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EVALUATION OF SOME CYTOKINES AND GROWTH FACTORS IN SERUM OF HEART DISEASE PATIENTS INFECTED WITH TOXOPLASMOSIS

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ABSTRACT

This study was carried out to investigate the immune status of heart disease patients infected with toxoplasmosis. One hundred fifty samples of both heart disease 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 heart disease 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 heart disease 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).

Keywords: Cytokines, Growth Factors, Heart Disease, Toxoplasmosis

Introduction

Toxoplasmosis is a coccidian and international disease in humans and animals caused by the opportunistic parasite named *Toxoplasma gondii*, it is an obligate intracellular parasite caused various disease for example toxoplasmic encephalitis and congenital birth deficiencies (Zhou *et al*., 2011). Cats and other members of felidae are the definitive hosts, whereas human and wide range of animals, birds and rodents act as intermediate hosts (John *et al*., 2006). Human infection is acquired through intake of undercooked meat infected with tissue cyst , or sporulated oocysts or food and water contaminated, also by mother-to-child transmission, or via an infected allograft during organ transplantation. Acute infection is typically asymptomatic in immunocompetent individuals, but cervical lymphadenopathy or ocular disease can occur and in immunocompromised patients AIDS, (cancer and other diseases) may became seriously ill or fatal (Ryan *et al*., 2004).

Can be diagnosed by different techniques by Latex agglutination test, ELISA, and PCR (Dubey, 2016). The specific acquired immunity begins to develop few days after infection and persists for a prolonged period, perhaps for life. Both cell-mediated immunity and humeral immune response are elicited by *T. gondii* infection (Gillespis *et al*., 2003). The major elements play role in immune response to parasitic infection were Natural killer cells, T lymphocytes and Macrophages in combination with cytokines (Cordeiro *et al*., 2008). The immune system includes pro-inflammatory cytokines that can enhance the functions of other cytokines and the immune response and anti-inflammatory cytokines that suppress this response; various interleukins (ILs) stand out in these responses (Shankaran *et al*., 2001). They are primarily synthetized by T cells, monocytes, macrophages and endothelial cells. The functions of ILs include the facilitation of communication among immune system cells, regulation of transcription factors, and control of inflammation, cell differentiation, proliferation and antibody secretion (Salazar-Onfray *et al*., 2007).

The function of IL-23 and a new member of the IL-12 family of regulatory cytokines produced by activated macrophages and dendritic cells. IL-12 and IL-23 promote two distinct immunological pathways that have separate but complementary functions. IL-12 is required for antimicrobial responses to intracellular pathogens, whereas IL-23 is likely to be important for the recruitment and activation of a range of inflammatory cells that is required for the induction of chronic inflammation and granuloma formation. Transforming Growth Factor-β (TGF-β3) family of proteins that have attracted much attention because of their ability to control cellular functions. TGF-β3 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 (Strack *et al*., 2002) .

Infections with *T. gondii* may manifest in the heart in humans and animals with myocarditis, pericarditis with myocarditis and acute heart failure. Acute and latent chronic *T. gondii* infections may seriously affect heart function and clinical course of cardiac disease leading to development of cardiovascular diseases in patients with autoimmune disease, particularly in those with impaired innate and, or acquired immunity (Dubey, 2013).

Material and Methods

The present study included collected samples from Ibn AL-Bitar (Specialist Center for Cardiac Surgery). The patients with heart disease ages ranged from 20 to 70 years old. Samples of five milliliter were collected from venous blood from each patient into test tubes, free from EDTA, left it at the room temperature to clot and the centrifuged at 3000 rpm for five min. The serum was separated and preserved at deep freeze (-20 ºC) till it is used.

The (IgG, IgM) antibody level against *T. gondii* was read through ELISA technique using the available kits (Foresight kit) which prepared according to company manufacture by using ELISA techniques.

Serum Level of Cytokine and Growth factor:

Sera of heart disease patients that have a positive IgG antibody against *T. gondii* and control group were enrolled to measure levels of cytokines IL-12, IL-23 and growth factor TGF β-3 by using ELISA test. All kits were provided from MyBioSource; USA. The manufacturer's protocols has been followed for each kit and recombinant reference cytokine and chemokine samples has been served as positive control for calibration.

