Evaluation of Toxoplasmosis Infection by Using Latex and Eliza Tests and it's Relation with C-reactive Protein

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Abstract

This study was conducted to evaluate the prevalence rate of toxoplasmosis among 79 healthy students (51male,28 female) their age ranged between 18-22 years from AL-Mustansiriya University. Blood samples were collected and the presence of *T.gondi*i IgG antibodies was determined by using two methods, Latex agglutination test LAT and Enzyme link immunosorbent assay ELIZA, C-reactive protein CRP was measured as an inflammatory marker.

The seroprevalence of toxoplasmosis by LAT and ELIZA were 35(44.3%), 29(36.7%) respectively. High significant difference ($P<0.01$) was found between LAT and ELIZA tests. There was no difference ($P>0.05$) in CRP positive rat of students (17 male, 10 female).When compare CRP result with LAT, ELIZA results 17 samples were found positive in both CRP and LAT, while 19 samples were found positive in both ELIZA and CRP.

الخلاصة

أشارت هذه الدراسة الى تقييم أنتشار داء المقوسات عند 79 طالب (51ذكر,28 أنثى) وبعمر يتراوح بين18 -22 سنة من طلاب الجامعة المستنصرية , فقد جمعت عينات الدم واجري الكشف عن الاجسام المضادة من نوع (ج) لطفيلي التوكسوبلازما باستخدام اختباري اللاتكس والاليزا, كما تم قياس البروتين التفاعلي من نوع سي كدليل لحالة الالتهاب في الجسم. بينت النتائج ان نسبة الاصابة بهذا الطفيلي في اختباري اللاتكس والاليزا كانت 35(44,3%) ,29(36,7%) على التوالي, وعند المقارنة بينهما كان هناك فرق معنوي عالي بين نتائج الاختبارين($P<0.01$). وأظهرت نتائج قياس البروتين عدم وجود فرق معنوي($P>0.05$) حيث وجد فقط في (17ذكر و 10اناث) , وعند مقارنة نتائج

اختباري اللاتكس والاليزا مع نتائج قياس البروتين, وجد أن (17) عينة فقط كانت موجبة في كلا فحصي اللاتكس والبروتين بينما (19)عينة وجدت موجبة في كلا فحصي الاليزا والبروتين .

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with a global distribution in humans and other warm-blood animals. 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 (1).

About one-third of the world's population is estimated to carry toxoplasma infection (2), there are large variations in prevalence and within different countries in animals and humans (3). The prevalence of infection is related to several factors including nutritional habits, contact with soil, age, rural or urban settings (4) and frequency of contact with domestic animals and climatic condition such as humidity (5).

The diagnosis of toxoplasmosis is most commonly made by detecting of the immunoglobulin (IgG and IgM) antibodies in the serum samples of patients using methods Latex agglutination test (LAT), Enzyme linked immunosorbent assay (ELISA), Indirect immunofluorescent test (IFT)... etc (6).IgG is only indicates of previous exposure to toxoplasma (recent or past) and the absence of IgG indicates the absence of infection (7).

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 (8).

The aim of this study was to evaluate the seroprevalence of toxoplasmosis in healthy students of Iraqi individuals using Latex and ELIZA tests to determine the level of IgG antibodies. The C-reactive protein also measured for the detection of inflammatory response.

Materials and Methods

This study was carried out on sera of 79 healthy students (51 male, 28 female) age ranged between 18-22 years. 5 ml of blood was collected from each of the students, the tubes were centrifuged at 2,000 rpm for 5 min and the sera were liquated in several vials and kept frozen at -20 C^{o} for further study.

Latex agglutination test (LAT) for toxoplasmosis was performed using the Toxotest-kit (BIOKIT, S.A.BARCELONA-SPAIN) following the manufactures instructions. Enzyme-linked Immunosorbent Assay (ELIZA) kit (BIOChek,Inc,Foster City, CA) according to the manufactures instructions.

C-reactive protein was estimated by latex fixation method via a kit (Spinreact SA, Spain) following the manufactures instructions. All results allowed classifying the sera as positive or negative for all testes.

Statistical Methods

Statistical analysis of the results was performed using SPSS for Windows. All data are expressed as the mean value±SE. The differences of parameters were tested by Student's t-test. P value <0.05 was considered as statistically significant.

Results

Blood samples of 79 students were analyzed for *T.gondii* IgG antibodies using Latex and ELIZA tests. Table (1) shows that, 35(44.3%) of students (41% male, 50% female) were found to be positive for toxoplasmosis test (Latex) which was not statistically significant ($p>0.05$). The distribution of IgG antibodies measured by ELIZA test is shown in Table (2). The prevalence of *T.gondii* IgG was found in 29(36.7%) students (31.4% male, 46.4% female), no statistically significant difference was found ($p>0.05$). By comparing the tests of Latex and ELIZA, there is high significant difference between them ($p<0.01$) as shown in Table (3).

