

Immunological Study for *Toxoplasms gondii* Parasite in College of Science for Women

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Abstract

In this study, the collection of blood samples from 100 students in Babylon university college of Science for women for the period from 11/2012 to 10/2013 and separated serum for the purpose of diagnosis of infection with *Toxoplasma gondii* and immunological testing. The current study showed that the infection rate reached 46% of the total samples tested were highest infection rate in the age group 19-23 years and have had the lowest rate in the age groups 24-28 and 29-33 years, which amounted to 4.34%, while the proportion was the positive result to C-reactive protein 17% of the total samples were infected with the parasite and the highest concentration of him when quantification 384 mg / dL in the age group 24-28 years with significant differences in all age groups. The immunological study included positive samples for the latex test to measure the concentrations of each of the serum proteins and immunoglobulin IgM, IgA and IgG, the concentrations of highest 2933.333 ± 490.747 , 1421.466 ± 1801.164 568.666 ± 490.679 , mg / dL, respectively, for different age groups and no significant difference compared with control, either for the concentrations of serum proteins has been observed the highest concentration of 3054 ± 0.748 mg / dL in the age group 24-28 years, and by the results obtained in the present study, we infer the existence of an immune systematic and local response against infection with toxoplasmosis.

Keyword: Immunological state with *Toxoplasms gondii* , *Toxoplasms gondii* in women

الخلاصة

تم في هذه الدراسة جمع 100 عينة دم من طالبات جامعة بابل/ كلية العلوم للبنات للفترة من 11/2012 ولغاية 10/ 2013 وفصلت الامصال لغرض تشخيص الاصابة بطفيلي *Toxoplasma gondii* واجراء الاختبارات المناعية. بينت الدراسة الحالية ان نسبة الاصابة بلغت 46% من مجموع العينات المفحوصة وكانت اعلى نسبة اصابة في الفئة العمرية 19-23 عام واقل نسبة لها كانت في الفئات العمرية 24-28 و 29-33 عام والتي بلغت 4.34%، في الوقت الذي بلغت فيه نسبة النتيجة الموجبة لبروتين طور الحاد 17% من مجموع العينات المصابة بالطفيلي وبلغ اعلى تركيز له 384 ملغم /ديسيلتر في الفئة العمرية 24-28 عام مع وجود فروق معنوية ولجميع الفئات العمرية.

اشتملت الدراسة المناعية للعينات الموجبة لاختبار اللاتكس على قياس تراكيز كل من بروتينات مصل الدم والكلوبولينات المناعية IgM , IgA , IgG اذ بلغت التراكيز 2933.333 ± 490.747 , 1421.466 ± 1801.164 , 568.666 ± 490.679 ملغم / ديسيلتر على التوالي باختلاف الفئات العمرية وبدون فرق معنوي مقارنة بالسيطرة ، اما بالنسبة لتراكيز بروتينات المصل فقد لوحظ اعلى تركيز 3054 ± 0.748 ملغم /ديسيلتر في الفئة العمرية 24-28 عام ، ومن خلال النتائج المستحصلة في الدراسة الحالية نستدل على وجود استجابة مناعية جهازية وموضعية ضد الاصابة بداء المقوسات. الكلمات المفتاحية: الاختبارات المناعية، طفيلي داء المقوسات، داء المقوسات عند النساء.

Introduction

Toxoplasma gondii is a coccidian parasite of the cat and its infection may lead to major public health problems (El-Awady *et al*, 2000). Transmission to humans occurs through ingestion of *T. gondii* oocysts shed into the environment by cats, or by eating meat of infected animals. Under normal immune conditions, *T. gondii* infection is frequently asymptomatic, but in individuals who are immunocompromised, such as in patients with AIDS, the parasites can become widely disseminated, causing severe toxoplasmosis and encephalitis. Primary infections acquired during pregnancy may also result in severe damages to the fetus, manifested as mental retardation, seizures, blindness, and death (Ajioka and Soldati, 2007). *T. gondii* causes *toxoplasmosis* ,The definitive host is the domestic cat and other felines. Humans and other mammals are

intermediate hosts (Dawit et al, 2004). About one-third of the world's population is estimated to carry toxoplasma infection (Joseph and Schawartzman, 2001). There are large variations in prevalence and within different countries in animals and humans (Nester et al, 2004).

The disease exhibits various clinical manifestations and therefore, poses difficulty in diagnosis (Cook *et al*, 2000). The natural site of *T. gondii* infection is the mucosal surface of the intestine where in immunoglobulin A (IgA) specific for *T. gondii* is synthesized. Protective immunity acquired after natural infection with *T. gondii* points to the importance to develop a vaccine that can stimulate mucosal defenses (Bourguin *et al*, 1993). There is considerable interest in developing a vaccine against *Toxoplasma gondii* infection due to the impact of toxoplasmosis on humans and animals. An effective vaccine should protect against both acute and chronic infection (Haumont *et al*, 2000).

