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Serological study of toxoplasmosis spread among unmarried female university students using LAT, ELISA and IgG avidity

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Abstract:

Toxoplasma gondii has a worldwide distribution and it is one of the most prevalent infectious agents in Iraq. The study was conducted on 200 serum samples of unmarried female university of students age ranged between 18 to 26 years to detect *Toxoplasma gondii* antibodies. The aim of this study was to detect *T. gondii* antibodies among unmarried female students in Iraqi universities using different serological tests. Seventy six (38%) serum samples out of 200 subjects were positive for toxoplasma antibodies by Latex agglutination test (LAT). Among 76 LAT sera positive ,only 58 (29%) serum samples were positive with toxoplasma IgG ELISA test , however , the results of IgM ELISA assay were positive only for 3 (1.5%) unmarried female sample .None of negative LAT serum samples gave positive results with neither IgG nor IgM ELISA.

Key words: *Toxoplasma gondii*, LAT, ELISA, IgG and IgM.

Introduction:

Toxoplasmosis is becoming a global health hazard as it infects (30-50) % of the world human population [1]. Also it is among the global major zoonotic diseases [2, 3].

The disease is caused by a protozoan parasite called *Toxoplasma gondii* (*T. gondii*) which an obligate intracellular parasite able of infecting all warm-blooded species, including humans, domestic mammals, and birds, but cats are the only definitive host [4, 5]. Transmission of *T. gondii* in human is usually by ingestion of cysts infected and undercooked or raw meat or by accidental ingestion of oocysts that may contaminate soil, water, and food Meat is one of the most important sources of the infection in individuals [6,7].Also can be transmitted through placenta during pregnancy [8]. A serious problem in immunocompromised patients. [9, 10].

Most humans with normal immune systems are asymptomatic, but there are still reports of fever, weakness and debilitation, ophthalmitis, multisystem infections [11, 12, 13]. Recently the diagnosis of toxoplasmosis has been drawing close attention, Several

serological techniques have been applied and have shown good sensitivity, specificity, and are quickly carried out, among different diagnostic test such as Latex Agglutination Test (LAT) and Enzyme-linked Immuno Sorbent Assay (ELISA)[14].

Toxoplasmosis has a worldwide seroprevalence measured by specific anti-Toxoplasma IgG varies between (1-100)% , this variability is related to various factors such as, age, sociocultural, environmental and socioeconomic conditions including nutritional forms in addition of health related practices, contact with domestic cats, climatic and geographical conditions[15,16] .Flegr *et al*., 2013 [17] studied the Prevalence of latent toxoplasmosis in women of childbearing age in 88 countries (Iraq was recorded 49% in pregnant women), and they concluded that the seroprevalence of toxoplasmosis correlated with various disease burden.

In Iraq, there are many studies on the toxoplasmosis prevalence among married women especially the pregnant, ranging from 35 to 60 % [18,19] .But only three studies were undertaken reported prevalence in pre-marital females by AL-Shikhly (2012) [20], AL-Shikhly *et al*., (2013)[21] and Obaid (2014) [22] conducted study among different groups of students (one of them pre-marital female). In Iraq toxoplasmosis is a neglected disease and infection in women of child-bearing age especially at unmarried, in the same time, the health system haven't a routine surveillance program for that group and the only data available are laboratory-confirmed clinical cases that are not sufficient to assess the true disease burden .

Aim of Study:

Detection of the seroprevalence level (IgM and IgG) of *T.gondii* infection among apparently healthy unmarried females students in Baghdad universities using serological methods (LAT and ELISA). Identify the females at risk of acquiring infection.

Materials and Methods:

A total of 200 unmarried female students were included in this study from five Universities in Baghdad (University of Baghdad, University of Al-Mustansiriya, University of Technology, University of Nahrin and University of Iraqi) 40 students from each one at age ranging from (18 to 26) years were sampled during the period at December 2013 till May 2014.

Before blood sampling, each female students asked some information according to a questionnaire sheet (Age ,college, contact with cats, working in the garden, restaurants food intake, clinical signs, a previous examination of toxoplasma test ,health information of toxoplasma disease).

Collection of Blood Sample:

Five ml of venous blood were collected from each female student under sterile condition by using disposable syringe.

