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The Relationship between Cytokines and *Toxoplasma* Infection in Heart Disease Patients

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Abstract

Toxoplasmosis is the general term for infection and disease in man and animal caused by a parasite called *Toxoplasma gondii*, this disease is prevalent among different categories of people, Heart disease are one of them. Because of the dormancy status of *T. gondii*, there is ambiguity and variation in the immunity of patients. This study aimed to investigate the immune status of heart disease patients infected with toxoplasmosis. Two hundred and fifty samples of both heart disease patients and controls had been tested by ELISA technique to detect anti-*Toxoplasma* Abs (IgG and IgM). The positive samples with toxoplasmosis were tested to detect the level of interleukins (IL-10, IL-12 and IL-17).

The result of this study revealed the prevalence of toxoplasmosis among heart disease patient in about (43.33% IgG and 0.66% IgM). The results of interleukins appeared high level of IL-12 (82.85 pg./ml) in heart disease patients infected with toxoplasmosis when compared with control (60.97 pg./ml) with significant difference between them at $p \leq 0.05$, while there was no significant difference in IL-10 between patients and controls. The results also showed decreased level of IL-17 in patients with heart disease. The increased level of IL-12 in patient with heart disease infected with toxoplasmosis indicate to the activity of cell mediated immunity in those patients and may be lead to provoke an inflammatory status in the heart valves and muscle then worse heart disease to be sever and need complicated treatments.

Key words: Toxoplasmosis, Interleukin, Heart disease.

دراسة الأرتباط بين بعض السيتوكينات وداء المقوسات في مرضى القلب

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داء المقوسات هو مصطلح عام للأصابة والمرض في الإنسان والحيوان يسببه طفيلي يعرف بالتوكسوبلازما، هذا المرض منتشر بين فئات مختلفة من البشر، ومن ضمنهم مرضى القلب . بسبب الحالة الساكنة (الكامنة) للطفيلي هنالك اختلاف وغموض في الحالة المناعية للمرضى المصابين بالطفيلي . هدفت هذه الدراسة إلى التحقيق في الحالة المناعية لمرضى القلب المصابين بداء المقوسات , وقد تم اختبار مائتين وخمسون عينه من كل من مرضى القلب والسيطرة(الأصحاء) بأستعمال تقنية اللأليزا (تقنية الانزيم المرتبط) للكشف عن الأجسام المضادة لطفيلي التوكسوبلازما , كذلك تم اختبار العينات الموجبة للتوكسوبلازما لتحديد مستويات الأنترلوكينات (IL-10, IL-12, IL-17, IL-1) . أضهرت نتائج هذه الدراسة انتشارطفيلي التوكسوبلازما بين مرضى القلب بنسبة 43.33% للجسم المضاد (IgG) و0.66 % بالنسبة للجسم المضاد(IgM) . أما نتائج اختبار الأنترلوكينات أضهرت أرتفاع مستوى الأنترلوكين (IL-12) 82.85 pg/mL) في مرضى القلب المصابين بطفيلي التوكسوبلازما عند مقارنتها مع عينات السيطرة الأنترلوكين (IL-12 ml) 60.97 pg / IL) حيث اضهرت فرق معنوي بينهم عند$p\leq0.05$, بينما لا توجد اختلافات واضحة في مستوى الأنترلوكين IL-10 بين مرضى القلب وعينات السيطرة , كذلك أضهرت النتائج أنخفاض في مستوى الأنترلوكين 17 في مرضى القلب. زيادة مستوى الأنترلوكين 12 في مرضى القلب المصابين بداء المقوسات يشير الى نشاط المناعة الخلوية في مرضى القلب ، ويمكن أن يؤدي إلى إثارة حالة التهاب في صمامات القلب والعضلات ثم أمرض القلب الأكثر شدة والحاجة للمعالجة المعقدة.