Table (4) shows that the C-reactive protein was found positive in 27 (34.2%) students (33.3% male, 35.7% female), no statistically significant difference was found ($p>0.05$). When Latex and CRP results were compared, the differences between them were statistically significant ($p<0.05$) as illustrated in Table (5). A high significant difference was observed when comparing ELIZA with CRP results ($p<0.01$) as shown in Table (6).

Table 1. Seroprevalence of toxoplasmosis by Latex test

Latex test	Male	%	Female	%	Total	%
Positive	21	41.1 %	14	56%	35	44.3%
Negative	30	58%	14	50%	44	55.6%
Total	51	100%	28	100%	79	100%

P-value > 0.05

Table 2. Seroprevalence of toxoplasmosis by ELIZA test

Latex test	Male	%	Female	%	Total	%
Positive	16	31.4%	13	46.4 %	29	36.7%
Negative	35	68.6%	15	53.6 %	50	63.3%
Total	51	100%	28	100 %	79	100%

P-value > 0.05

Table 3. Comparison of Latex and ELIZA tests

Latex	ELIZA				Total	%
	Positive	%	Negative	%		
Positive	22	75.8%	13	26%	35*	44.3%
Negative	7	24.1%	37	74%	44	55.7%
Total	29*	100%	50	100%	79	100%

*P-value < 0.01

Table 4. Seropositive C-reactive protein obtained from individuals

C-reactive Protein	Male	%	Female	%	Total	%
Positive	17	33.3%	10	35.7%	27	34.2%
Negative	34	66.7%	18	64.3%	52	65.8%
Total	51	100%	28	100%	79	100%

P-value > 0.05

Table 5. Comparison of C-reactive protein and Latex tests

Latex	C-reactive protein				Total	%
	Positive	%	Negative	%		
Positive	17	62.9%	18	34.6%	35*	44.3%
Negative	10	37.0%	34	65.4%	44	55.7%
Total	27*	100%	52	100%	79	100%

*P-value < 0.05

Table 6. Comparison of C-reactive protein and ELIZA tests

ELIZA	C-reactive protein				Total	%
	Positive	%	Negative	%		
Positive	19	65.5%	10	34.5%	29*	100.0%
Negative	8	16.0%	42	84.0%	50	100.0%
Total	27*	34.2%	52	65.8%	79	100.0%

*P-value < 0.01

Discussion

In this study, two different methods (Latex & ELIZA) were used for increasing the accuracy of diagnosis; seropositive rates from each test were 44% and 36% respectively. ELIZA demonstrates great sensitivity, and may be considered a good tool for epidemiological studies of Toxoplasma infection (9), the increase of infection rate in Latex test is due to decrease of it's specificity, also the false positive samples were increased in this test (10).

When comparing the seropositivities of Latex with ELIZA, only 22 samples were detected positive by both methods, the difference in seropositivity according to assay method may have resulted partly from different antigenic determinant (epitopes) being recognized in each method, and partly from different limits of sensitivity of each test (11).

The positive rate in female (LAT 50%, ELIZA 46%) was slightly higher than in male (LAT 41%, ELIZA 31%) but the difference was not significant. This difference is due to many factors. First females traditionally take more care of pet animals including cats at home, and secondly, females handle raw meat frequently than men and spend more time cooking at home (12). Some results demonstrate that females and males have the same probability of contracting *T.gondii* infection (13), while in some studies showed higher antibody prevalence in males than in females (14).

Twenty seven samples had positive CRP (17 male and 10 female), this gives an indication of possible interaction of *T.gondii* with immune system and CRP is an inflammatory marker and play a key role in the host's defense against infection (15) When comparing CRP with LAT & ELIZA results, 17 samples were found positive in both Latex and CRP tests, while 19 samples were found positive in both ELIZA and CRP tests, this indicating that *T.gondii* may related to systemic inflammation , Birgisdottir *et al* (16) found a weak but statistically significant association between *T.gondii* and elevated CRP(17). The seroprevalence of *T.gondii* by LAT and ELIZA were negative with positive CRP, a negative result is may be due to the very recent infection, IgG antibodies appear after one to two weeks of infection (18), and the positive rate of CRP could be resulted from obesity, autoimmune disease drug allergies or other disease (19). In contrast the seroprevalence of *T.gondii* were found positive by LAT and ELIZA with negative CRP, this suggests that IgG positive samples were infected with latent toxoplasmosis, after a cute infection in the past and IgG antibodies remain detectable in blood for life (20) latent toxoplasmosis is mostly diagnosed by detection of specific IgG antibodies (21) .

