The Effect of Toxoplasmosis Infection on Interleukin-12Level During Human Maturity in Baghdad Province

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Abstract

The goal of this study is to perceive the immunological sex-differences in puberty age who have positive anti-*Toxoplasma* Immunoglobulin-G (IgG) specific antibodies. From the first of November 2012 till the end of April 2013, 303 blood samples were collected from students in both genders (150 males and 153 females) were divided into two age groups: group (A) which included subjects with age range (12-15) years old and group (B) which included subjects with age range (16-19) years old. Serum samples were tested for toxoplasmosis infections by using Enzyme Linked Immunosorbent Assay (ELISA) for anti-*Toxoplasma* IgG antibodies test. The results revealed that 107/303 (35.31%) of the studied subjects showed seropositive toxoplasmosis, 60 males and 47 females of them with a high significant differences between toxoplasmosis infected and uninfected subjects. All serum samples were tested by using ELISA technique for detection of serum mean concentration of IL-12. This study showed high significant level of IL-12 in both males and females with latent toxoplasmosis in comparison with free-toxoplasmosis groups.

Keywords: Toxoplasmosis, IL-12, *Toxoplasma gondii*, *IgG*.

تأثير الاصابة بداء المقوسات الكوندية على المدورات الخلوية نوع (IL-12) خلال فترة بلوغ الانسان في محافظة بغداد

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الخلاصة:

إن الهدف الرئيسي لهذه الدراسه هو فهم الفروقات الجنسيه والمناعيه في سن البلوغ في الاشخاص الذين لديهم اجسام مضاده نوع (ج) لطفيلي المقوسات الكونديه. تم جمع 303 عينة دم من الطلاب في كلا الجنسين (150 ذكر و153 انثى) من الأول من تشرين الثاني 2012 وحتى نهاية شهر نيسان عام 2013. قسمت العينات الى مجموعتين عمريه، المجموعه (أ) تتضمن افراد تتراوح اعمارهم من 12–15 سنه ، والمجموعه (ب) تتضمن افراد تتراوح اعمارهم بين 16-19 سنه. فحصت العينات المصليه للكشف عن الاصابه بداء المقوسات الكونديه بأستخدام تقنية الامتزاز المناعي المرتبط بالانزيم (الاليزا ELISA) للضد النوعي IgG. أظهرت النتائج

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بأن 303/107 (35.31%) من العينات المدروسه سجلت إصابه موجبه لطفيلي المقوسات الكونديه 60 ذكر و 47 أنثى مع وجود فروقات معنويه عاليه بين المصابين وغير المصابين. كما تم قياس معدل تركيز الانترلوكين-12 في مصول العينات المدروسه باستخدام تقنية الاليزا ايضا وقد أظهرت النتائج مستوى مرتفع معنويا من الانترلوكي-12 في كل من الذكور والاناث المصابين بداء المقوسات الكونديه المزمن بالمقارنه مع المجاميع غير المصابه.

Introduction:

Toxoplasmosis is a cosmopolitan disease in humans and most mammals. It is caused by the opportunistic protozoan parasite *Toxoplasma gondii* mainly through peroral, bloodstream and congenital acquired infections. It has been estimated that one third of the world population has been infected [1]. *T. gondii* needs both definitive and intermediate hosts to complete its sexual and asexual replication phases in life cycle. The sexual phase only occurs in the intestine of the definitive host, felids. All the warm-blooded animals, the intermediate hosts, become infected mainly by consuming food or drink contaminated by oocysts evacuated from felids and tissue cysts from other intermediate hosts [2]. Acute infection happens in the initial few days it depends on the dose and immunity of the host, with the rapidly growing replication of the tachyzoites. Tachyzoites switch to bradyzoites (an intracellular crescent resistant form persisting in the muscle and brain tissues) as time goes by and form tissue cysts parasitizing in host cells. It would be lethal in *Toxoplasma* infected immune-compromised patients if bradyzoites revert to tachyzoites. In addition to felids, intermediate hosts carried with tachyzoites or tissue cysts are also responsible for the spread of *T. gondii* [3]. Our study showed that the sera of all people exposed to *T. gondii* contain high level of IgG antibodies directed against antigens in the chronic phase of infection. Cell mediated immunity (CMI) is considered one of the most distinctive immunological features of *T. gondii* infection. CMI is elicited by the parasite, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes [4].The parasite causes a very strong response of T helper 1(Th1). This immune response limits the tissue extension of the parasite, and leads the parasite to convert to bradyzoite [5]. Interleukin 12 (IL-12), which is secreted by the macrophages and the dendritic cells during antigen stimulation, appears to play a major anti-*Toxoplasma* role during the acute phase of the infection. It activates the production of IFN-γ by NK cells and CD4+, CD8+ TL [6] IL-12 is also essential during the chronic phase of the infection, when it is responsible for maintaining a long-term immune response [7].

Materials and methods:

From the first of November 2012 till the end of April 2013, 303 blood samples were collected from students in both genders (150 males and 153 females) from Al-Erfan, Ignadeen and Algawahery schools and Baghdad University. The student's ages ranging between 12 and 19 years.