1. Introduction :

Toxoplasma gondii is a parasite and is a member of the phylum Apicomplexa, is an obligate intracellular parasite which is capable of infecting virtually human and other warm-blooded animals (Steven *et al*.,2008). The infection of *T. gondii* is distributing in all global population, serological data indicates that about one – third of all population has been infected with *T.gondii* (Rosso *et al*., 2008). Human may be infected for long period of life but without any symptoms unless immunosuppression happens (Herrmann *et al*.,2010). Birds and humans considers as intermediated hosts, while cats (Felidae) are definitive host, goats and sheep are very important intermediated host sources for infection with toxoplasmosis (Sevgili *et al* .,2005). The infections of *T.gondii* is variable and related with many of factors including age , nutritional habits, sociocultural, contact with cats and environmental conditions (Barbosa *et al* .,2005). *T. gondii* infection stimulates both cell mediated immunity (CMI) in addition to humoral immune response as antibody production, which includes IgM and IgG antibody. (Phuangphet ,2008). Cytokines have been described (McDermott ,2001), as a huge array of relatively low molecular weight, pharmacologically active proteins that are secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect) (Patrizi, 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). A number of different host cells and compartment are involved in innate response to *T,gondii* and interplay between these cell, particularly with regard to production of Interleukin 12 (IL-12), Interferon gamma (IFN-y), Tumour Necrosis Factor (TNF), Nitrogen monoxide (NO), an Reactive Oxygen Intermediates (ROI) and other factors , IFN- γ and α (which activate macrophages function) are important for controlling tachyzoite replication during both acute and chronic phases of infection, IL-10 and IL-12 appear to be crucial at the initial phase of infection and less important during chronic toxoplasmosis. IL-12 is clearly important in initiating a strong and effective cell -mediated immunity against *T. gondii* tachyzoite, IL-10 appears to modulate both IL-12 and IFN -γ synthesis in *vivo*, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage. IL-10 and IL- 12 are two major antagonist involved in regulating IFN- γ synthesis during the initial phases of infection (Butcher *et al*.,2005). Myocarditis has rarely been reported as a manifestation of acute toxoplasmosis. This

manifestation may occur in isolation or as part of a broader clinical spectrum of illness. Pericardial effusion, constrictive pericarditis, arrhythmias, and congestive heart failure in patients with *T. gondii* myocarditis have been described (Hidron ,2010). During the inflammatory reaction, anti-inflammatory cytokines are also formed and tend to modulate the inflammatory process. Whereas a large body of evidence exists to support a role for proinflammatory cytokines in atherosclerosis (ROSS ,1999).

2. Materials and Methods:

2.1. Sample Collection

One hundred and fifty samples of blood had been collected at period from August 2016 till November 2016 from patients with heart disease, they lying or visit Heart Medical Centre in Thi - Qar province. According to gender, the patients divided in to 81 males and 69 females with age range from18 to 82 years old. One hundred samples of blood had been collected from apparently healthy persons as negative control, dividing according to gender into 60 males and 40 females their age from 19 to 56 years old.

6ml of venous blood were collected in sterile conditions, and then the blood was placed in gel tubes and allowed to clot at room temperature, centrifuged at 3000 round per minute (rpm) for 10 minutes and sera were dispensed into 4 Eppendorf tubes, and stored at -20 ° C.

2.2. Detection of *T. gondii*:

This assay was performed according to manufacturer´s procedure using the commercial kits (Foresight, USA) for the detection of anti-*Toxoplasma* IgG and IgM antibodies. The results were read by ELISA reader.

2.3. Detection of IL-10 , IL-12 and IL-17 :

Enzyme-Linked Immunosorbent Assay this assay was performed using the commercial kits (Elabscience, Chines) for the detection of IL-10, IL-12 and IL-17. The was done according to the manufacturer´s instructions. The optical density (OD) is measured by ELISA reader at a wavelength of 450 nm.