Statistical Analysis

The data were analyzed by using the IBM SPSS computer program version 25.0 for the parametric data by calculating the mean and standard error of the mean, the probability was calculated by using T-test and ANOVA table. While, Pearson's chi-square was used to calculate the probability of the non-parametric data.

Results

In the present study of 150 samples from patients and healthy male and female were investigated for toxoplasmosis using *Toxoplasma* IgG, IgM. Table (1) showed that 64 (64%) from 100 patients have positive response for ELISA test which have heart disease and toxoplasmosis, while 36(36%) from 100 patients have a negative response for ELISA which have heart disease only, and 50 healthy (control) showed that 20 (40%) volunteers have positive response for ELISA while 30 (60%) healthy have negative response for ELISA. Such difference was significant between these groups($P < 0.05$).

Table 1 : Prevalence *T. gondii* infection according to ELISA IgG test in studied groups

Groups	No. of tested Samples	No. (+) for toxoplasmosis (%)
Heart disease with Toxoplasmosis	100	64 (64%)
Healthy controls	50	20 (40%)
P. Value	≤ 0.05	*

The results showed presence of anti-*Toxoplasma* IgM Abs in 1 of 100 (1%) in the Heart Diseases patients group. However, the current results indicate no significant differences showed in *Toxo.* IgM ELISA test table (2). Most of antibody of IgM was least than cut-off value (< 9.0 pg/mL) in addition, the life span of IgM is lower than 2 weeks, thus it might not reflect the real size of the problem in our society.

Table 2: Distribution *T. gondii* infection according to *Toxo.*IgM (pg/ml) by ELISA test in studied group

Diagnosis	Response for Toxoplasmosis	ELISA Test						P. Value
		Heart Diseases		*T. gondii*		Control		
		No.	%	No.	%	No.	%	
Toxo. ELISA Kit IgM	+Ve	1	1	0	0.0	0.0	0.0	NS
	-Ve	99	99	20	40	30	60	
Total		100		20		30		150

Table (3) shows the distribution of toxoplasmosis according to the age of study group, considering the age groups and it is relation with the heart disease and toxoplasmosis infection. The results of this study showed that presence of anti-*Toxoplasma* Abs (IgG) in all age groups of this study, the highest value of anti-*Toxoplasma* IgG Abs was between 56-62 years of heart disease infected with toxoplasmosis which was 17 (26.6%). While the lowest seroprevalence percentage was 2 (3.1%) in the age between 70-76 year. There were differences of a statistical significant among the groups age.

Table 3 : Prevalence of Toxoplasmosis and heart disease with the age groups

Age years	No. Heart disease with toxoplasmosis	No. Heart disease only
20 -26	6 (9.4)	1 (2.8)
27 – 34	7 (10.9)	2 (5.6)
35 – 41	5 (7.8)	2 (5.6)
42 – 48	8 (12.5)	7 (19.4)
49 – 55	11 (17.2)	8 (22.2)
56 – 62	17 (26.6)	7 (19.4)
63 – 69	8 (12.5)	8 (22.2)
70 – 76	2 (3.1)	1 (2.8)

Table (4) shows the distribution of study groups according to gender, the higher value of anti-Toxoplasma IgG Abs was 38 of 57(66.66%) in male of heart disease patients and the lower value of seroprevalence of anti-

Toxoplasma Abs 26 of 43 (60.46 %) was in female in heart disease patients, while the value of anti-Toxoplasma IgG Abs in apparently healthy was similar in both male and female 10 of 25 (60%) for each gender. However, the results of current study indicate to non- significant differences at ($p \leq 0.05$) between male and female in both groups (heart disease patients and apparently healthy).

Table 4 : Distribution of study groups according to gender

Groups	Gender	IgG Positive		IgG Negative		Total	P. Value
		N	%	N	%		
Heart disease	Male	38	66.66	19	33.33	57	
	Female	26	60.46	17	39.53	43	NS
Control	Male	10	40	15	60	25	
	Female	10	40	15	60	25	NS

Results showed a statistically significant elevation in the mean level of the cytokine (IL-12) in heart disease patients infected with toxoplasmosis, the mean level of the cytokine (IL-12) was (275.4 ± 42.3 pg/ml) comparing with production in the control group and mean level for heart disease only was (39.7 ± 9.9 pg/ml). However the mean level of (IL-12) was (29.8 ± 4.2 pg/ml) in group infected with *Toxoplasma gondii,* and as showed there are significant differences between male and female samples in heart disease patients infected with toxoplasmosis compared with control (Table 5).

