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Some Serological Diagnostic Methods of *Toxoplasma* Infection Correlated with Some Risk Factors among College Students

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Abstract

Background: *Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite. **Objective**: We performed this study to evaluate the seroprevalence of toxoplasmosis and investigate the possible risk factors among students in the College of Pharmacy of University of Mosul (CPUM).**Materials and Methods**: A cross-sectional study was designed to contain 112blood specimens had been amassed among students in CPUM, from February to May in 2018.The blood specimens have been examined for anti-*T. gondii* antibodies using Enzyme Linked Immuno-Sorbent Assay (ELISA) and Toxo Rapid Test-Cassette (TRT-C).Univariate and multinomial logistic regression analysis had been used to identify the risk factors for *T. gondii* infection. **Results**: The overall seroprevalence of *T. gondii* by using TRT-C and *Toxoplasma* ELISA among students in CPUM have been 19.64% and 18.75%, respectively. Significant correlation used to be seen among *T. gondii* seropositivity and married students (OR=0.081, 95%CI 0.008–0.803, P=0.032) by using TRT-C. Also, significant association used to be showed between *T. gondii* seropositivity and students lived in urban areas (OR=0.116, 95%CI 0.022–0.619, P=0.012), no cat holders(OR=0.127, 95%CI 0.020–0.831, P=0.031), no smokers (OR=0.074, 95%CI 0.007–0.788, P=0.031), having a habit of eating away from home (OR=0.171, 95%CI 0.036–0.816, P=0.027) and married students (OR=0.017, 95%CI 0.001–0.342, P=0.008) by using *Toxoplasma* ELISA. No significant association used to be found between *T. gondii* seropositivity and other categorical variables by using TRT-C and *Toxoplasma* ELISA like gender, socioeconomic level, visual impairment, and blood groups. **Conclusion**: The total seroprevalence of toxoplasmosis among students in CPUM in Nineveh province used to be relatively moderate and the odds ratio together with associated *T. gondii* seropositivity have been less likely happening with significant categorical variables. Awareness introduction about the infection using health extension employees will may additionally be forestalling the infection in the future.

Keywords: Serological methods, *Toxoplasma*, Prevalence, Correlation, Risk factors, College students

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Introduction

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Toxoplasma gondii(*T. gondii*) is a protozoan pathogen (parasite).[1],[2]Cats are ultimate hosts, whilst a large range of snug blooded animals, consisting of human beings serve as an intermediate host.[3]*Toxoplasma* parasites maybe transmitted orally to human beings via *Toxoplasma* tissue cysts or oocysts from contaminated meals and water, or vertical disseminate to posterities through transplacental transmission.[4]Infection with the *Toxoplasma* parasites has been observed globally in almost 0.33% of the human and varies extremely amongst different countries, geographical regions among the identical land.[5]*Toxoplasma* parasites identified via revealing anti-*T. gondii* precise (IgM and IgG antibodies) serologically. Serological tests, such as the Enzyme Linked Immuno-Sorbent Assay (ELISA) and Toxo Rapid Test-Cassette (TRT-C) are applied in many medical laboratories.[6]

The overall seroprevalence of *T. gondii* infection has been documented in distinctive, worldwide and neighboring countries of Iraq; 24.1% from seroepidemiological analysis among college students of Brazil, [7] 27.63% in Ethiopia; [8] 5.87% in pregnant women attending antenatal clinic at the university teaching hospital in Zambia, [9] 66.5% among undergraduate university female students in Jordan; [10] 43.3% in diabetic patients in Saudi Arabia, [6] and 16.8% in blood donors in Iran. [11]

Also, toxoplasmosis prevalence has been evaluated in Iraq in some populations; 21.5% from a survey study among Kirkuk university students, [12] 9.8% from a survey in pregnant women and university staff of Kirkuk province;[13] 32.81% and 47.1% among aborted women in Thi-Qar and Al-Najaf provinces, respectively. [14],[15]Moreover, there are no documented records about the prevalence of the disease and related risk factors in the study area. Therefore, we performed this study to evaluate the seroprevalence of toxoplasmosis and investigate the possible risk factors among students in the College of Pharmacy of University of Mosul (CPUM).