The blood sample was placed in a plain tube and left stand for 20 min. at room temperature to clot. Serum was separated from clot by centrifugation at 3000 r. p.m. for 10 min., and the obtained serum was divided into three parts in different Kan tubes to avoid repeated freezing and thawing, and then stored at (-20) ºC until being analyzed for detection of *Toxoplasma* antibodies by different methods.

Two serological tests used for detection of Toxoplasma antibodies were latex agglutination test (LAT) and (ELISA IgM, IgG test).

The latex kit received from (Linear) Spain and Toxo ELISA IgM and IgG kit were received from (Vircell) Spain.

Statistical analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage and T-teat Least was used to significant compare between means in this study.

Results and Discussion:

Detection of *T. gondii* antibodies using Latex Agglutination test (LAT).

The total percentage of LAT sero (+ve) Toxoplasmosis in all five universities in Baghdad was recorded in 76(38%) out of 200 unmarried female students at age of (18-26) years, while the sero (-ve) was recorded in 124(62%). there were highly significant ($p \leq 0.01$) between LAT sero(+ve) and LAT sero(-ve).Table (1).

Table (1): Distribution of Toxoplasma by LAT throughout the universities among unmarried female students.

University	LAT				P-value
	LAT(+ve)		LAT(-ve)	Total	
	No.	%	NO. %	NO.	
T	20	50.00	20 50.00	40	1.00 NS
I	17	42.50	23 57.50	40	0.0271 *
M	14	35.00	26 65.00	40	0.0042**
B	13	32.50	27 67.50	40	0.0037 **
N	12	30.00	28 70.00	40	0.0001 **
Total	76	38.00	124 62.00	200	0.0044 **
P-value	0.0149 **		0.0149 **	----	----

*** (P≤0.05), ** (P≤0.01), NS: Non-significant.**

B=University of Baghdad M=University of Al Mustansiriya
T=University of Technology N= University of Nahrin I=Iraqia University

Table (1) showed that the higher percentage of LAT sero (+ve) *T. gondii* was recorded in University of Technology, then in the universities (Iraqia, Mustansyria, Baghdad and Nahrin) was 20(50%), 17(42.5%), 14(35%), 13(32.5%) and 12(30%), respectively. There were significant differences between LAT sero(+ve) and LAT sero(-ve) within each university .

The present study concentrated only on the unmarried female students when comparing with AL-Shikhly (2012)[20] who recorded *T. gondii* infection in pre-marital female 100(22%) out of 450 universities students (females and males) at age of (19-28) years while recorded low level of infection in married female using LAT.

Our results were in agreement with AL-shikhly *et al.*, 2013) [21] who recorded high percentage 271(51.5%) of latex sero(+ve) in apparently premarital female at age (15-35) years distributed as 147(54.2%) in AL-Rusaffa and 124(45.8%) in Karkh regions of Baghdad province. But higher percentage of this result was obtained by Obaid (2014)[22] who recorded 8(16%) out of (50) unmarried female at age (13-29) years in KirKuk city using LAT.

In Syria Ismail and AL koutayni, 2012 [23] recorded high latex sero positive (47%) out of 313 female students among one universities at age (19-23) years.

Numerous studies have shown that routine serological screening for *T. gondii* antibodies using LAT gives excellent results in the overall investigation, because of the high quality, simple, rapid, specific and inexpensive cost. It also is useful tests in suspicious cases in other methods .This test have used to comprehensive detect for toxoplasmosis antibodies in several regions of the world, among them South-East Asia[24] and Africa[25] .

Detection of *T. gondii* antibodies using ELISA IgM and IgG

Out of 200 unmarried female students there were 61(30.5%) sero (+ve) for IgM and IgG sero(+ve) by ELISA, while more than two third (69.5%) was sero (-ve). Only 3 (1.5%) were had positive IgM and was recorded as one case in each of (Mustansyria, Technology and Iraqia) was (0.5)%, There are negative results in other universities. . The statistical analysis showed highly significant p(≤0.01) differences between total sero (+ve) IgM and IgG. As shown in Table (2).

Table (2): Distribution of Toxoplasmosis by ELISA IgG and IgM according to universities among the study groups.