Table 5 : Descriptive Statistics for All Groups for IL-12

Groups	(mean IL-12 level ± SE) pg/ml			
	Male	Female	P.Value	Total
Heart disease with toxoplasmosis	212.6 ± 50.4 ^{A}	359.3 ± 68.1 ^{A}	P < 0.05	275.4 ± 42.3 ^{A}
Heart disease only	38.7 ± 13.0 ^{B}	41.0 ± 16.0 ^{B}	P > 0.05	39.7 ± 9.9 ^{B}
T. gondii	46.7 ± 4.1 ^{B}	21.4 ± 3.8 ^{B}	P > 0.05	29.8 ± 4.2 ^{B}
Control	4.2 ± 2.5 ^{B}	8.8 ± 3.6 ^{B}	P > 0.05	5.5 ± 2.1 ^{B}

Result showed no significant differences in IL-23 levels in all groups compared with control group .but there are significant differences between females samples in Heart disease patients infected with Toxoplasmosis group compared with control and *Toxoplasma gondii* groups as shown in table (6).

Table 6 : Descriptive Statistics for All Groups for IL-23

Groups	IL-23 level (mean ±SE) pg/ml			
	Male	Female	Probability	Total
Heart disease with toxoplasmosis	23.8 ± 7.7 ^{A}	155.7 ± 96.6 ^{A}	P < 0.05	80.3 ± 42.3 ^{A}
Heart disease only	1.4 ± 1.0 ^{A}	0.0	P > 0.05	0.8 ± 0.6 ^{A}
T. gondii	1.8 ± 1.7 ^{A}	7.0 ± 2.9 ^{B}	P > 0.05	5.3 ± 2.1 ^{A}
Control	4.4 ± 4.3 ^{A}	3.6 ± 3.5 ^{B}	P > 0.05	4.1 ± 2.9 ^{A}

Serum growth factor TGFβ-3 mean levels in heart disease patients with toxoplasmosis group was (381.8 ± 44.0 pg/ml) differed from that of control group, the mean level of TGFβ-3 was (108.8 ± 32.0 pg/ml), which is highly statistically significant ($P < 0.05$), while in group heart disease only the mean level was (220.8 ± 36.3 pg/ml) and *Toxoplasma gondii* group the level was (159.0 ± 34.6 pg/ml), also there are significant differences between males and females samples in Heart disease patients infected with Toxoplasmosis group compared with control (Table 7).

Table 7 : The levels of TGF β-3 in the sera of the studied groups

Groups	TGFβ-3 level (mean ±SE) pg/ml			
	Male	Female	P.Value	Total
Heart disease with toxoplasmosis	269.3 ± 43.1 ^{A}	531.8 ± 69.4 ^{A}	P < 0.05	381.8 ± 44.0 ^{A}
Heart disease only	229.5 ± 48.4 ^{AB}	209.6 ± 57.8 ^{B}	P > 0.05	220.8 ± 36.3 ^{B}
T. gondii	132.5 ± 42.3 ^{AB}	198.7 ± 59.8 ^{B}	P > 0.05	159.0 ± 34.6 ^{B}
Control	48.4 ± 32.9 ^{B}	138.9 ± 43.0 ^{B}	P > 0.05	108.8 ± 32.0 ^{B}

Discussion

High distribution rates of heart disease and toxoplasmosis in Iraq due to many factors like genetically or environment conditions and numerous nations around the whole world. Also keeping in mind the essential role of the complications and impairment of the cellular and humoral immunity resulted from heart disease, the present study aimed to determine the levels of sera of Toxoplasma antibodies in heart disease patients and measured the levels of cytokine (IL-12,IL-23) and growth factors TGFβ-3.