Materials and Methods

Study design and information collection

The study design was cross-sectional. It was performed in male and female students in CPUM, Nineveh province, Iraq. The find protocol was authorized by ethic committee in CPUM and an informed consent used to be obtained from each participant. Students who had been unable to communicate and those who have been now not inclined to furnish information and blood specimens were excluded from the study; therefore, a total of 112 blood specimens were obtained from the students in CPUM, from February to May in 2018. About 3 milliliters of venous blood used to be gathered aseptically from all participants into Eppendorf tubes. Blood specimens were analyzed in the microbiology laboratory in the department of clinical laboratory sciences in CPUM. Blood specimens were obtained by centrifugation of fresh whole blood from all participants at 3000 RPM for 3 minutes and stored frozen at −20 °C until processing. Sociodemographic traits have been notarized for every study participant. Using arranged questionnaire, information on exposure to feasible risk factors have been gathered, and contained gender, residence area, socioeconomic level, cat holders, smokers, eating away from home, marital status, visual impairment, and blood groups.

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Serological tests

ToxoIgG/IgM Rapid Test-Cassette is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG and IgM anti-*T. gondii* in human serum. The test was carried out in accordance to the manufacturer's instruction (Spectrum diagnostics, Egypt).*Toxoplasma* (IgG and IgM) ELISA is used for the detection of IgG and IgM antibodies to *Toxoplasma* in human serum. The test used to be executed according to the manufacturer's preparation (MyBioSource, USA). The ABO phenotypes had been recognized through using Anti-A (monoclonal reagent), Anti-B (monoclonal reagent) is used in the red blood cell determination of the ABO blood group and Anti-D (blend reagent) is used to determine the Rhesus (Rh) type. The test used to be achieved in accordance with the manufacturer's guidance (Atlas Medical, UK).

Statistical data analysis

Data from the questionnaire and serological checks were analyzed using SPSS version 24.Univariate and multinomial logistic regression analysis was used to analyze the association among variables and toxoplasmosis. P-value <0.05 used to be represented a significant in the analysis. Chi-square (χ^2) test, odds ratio (OR) with 95% confidence interval (CI), the degree of freedom (DF) had been studied.

Results

The overall seroprevalence of *T. gondii* by using TRT-C (screening test) and *Toxoplasma* ELISA (confirmatory test) among students in CPUM was 22 (19.64%) and 21 (18.75%), respectively. The result showed that the seropositive rate of TRT-C of anti IgG is 22 (19.64%) while *Toxoplasma* ELISA revealed that 20 (17.86%) of students have been positive for anti-IgG. The result did not exhibit seropositivity to IgM whether by TRT-C or by *Toxoplasma* ELISA. Of them, 0.89% students were positive for each IgM and IgG in accordance with *Toxoplasma* ELISA, while TRT-C does not reveal any positive cases for IgM with IgG together (Table 1). Referring to univariate analysis via using TRT-C, married students (OR=0.081, 95%CI 0.008–0.803, P=0.032) had a significantly higher seroprevalence and the odds ratio together with correlated *T. gondii* seropositivity was less likely to occur whilst nearly categorical variables by the use of TRT-C such as gender, residence area, socioeconomic level, cat holders, smokers, eating away from home, and visual impairment did not exhibit significant association with *T. gondii* seropositivity (Table 2).

Depending on univariate analysis by using *Toxoplasma* ELISA, students lived in urban areas (OR=0.116, 95%CI 0.022–0.619, P=0.012), no cat holders (OR=0.127, 95%CI 0.020–0.831, P=0.031), no smokers (OR=0.074, 95%CI 0.007–0.788, P=0.031), having a habit of eating away from home (OR=0.171, 95%CI 0.036–0.816, P=0.027) and married students (OR=0.017, 95%CI 0.001–0.342, P=0.008) had significantly higher seroprevalence and the odds ratio together with associated *T. gondii* seropositivity have been less likely to occur, whereas sociodemographic variables by the usage of *Toxoplasma* ELISA such as gender, socioeconomic level, and visual impairment did not show a significant association with *T. gondii* seropositivity (Table 3). We found no significant correlation between ABO phenotypes amongst students of CPUM (toxoplasmosis mostly prevalent in O+ whereas

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less prevalence an AB+ blood group) and *T. gondii* seropositivity using TRT-C ($\chi 2$=3.951; DF=5; P=0.557) (Table 4) and the absence of significant connection between ABO phenotypes among students of CPUM (also observed toxoplasmosis mainly familiar in O+ while less prevalence in an AB+ blood group) and *T. gondii* seropositivity using *Toxoplasma* ELISA ($\chi 2$=5.206; DF=5; P=0.391) (Table 5).

Discussion

The present study is one of the little researches completed in Nineveh province to evaluate the total seroprevalence of toxoplasmosis, specifically amongst students of CPUM. According to TRT-C (screening test) the overall seroprevalence in the current study was 19.64%, which was higher than the reported studies [6],[9] whereas lower than the previous studies [16],[17] and when confirmation the results by using *Toxoplasma* ELISA the overall seroprevalence was 18.75%, which used to be higher than the recorded studies [11],[13] while lower than the prior studies. [6],[10],[12],[14],[15],[18] Such seroprevalence variation in the rate of seropositivity of *T. gondii* in different areas of the world can be attributed to climatic status for the existence and steadiness of *Toxoplasma* oocysts in the ground, exceptional nourishment habits, geographical location, immune response of the host, sample measurement and strain of the parasite.