2.4. Statistical Analyses:

The significance of differences in proportions was analysed by Fisher's exact test and Chi square test . Data were entered into SPSS version 20 and $P \leq 0.05$ considered statistically significant.

3. Results:

The results of the present study showed presence of anti-*Toxoplasma* IgG Abs in 65 of 150 (43.33%), and the result of ELISA- IgM test was 1 of 150 (0.66 %). The results also showed presence of anti - *Toxoplasma* Abs in apparently healthy subjects (negative controls), 17 of 100 (17%) were IgG positive while 2 of 100 (2%) were IgM positive, table (1) explain the details. However, the results indicate to the presence of significant differences ($p< 0.05$) between heart disease patients and controls in the seropositivity of anti-*Toxoplasma* Ab.

The results of measurement level of interleukins in the present study showed elevation in the level of IL-12 (82.85 pg./ml) in heart disease patients comparing to controls group(60.97 pg./ml) and there was significant difference $P < 0.05$, the results of IL-10 showed no significant difference between heart disease patients and controls, while IL-17 level was decreased (36.72 pg./ml) in patients of heart disease infected with toxoplasmosis as in table(2). For specify the role of interleukin in heart disease patients infected with toxoplasmosis, the interleukins measured in controls whose have anti-*Toxoplasma* Abs, table (3) and the results indicate to the presence of significant difference at ($p<0.05$) in the level of IL-12 between controls whose anti-Toxoplasma Abs and controls whose not. While there was no significant difference in IL-10 and IL-17 levels.

The results of correlation coefficient indicate to little correlation between anti-*Toxoplasma* IgG and IL-10 and same one between anti-*Toxoplasma* IgM and IL-12 and there was no correlation between anti-toxoplasma Abs and level of IL-17.

Table 1: Seroprevalence of anti-*Toxoplasma* Abs in heart disease patients and apparently healthy controls using ELISA

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Number of samples	Age	ELISA test			
		IgG Positive (%)	IgM Positive (%)	IgG Negative (%)	IgM Negative (%)
Heart disease patients (150)	18-82	65 (43.33%)	1 (0.66 %)	85 (56.66%)	149 (99.33%)
Control(apparently healthy) (100)	19-56	17 (17%)	2 (2 %)	83 (83%)	98 (98%)
Total		82 (60.33 %)	3 (2.66 %)	168(100)%	247(100%)
		X^2=18.78, Df=1, P≤0.05	X^2=0.900, Df=1, P≤0.05		

Table 2: The interleukins level in heart disease patients with seropositive toxoplasmosis and in apparently healthy controls

Groups		Mean	Std. Deviation
IL10	patients	7.18	3.71
	control	8.14	2.30
	t= -1.299, significant P value =0.199		
There was no significant difference between groups at α 0.05			
IL17	patients	16.68	11.62
	control	36.72*	41.48
	t= -2.127, significant P ≤0.0.5		
*There was a significant difference between groups at α 0.05			
IL12	patients	82.85*	58.95
	control	60.97	17.23
	t= 2.382, significant P ≤0.05		
*There was a significant difference between groups at α 0.05			

Table 3: The interleukin levels among apparently healthy subjects whom have anti-Toxoplasma Abs and who was not have anti-*Toxoplasma* Ab.

Groups		Mean	Std. Deviation
IL10	Control have Ab.	9.14	2.74
	Control Not have Ab.	8.14	2.30
	t= 1.207, significant P value =0.236		
There was no significant difference between groups at α 0.05			
IL17	Control have Ab.	64.03	46.84
	Control Not have Ab.	36.72	41.48
	t= 1.893, significant P value =0.067		
There was no significant difference between groups at α 0.05			
IL12	Control have Ab.	220.24**	165.96
	Control Not have Ab.	60.97	17.23
	t= 4.052, significant P value =0.001		
*There was a significant difference between groups at α 0.05,0.01			