The our results showed that 64 cases out of 100 had a heart disease with a positive IgG antibodies of Toxoplasma. This results was match to (Yazar S *et al*., 2006) results (11), who found that 68 % cases in the patient group were positive for IgG antibodies. While did not agreed with Abdulhussein HS 2017(12), in his study the presence of anti-Toxoplasma

IgG Abs was 43.33%. The seroprevalence estimated for human population varies greatly among different countries, among different geographical regions in the same country, even within, same city (Jones JL and Dubey JP2010) .This may explain the variation in seropositivity. Also the similarities and variation in the results may be related to several factors, including climatic, nutrition habits, cultural patterns, sample size, target population, age , sampling method, types of laboratory tests and tools or may due to different manufacture origin of the kits used (Al-Saadii 2013).

The current study results showed that distribution of IgM- antibodies of *Toxoplasma* in heart disease patients was 1%, this result reflects that infected one heart disease patient had acute toxoplasmosis, while for IgM antibodies was less than cut-off value which might be referred to the infection nature in which most of patient infected didn't attended the hospital through the acute stage, the life span for IgM less than 14 days. The results of ELISA-IgM test in our current study was matched with (Zghair *et al*., 2015) which recorded that the seroprevalence IgM *Toxoplasma* antibodies was 1.7%. In contrast, the seroprevalence IgM *Toxoplasma* in our study was lower than the seroprevalence IgM that found by (Al-Ghezy, 2012) who indicated that the seroprevalence IgM *Toxoplasma* antibodies was 3.4%.

In our present study, the results showed elevating in the level of IL-12 in heart disease patients whom infected with toxoplasmosis comparing with heart disease patients whom non-infected with toxoplasmosis, and controls, this results was matched with results of other studies by (Gomes *et al*,. 2014) who scored increased in the level of IL-12 in persons infected with Toxoplasma in skeletal muscle cells (SMC). Production of IL_12 and IFN_γ is important to control infection by *T. gondii*, IFN_γ synergizes with IL-12 driven to the differentiation of Thp to Th1 phenotype, express IL_12 receptor on T cells, and constrain the antagonist IL4 to avoid the differentiation of the Thp towards Th2 phenotype (Cordeiro *et al*., 2008).

In this study the level of IL-23 it was studied to exposure a possible relationship between IL-23 and heart disease patients whom infected with toxoplasmosis ,as the results showed elevating in the level of IL-23 in both gender with significant difference at $P<0.05$ in heart disease patients whom infected with toxoplasmosis comparing with heart disease patients whom non-infected with toxoplasmosis and controls ,but there no significant difference between heart disease and control. The results of our recent study agreed with (McGovern and Powrie, 2007), who indicate to associated chronic inflammatory diseases such as IBD, psoriasis and myocardial infarction with polymorphisms of the IL-23 receptor complex. At present it is unclear what determines the dominance of IL-12 vs IL-23 in different experimental models of infection or autoimmunity, but it is unlikely to be simply a consequence of the preferential induction of one cytokine. Rather, the context and type of stimulus is likely to determine the relative role of these cytokines. It is possible that the observation that the protective effects of IL-23 are secondary to those of IL-12 during toxoplasmosis, also be the case with other intracellular infections.

TGFβ-3 result agrees with the study by (Passos *et al*., 2010), who demonstrated that increased the levels of TGFβ-3 and corporation with IL-6 and IL-23 promotes NK cell production of IL-17 and also development of Th17 lymphocytes during toxoplasmosis leading to the eradication of the parasite. The current study showed that there is significant increase of TGFβ-3 in the serum of heart disease patients with toxoplasmosis infection compared with control group. This increase of TGFβ-3 levels referrer to the role of TGFβ-3 against Toxoplasma by induction of immune responses by development of Th17 lymphocytes and mucosal immunity and suppression of immune responses by way of direct and indirect pathways.

Conclusion

There are high values of anti-Toxoplasma Abs (IgG) in compare with IgM through tested by ELISA technique. Increasing of IL-12 in heart disease patients with toxoplasmosis indicate to an inflammatory status may be included in heart disease pathogenesis. The lower value of anti-inflammatory interleukin IL-23 in patients with toxoplasmosis indicates the secondary role of IL-23 to IL-12 in immune response against toxoplasmosis. TGFβ-3 is a key cytokine for inducing appropriate mucosal immune responses against parasites . However, its anti-inflammatory effects are used by *T. gondii* to suppress immune responses. Therefore, TGFβ-3 can be considered as a high risk factor for toxoplasmosis. There is significant association between toxoplasmosis and heart disease patients, also there is relationship between toxoplasmosis and risk factors.

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