The diagnosis of toxoplasmosis is most commonly made by detecting the immunoglobulin (IgG, IgM and IgA) antibodies in the serum samples of patients using methods Latex agglutination test (LAT), Enzyme linked immunosorbent assay (ELISA), Indirect immunofluorescent test (IFT)... etc (7). The tendency of IgM to persist for a long time even at high levels has been verified in several studies (Del Bono et al, 1989). The measurement of IgG has proved to be a highly useful procedure (Hedman *et al*, 1989), IgG is only indicates previous exposure to *Toxoplasma* (recent or past) and the absence of IgG indicates the absence of infection (Juan *et al*, 2009). C-reactive protein (CRP) is a protein found in blood is predominantly made in the liver and is secreted in increased amount within six hours of an acute inflammatory stimulus. It had physiological role by binding to phosphocholine expressed on the surface of dead or dying cells (Thompson *et al*, 1999).

Aim

The aim of this study was to evaluate the seroprevalence of toxoplasmosis in healthy women using latex and single radial immunodiffusion (SRID) tests to determine the levels of IgG, IgA and IgM. The C-reactive protein also measured the detection of inflammatory response.

Materials and Methods

1- Patients

This study was carried out on sera of 100 healthy female students age ranged between 14-33 years at science for women college from 11 - 2012 to 10 - 2013. 5 ml of blood was collected from each of the students and serum separated, and kept frozen at -20 C. for further study.

2-Immunological Assays:

A-Latex agglutination test (LAT) for toxoplasmosis was performed using the Toxotest kit following the manufactures instructions.

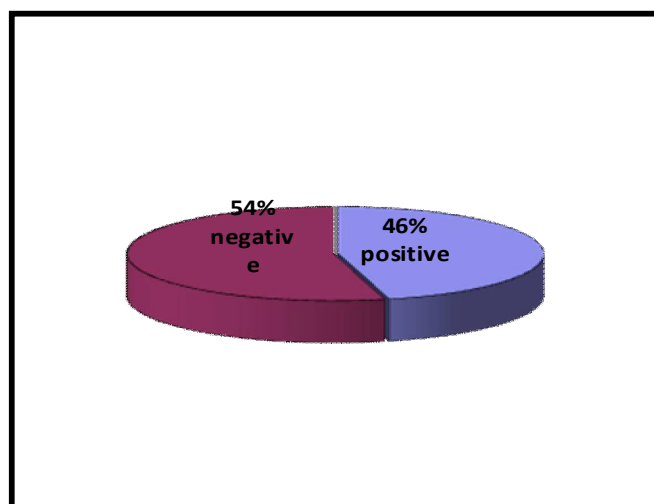
B-Immunological study including detection each of serum proteins by Biuret methods (Bishops *et al*, 1985), and CRP was estimated qualitatively and quantitatively according to the manufactures instruction of CRP kit. As well as measuring the concentration of serum Immunoglobulins (IgG, IgM and IgA) quantitatively by single radial immunodiffusion (SRID) or (Mancini method).

Statistical Analysis:

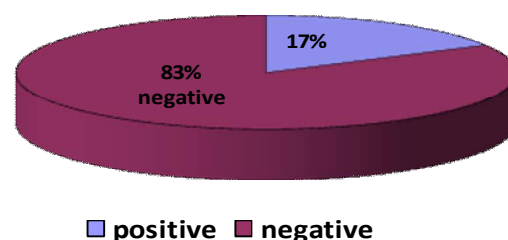
Use the U.S. Census (spss 11) to perform statistical analysis, as analyzed the results using the design random full-scale analysis of variance and adopted the test less significant differences Least significant difference test (LSD) and table analysis of variance (ANOVA Table) below the level of significance 0.05 (Niazi, 2004).

Results

Between **November 2012 and October 2013** toxoplasmosis test was carried out on 100 human females serum: 46% were positive, 54% negative as revealed in (figure 1) also, the positive results for CRP more 17% as illustrated in (figure2). This study showed that toxoplasmosis appears in age ranged between 14-33 years, however, the incidence of inflammation increased in the rate 19-23 year (table 1).



Figure(1): percentage of toxoplasmosis in sample study



figure(2): percentage of positive result for CRP test

Table 1. Seroprevalence of toxoplasmosis by Latex test

Age group	No.	Toxoplasma / positive	percentage
14-18	16	4	8.695 %
19-23	74	38	82.606%
24-28	5	2	4.347%
29-33	5	2	4.347%
Total	100	46	

low percentage for CRP test which appears in the rate 24-28 year (table 2), while the positive number in age group 29-33 year with different significance ($P < 0.05$) in other groups to the CRP titer (table 3).