Table 4: The Correlation coefficient between interleukins level and anti-Toxoplasma Abs IgG and IgM in patients with heart disease

Parameter	IL10	IL17	IL12	IgG	IgM
IL10	1				
IL17	0.309	1			
IL12	-0.024	0.274	1		
IgG	0.215	- 0.040	- 0.032	1	
IgM	0.009	- 0.003	0.032	0.196	1
*. Correlation is significant at the α 0.05 level					

4. Discussion:

In the present study, the results showed elevating in the level of IL-12 in heart disease patients whom infected with toxoplasmosis comparing with controls , this was matched with results of other studies (Abdul-lateef and Sabah,2012; Alkhanak *et al* .,2015), and different from others (Al-Khafajhi *et al*., 2011). Production of IL-12 and IFN-γ is essential to control infection by *T. gondii*, IFN-γ synergizes with IL-12 to drive the differentiation of Thp to Th1 phenotype, express IL-12 receptor on T cells, and inhibit the antagonist IL-4 to prevent the differentiation of Thp towards Th2 phenotype (Cordeiro *et al* .,2008). The role of IL-12 in toxoplasmosis including induces production of IFN-γ from natural killer (NK) cells and T cells, IFN-γ signaling promotes the development of a number of activator of transcription 1 (STAT1)-dependent anti-parasitic effector mechanisms, including reactive oxygen intermediates (ROI) production and p47 GTPase upregulation (Tait and Hunter, 2009). Results of current study showed a decrease in the mean serum level of IL-17 in heart disease patients with toxoplasmosis seropositive when compared with its serum levels in control. The role of IL-17 is controversial, some studies indicate the role of IL-17 in toxoplasmosis involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection (Yisong and Richard,2009). However, in the current study the

role of IL-17 may be dysregulated and provoke toxoplasmosis in heart disease patients towards chronicity. On the other hand, the results of IL-10 showed no significant difference between heart disease patients and controls and this may be because the increasing level of IL-12 that have antagonist effect with IL-10. The results of correlation coefficient indicate to little correlation between anti-*Toxoplasma* IgG and IL-10, this low level of IL-10 during chronic or past infection with toxoplasmosis in the current study match the results of other studies (Al-Khafajhi *et al* .,2011), IL-10 plays a vital role in controlling the inflammatory response during chronic phase of *T. gondii* infection(Wilson *et al* .,2005). IL-10 serves a dual important role during acute toxoplasmosis, by it inhibits IFN-γ production and the proliferation of T-lymphocytes, thus preventing a potentially protective Th1 immune response. Such T-cell-dependent immune suppression exerted by IL-10 primarily appears to avoid overwhelming inflammation which eventually leads to death. IL-10 may also deactivate macrophages, thus reducing IFN-γ-induced toxoplasmacidal activity and facilitating intracellular parasite survival. Hence, IL-10-induced immune suppression following infection with *T. gondii* is beneficial for both the parasite and the host and favors a stable host - parasite relationship (Ellis-Neyer *et al*.,1997). Concerning relation of heart disease and toxoplasmosis, the present finding was compatible with finding of Hidron *et al*. (2010) ,who found that *T.gondii* is the reason for myocardiasis and pericardiasis, *T.gondii* is the second relevant pathogen in protozoan myocarditis (Feldman and McNamara, 2000 ; Kirchoff *et al* .,2004), whearas this finding is incompatible with finding of Tallab, Al-Autabbi, *et al*. (2012) , who found there is no any relationship between *T.gondii* and athersclerosis.

In case of relation of IL-17 and IL-12, the present study revealed that there was an association between increase serum level of Il-17 and IL-12 whereas there was an inverse relationship between serum level of IL-17, IL-12 and IL-10, that mean there was a concomitant increase of both IL-17 and IL-12 in the sera of samples with toxoplasmosis, while the level of IL-10 was low in the same samples, this results are compatible with finding of Kareem *et al*. (2012).

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