Test	ELISA(Abs)							P-value
	T NO. %	M NO. %	B NO. %	N NO. %	I NO. %	Total.(+ve) NO %	Total.(-ve) NO %	
ELISA IgG (+)ve	13 (6.5)	12 (6)	11 (5.5)	11 (5.5)	11 (5.5)	58 (29)	142 (62)	0.617 NS
ELISA IgM (+)ve	1 (0.5)	1 (0.5)	0 (0.00)	0 (0.00)	1 (0.5)	3 (1.5)	197 (98.5)	0.962 NS
Total	14	13	11	11	12	61	--------	---
P-value	0.093 NS	0.093 NS	0.328 NS	0.328 NS	0.307 NS	0.013 **		

**** (P≤0.01), NS: Non-significant.**

B=University of Baghdad M=University of Al Mustansiriya
T= University of Technology N= University of Nahrin I=Iraqia University

Table (2) also showed that 58(29%) of students had positive results distributed in the universities (Technology and Mustansiriya) of 13(6.5%) then 12(6%) respectively and 11 case in each of (Baghdad, Al-Nahrin and Iraqia) of (5.5%).

The statistical analysis showed non-significant differences between ELISA IgM and IgG within each university.

IgM is an indicator of recent infection and becomes negative within (4–12) weeks[26] The detection of specific IgM is the most common method used to determine the time of primary infection and it is crucial for the clinical management of pregnant women [27] .While a single positive IgG test indicates chronic infection, IgG antibodies usually appear within one to two weeks of acquisition of the infection and peak within one to two months, then fall at a variable rates, and usually persist for life which might have been acquired before conception. [28].

The current results showed higher percentages obtained by AL-shikhly (2012) [20]who recorded positive IgM 2(1%) and IgG 65(33.3%) among premarital females respectively using ELISA . In addition of Obaid (2014)[22] who recorded IgM 1(9.09%) , IgG 9(81.8%) and both IgG-IgM 1(9.09%) out of 50 unmarried women.

Generally IgM are detected within the first 2 weeks of infection and reduce to negligible levels within 6 months after exposure, However in toxoplasmosis, low levels of IgM can remain for many months, even year after acute infection. Thus, the more presence of IgM is not diagnostic of an acute toxoplasmosis infection[28,29].

Because antibody titers to *Toxoplasma* IgG may remain elevated for significant periods of time, an increase in IgG antibody may reflect an active primary infection, reactivation of infection, or a persistent immune response to a dormant infection [30].

Immunocompetent mothers infected before pregnancy do not pass the parasite to their offspring in uterus, even if re-exposure to the parasite occurs

during the critical period of the pregnancy [31].

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دراســة مصليـة لانتشار داء المقوسات بين طالبــات جامعيــات غير متزوجـــات باستخدام فحص التلازن، الاليزا وفحص الرغابـــة

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الخلاصة:

المقوسات الكوندية *Toxoplasma gondii* طفيليات واسعة الانتشار في العالم وهي احدى أكثر العوامل المرضيه أنتشارا في العراق. وقد أجريت الدراسة على 200 عينة مصل لطالبات جامعيات غير متزوجات تراوحت أعمارهم بين (18-26) سنة للكشف عن الأجسام المضادة للمقوسات ..ان الهدف من الدراسة الحالية هو دراسة مصلية لانتشار داء القطط بين طالبات اناث غير متزوجات باستخدام فحوصات مصلية مختلفة.اظهرت نتائج التحري ان 76(38%) حالة كانت موجبة للاجسام المضادة للمقوسات باختبار تلازن اللاتكس من اصل 200 عينة مصل. ومن بين76 حالة موجبة باختبار تلازن اللاتكس ،فقط 58(29%) حالة موجبة للاجسام المضادة للمقوسات نوع IgG باختبار الاليزا و3 (1,5%) حالات موجبة للاجسام المضادة للمقوسات نوع IgM. لم تعطي اي عينة سلبية باختبار تلازن اللاتكس نتيجة موجبة لاختبار الاليزا للاجسام المضادة للمقوسات نوع (IgG,IgM) .

الكلمات المفتاحية: المقوسات الكوندية، اختبار تلازن اللاتكس، اختبار الاليزا، الاجسام المضادة للمقوسات نوع (IgG,IgM) .