Sera were separated after centrifugation at 3000 rpm for 10 minutes, and stored at -20^{o} C until required. Information was recorded from patients by using questionnaire paper.

Serological technique: This assay was performed using the commercial kits (Human ELISA Toxo) for the detection of IgG anti-*Toxoplasma gondii* antibodies (Biocheck, Europe). Also we use an immunoenzymometric assay for the quantitative measurement of human IL-12 in serum using (Biosurce, Belgium) kit, which were performed following the manufacturer´s instructions.

Statistical analyses: Chi-square test was used and $P < 0.05$ were considered as significant and $P < 0.01$ as highly significant.

Results and discussion:

In the present study ELISA-IgG test was used to determine toxoplasmosis chronic infections. ELISA- IgG test revealed that 107/ 303 (35.31%) of the studied subjects showed seropositive toxoplasmosis for both males and females with significant difference ($p< 0.05$) between them, Also these results revealed that males in puberty age were more susceptible than females to get toxoplasmosis infection or may be some

of these infections occurred in earlier ages. Also these results revealed that males in puberty age were more susceptible than females to get toxoplasmosis infection or may be some of these infections occurred in earlier ages. The highest percentage of infection occurred in group B of males with age ranged 16-19 year, which was 34(41.46%) with no significant difference with group A, female groups A and B also didn't exhibit significant difference between them in toxoplasmosis seropositivity These results were higher than those obtained by [8], Mohammed and Al-Nasiry [9] and Al-Rawi [10], who showed that the rate of prevalence was 18%, 20.4%, and 25.83% respectively. Other previous studies done by Kareem [11], Khalil [12] and Al-Dalawi [13], recorded more elevated rates of toxoplasmosis prevalence, which were 30.4%, 27.1% and 29.2% respectively.

Table 1- Frequency of toxoplasmosis in sera of apparently healthy males and females in Baghdad city using ELISA test.

Subject (No)	**Age group year**	**ELISA test**		**Total (%)**
		Positive (%)	**Negative (%)**	
Male (150)	12-15 (A)	26(38.23%)	42(61.76%)	68(22.44%)
	16-19 (B)	34(41.46%)	48(58.53%)	82(27.06%)
Female (153)	12-15 (A)	23(30.66%)	52(69.33%)	75(24.75%)
	16-19 (B)	24(30.76%)	54(69.23%)	78(25.74%)
Total		107(35.31%)	196(64.68%)	303(100%)

The present study showed different levels of IL-12 in males and females for both groups. Group B revealed elevated levels of IL-12 in both genders with significant ($p \leq 0.05$) differences between males and females. This study showed a high level of IL-12 in both males and females with latent toxoplasmosis in comparison with free- toxoplasmosis groups. The mean concentration of IL-12 recorded (4.75 ± 0.88 pg/ml) and (4.12 ± 0.69 pg/ml) in toxoplasmosis seropositive male groups A and B respectively. There was significant ($p \leq 0.05$) differences between toxoplasmosis infected and un-infected males in both groups as shown in table -2. Also, toxoplasmosis seropositive female groups A and B revealed a mean concentration of (5.60 ± 0.12 pg/ml) and (6.04 ± 0.26 pg/ml) respectively with a significant ($p \leq 0.05$) differences between toxoplasmosis infected and uninfected females, as shown in table -3.

Table 2- The mean concentration of IL-12 in males according to *Toxoplasma gondii* infection by using ELISA test.

Test / Subject Male (year)	Mean conc. of IL-12(pg/ml)		Total Mean (pg/ml)
	Toxo. (IgG+ve)	Toxo. (IgG-ve)	
12-15 (group A)	4.75	2.86	3.80
16-19 (group B)	4.12	2.46	3.29

Table 3- The mean concentration of IL-12 females according to *Toxoplasma gondii* infection by using ELISA test.

Test / Subject Female (year)	Mean conc. Of IL-12 (pg/ml)		Total Mean (pg/ml)
	Toxo. (IgG+ve)	Toxo. (IgG-ve)	
12-15 (Group A)	5.60	3.32	4.64
16-19 (Group B)	6.04	4.27	5.15

However, there was no significant difference between the two sexes in level of IL-12. This result was disagreed with a study done by Walker *et al.*,[14] on the severe combined immunodeficiency (SCID) mice infected with *T. gondii* ,they found that there is a difference between male and female in IL-12 concentration and IL-12 level peak more rapidly in male than female, this compatibility may be due to differences in race. On the other hand there was significant ($p \leq 0.05$) difference between asymptomatic toxoplasmosis and free- toxoplasmosis in the same group males in IL-12 level, the same difference was between females with asymptomatic toxoplasmosis and free- toxoplasmosis females. This indicates the important role of IL-12 in established past infection infections, also reflects its importance in long term maintenance of IFN-γ dependent resistance against intracellular parasite [7]. According to this study we suggested that toxoplasmosis antibody test should be used as a preliminary test of marriage in Iraq courts and the procedure of court should remain unachieved unless the test has been made as AIDS, Presenting education to people specially women by tool mass media about being contact with animals, drink raw milk, handling raw meat, never tasting raw meat and gardening by using gloves and early detection and treatment combined with explanation of hazardous living with domestic animals specially cats.

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