Table 2. Seropositive C-reactive in sample study

Age group	No.	CRP / positive	percentage
14-18	16	6	35.294 %
19-23	74	10	58.823 %
24-28	5	1	5.882 %
29-33	5	0	0 %
total	100	17	

Table 3 : The concentration of C-Reactive protein in patient with toxoplasmosis

Age group	No.of case with CRP		Protein titer mg/ dL M $\pm$ S.D	
	positive	negative	Control	patients
14-18	6	11	42.666 $\pm$ 32.924	192 $\pm$ 0
19-23	10	60	51.333 $\pm$ 10.263	241.333 $\pm$ 80.829
24-28	1	8	28 $\pm$ 18.33	384 $\pm$ 0
29-33	0	4	28 $\pm$ 18.33	0
total	17	83		

L.S.D under ($p > 0.05$) = 53.70

The present study showed that age groups 19-23 and 14-18 year have a high percentage for two tests were the comparison between them 82.606% , 8.695% and 58.823% , 35.294% respectively (Table 4).

Table 4 . Comparison of C-reactive protein and Latex tes

Age group	No.	Toxoplasma +	%	CRP +	%
14-18	16	4	8.695 %	6	35.294 %
19-23	74	38	82.606%	10	58.823 %
24-28	5	2	4.347%	1	5.882 %
29-33	5	2	4.347%	0	0 %
total	100	46		17	

There is a significance difference between all the age groups below the level of significance ($P < 0.05$) for the concentration of serum proteins for toxoplasmosis as showed in (table5).

Table 5: The concentration of serum proteins for patients with toxoplasmosis

Age group	Group	concentration of serum proteins mg/dl M ± S.D
14-18	control	5.497 ±1.331
	patient	2.09 ± 0.551
19-23	control	4.121 ± 1.155
	patient	2.871 ±1.128
24-28	control	4.992 ± 1.599
	patient	3.054 ± 0.748
29-33	control	4.328 ± 1.401
	patient	1.948 ± 0.476

L.S.D under ($p > 0.05$) = 1.934

There is no significance difference between the age groups below the level of significance ($P < 0.05$) for the concentration of immunoglobulins IgG, IgM and IgA of toxoplasmosis sample when measured, has reached the highest concentration of IgG, IgA and IgM at the age group of 29-33, 19-23 and 24-28 years respectively (Table 6).

Table 6 : Effects of toxoplasmosis on serum immunoglobulin

Age group	Group	Concentration of IgG(mg/dl) M ± S.D	Concentration of IgM (mg/dl) M ± S.D	Concentration of IgA (mg/dl) M ± S.D
14-18	control	1095.3 ±534.51	274.4±53.598	128.6±19.909
	patient	3308±586.081	228.666± 98.895	400.766±258.430
19-23	control	939.1±403.029	240.95±100.904	47.066±23.000
	patient	2439.733±1836.435	315.333±134.522	1421.466±1801.164
24-28	control	1335.033±284.168	42.6±28.425	129.933±29.661
	patient	2247.733±1762.178	568.666±490.679	403.2±72.746
29-33	control	1388.5±391.645	140.65±40.941	494.8±95.05
	patient	2933.333±490.747	270.733±162.755	296.466±161.305

*no statistically significant difference under ($p > 0.05$)

Discussion:

The vast 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* 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 (Torda, 2001), and that the rise in this ratio between students of the college of Science for women, especially the age group 19-23 years can be attributed to eating food from a variety of sources which may be unhealthy.

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 climatic condition such as humidity (Kamani *et al*, 2009).

Although the immune response in *Toxoplasma*-infected has been well characterised, the mechanisms involved in reactivation in humans are still unclear. Knowledge of the anti-*T. gondii* immune response is important, especially in

immunocompromised individuals. We addressed this issue by examining the specific human parasite responses, 17 samples had positive CRP, this gives an indication of possible interaction of *T.gondii* with immune system and CRP is an inflammatory marker and plays a key role in the host's defense against infection (Black *et al*, 2004).

In this immunological study when measured the immunoglobulins found highest concentration in all of types of Ig compared with control. This indicates present immune response for primary and latent infection.

Acute infection can be diagnosed a single serum specimen, and further, primary infection can be distinguished from secondary (recurrent or reactivation) infection the quantity of Toxoplasma –IgG has been shown to be an unreliable indicator of recent infection (Remington and Desmonts, 1995). And the long-term persistence Toxoplasma-specific IgM- antibodies poses problems in timing of infection, especially in pregnant women.

Diagnosis of acute *T.gondii* infection should be based upon the demonstration of a rise in antibody titers in serial serum samples, further, Toxoplasma – specific IgM and IgA antibodies tend to persist long. IgG avidity has shown its usefulness in diagnosis of acute Toxoplasma infections in several studies. (De Ory *et al*, 1995)

The presence of elevated levels of *T.gondii* specific IgG antibodies indicates that infection has occurred but does not distinguish between recent infection and infection acquired in the distant past (Wilson *et al*, 1999).

Natural *T. gondii* infection leads to strong, lifelong non-sterile protective immunity. The persistence of memory T lymphocytes, stimulated by regular rupture of *T. gondii* tissue cysts or by recurrent contact with infected food, prevents reinfection in most individuals (Hunter and Reichmann, 2001).

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