According to these results, the measurement of IgM antibody was suggested with IgG for more accuracy in diagnosis of toxoplasmosis. Moreover it must be known if the patients are under treatment and it would be desirable that the antibodies status be known before, during and after treatment. The need to provide health education to citizens in order to prevent primary infection is very necessary.

References

1. Ajioka JW, Soldati D. *Toxoplasma*-Molecular and Cellular Biology Norfolk, UK. Horizon Bioscience. 2007, p37-58.
2. Torda A (2001) “Toxoplasmosis. Are cats really the source?” Aust Fam Physicians 30(8):743-747.
3. Nester EW, Anderson DG, Pearsall NN, and Nester MT (2004) Microbiology: a human perspective. 4th edn. McGraw Hill, New York. pp 755-757

4. Remington, J.S., R. McLeod, P. Thulliez and G. Desmonts (2006). Toxoplasmosis. In: Infectious Disease of the, Fetus and Newborn Infant, Remington, J.S., J Klein, C.B. Wilson and M.D. Baker (Eds.) Elsevier Saunders, Philadelphia, PA., pp: 946-1091.
5. Kamani, J., A.U. Mani, G.O. Egwu and H.A. Kumsh2009. Seroprevalence of human infection with, *Toxoplasma gondii* and the associated risk factors in Maiduguri, Borno state, Nigeria. Ann. Trop Med. Parasitol., 103: 317-321.
6. Evaluation of three immunoassays for detection of Toxoplasma-specific immunoglobulin G and M.(1996).Eur J Clin Chem Clin Biochem ,34:503-505.
7. Juan Hua Quan, Hassan Ahmed Hassan, Guang-Ho Cha, Dae-Whan Shin, and Young-Ha Lee (2009**).** Antigenemia and Specific IgM and IgG Antibody Responses in Rabbits Infected with *Toxoplasma gondii.* Korean J Parasitol; 47(4): 409–412.
8. Thompson D, Pepy MB, Wood SP, (1999).The physiological structure of human C-reactive protein and its complex with phosphocoline. Structure 7(2):169-77.
9. Shaapan RM, El-Nawawi FA, Tawfiq MA,(2008). Sensitivity and specificity of various serological tests for detection of T.gondii infection in naturally infected sheep. Vet Parasitol. ; 153:359-362.
10. Oshima T, (1982). Fales passive reaction due to nonspecific IgG in Toxoplasma indirect latex agglutination test. Igaku-no-Ayum: 121:482-7.
11. Evans R, Ashburn D, Chatterton J, Jossy A, Ho-Yen A? (2002).How to detecy current toxoplasma infection .Br. J. Biomed. Sci; 59:4-6.
12. Yue X, Jigang Y, Ning J, Hong S, Huiji X, Johan L. (2010). Seroepidemiology of human *Toxoplasma gondii* infection in china. BMC Infectious Disease ;10:4 .
13. Hatam GR, Shamseddin A, Nikouee F.(2005).Seroprevalence of toxoplasmosis in High School Girls in Fasa District, Iran. Iran J Immunol. ; 2:177-81.
14. Konishi E, Houki Y, Harano K, Rento SM, Yoes PD,(2000). High prevalence of antibody to *toxoplasma gondii* among human in Surabaya .Indonesia. Jpn. J. Infect. Dis.; 53:238-241.
15. Black S, Kushner I, Samols D, (2004).C-reactive protein. J. Biol. Chem.; 278:48487-90.

16. Birgisdottir A, Asbjorsdottir H, Gislason D, Olafsson I, Jogi R. (2006). Seroppprevalence of Toxoplasma gondii in Sweden, Estonia and Iceland. Scand J Infect Dis .38(8):625-31.
17. Alda B, Hulda A, Elisabeth C, Rian J, (2006).Seroprevalence of Toxoplasma gondii in Sweden , Estonia and Icland. Scad. J. Ifect. Dis.; 38:625-31.
18. Tuuminen T, Seppanen H, Pitkanen EM, Kapyaho K, (1999). Improvement of immunologlobulin M captures immunoassay specificity. J. Clin. Microbiol. ; 37:270-3.
19. Gabay C, Kushner I, (1999).Acute-phase proteins and other systemic response to inflammation .N. Engl. J. Med. ;340:448-54.
20. Carmen S, Gabriela B, Renata H, (2006). Serprevalence of *Toxoplasma gondii* antibodies in a healthy population from Slovakia. Eurp. J. Inter. Medic; 17:470-473.
21. Jones JL, Lopez A, Wilson M, (2001). Congenital toxoplasmosis: a review. Obest. Gynecol. Surv; 56:296-305.