with significant differences at ($P< 0.05$) (Hammadi and Hamad 2021).

In our study, T. gondii patients had a higher level of IL-12 as compared to other subjects included CMV and both infections, our outcomes were related to which seems to confirm the presence of an inflammatory state, interleukins production, may plays an essential role in the inflammatory response during acute T. gondii infection, since it inhibits the immune response of IL-12 and inflammatory responses in non-aborted female, this outcome were the same of Wilson et al. (2005). The results obtained by Khosroshahi et al., (2012) indicated that after challenging the mice with the fatal RH strain of T. gondii, the survival rates of mice immunized with pcROP2+pcSAG1, pcSAG1+pcROP2+alum, and pcSAG1+pcROP2+IL-12 in aborted mice were significantly greater than that of the control groups and non-abortion mice ($p < 0.05$). Moreover, measurement of total IgG antibody indicated the significant difference between the control and experimental groups ($p < 0.05$).

While current study data were not compatible with Evering and Weiss (2006) showed tha the cellular response, involved among others IL-12 and TNF-α, plays an important role in parasitic invasions. In our patients infected with T. gondii, the levels of IL-12 and TNF-α were comparable to those observed in healthy controls. IL-12 was found to increase the production of IgG but not to inhibit IgE. IFN-γ, IL-2, and IL-12 are involved in the protection against parasitic invasions. IL-12 stimulates the production of IFN-γ and TNF-α and activates lymphocyte cytotoxicity. In the course of toxoplasmosis the levels of TNF-α and IL-12 did not change (Mahmoudzadeh et al. 2021), also Denis et al. (2022) has been mentioned the same information in both aborted and not aborted cases. However, as reported by Lang et al. (2007) T. gondii inhibits production of TNF-α and IL-12. TNF-α is a cytokine of inflammatory and immune response and together with IL-6 can enhance proliferation and differentiation of B lymphocytes.

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