T. gondii can be transmitted to humans via vertical disseminate to posterities through transplacental conveyance. [4] In our study significant association was found between *T. gondii* seropositivity and married students, according to screening and confirmatory tests. A study from Baghdad province [19] found a significant rise in *T. gondii* seropositivity in married students. However, exceptional studies [20],[21] stated the lack of significant association among married students and seropositivity of *Toxoplasma* infection, while another study [9] recorded significant association between single (unmarried students) and seropositivity of *Toxoplasma* infection. The cause of this might be the married students in the current study of female college students, which are prone to acute toxoplasmosis in the course of their childbearing period, and their babies might be at risk of congenital toxoplasmosis, if the female students become infected all through their first pregnancy. Moreover, impact the seropositivity that the use of specific detection methods. In the present study, there was a significant distinction amongst *T. gondii* seropositivity and students living in urban areas who rely on confirmatory test. This end outcome is in accordance with a study into Thi-Qar province. [14] On the other hand, the study disagree with investigations of preceding studies, [11],[15],[20],[23] which did not found any significant association between urban areas and *T. gondii* seropositivity whilst reported significant correlation between rural areas and *T. gondii* seropositivity. [10],[18],[19],[23] The possible reasons for the spread of infection into urban areas have been linked to increased number of stray cats that will impact veggies where the elimination of oocyst might also not be achieved due to the current situation for not providing the water for several parts inside the urban. The other causes are increased consumption of meat which can also be infested with *T. gondii* tissue cysts or because of dealing with an excessive number of cases in urban areas, in contrast with to the rural areas.

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Although felines are the ultimate hosts that disseminate oocysts, [24] no cat holders were significantly associated with toxoplasmosis based on confirmatory test. This is in line with another study.[25]In contrast, some studies [6],[10],[25]reported the absence of a statistically significant relationship between no cat holders and *T. gondii* seropositivity. Different outcomes recorded significant correlation between cat holders and *T. gondii* seropositivity.[18],[21],[23]Consequently, this variant in one-of-a-kind research does not suggest cat holders is not a pool of toxoplasmosis, however, this diversity exhibit that human except having contact with cats are equally infected with *T. gondii*, in comparison with those who have cat contact. Also, the presence of cats as pets in Iraqi homes is much less than the rest of the world; therefore, they have much less contact with cat feces.

A significant connection was additionally determined amongst *T. gondii* seropositivity and no smokers, students relying on confirmatory test. However, although the finding disagree with the reviews of Cosme Alvarado-Esquivel et al. [26] who did not display a significant correlation amongst smokers and seropositivity of *T. gondii* while another study in Mexico [22]shows up significant connection between people who smoke and *T. gondii* seropositivity. This does not mean that smokers have no impact on the transmission of *T. gondii* infection by carrying the pathogen from the hands to the mouth while smoking..

The study additionally showed a statistical significant connection amongst seropositivity of *T. gondii* and having habit of eating away from home, according to confirmatory test. This is inconsistent with prior reports. [7],[11],[12],[25]On the contrary, in previous studies, statistical distinction was found an insignificant correlation between *T. gondii* seropositivity and having a habit [21] or did not have a habit [10] of eating away from home whereas another study [26] show a significant relationship between *T. gondii* seropositivity and did not have a habit of eating away from home. This variation might be related to the really worth understanding that the principal sources of toxoplasmosis the human being (especially in college students) are supposed to be undercooked meat containing tissue cysts, in addition; contaminated veggies by oocysts which more frequent when eating away from home (in restaurants and quick food outlets).

In the present study, loss of significant correlation amongst *T. gondii* seropositivity and ABO blood group phenotypes among students of CPUM depending on confirmatory test. This result is in agreement with preceding studies. [11],[27],[28] Furthermore, the finding disagree with the study of Mahmood *et al.*[19] who showed a statistical significant connection amongst seropositivity of *T. gondii* and ABO blood group phenotypes. The cause of this might be the possible that the molecular variability of strains in Iraqi patients, or using only male suffers in the previous study when evaluating with current study.

Conclusion

It may additionally be concluded that the total seroprevalence of toxoplasmosis among students in CPUM in Nineveh province used to be relatively moderate than that recorded from different countries. Also, the odds ratio together with related T. gondii seropositivity have been less likely happening with

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statistical significant categorical variables (married students, urban areas, no cat holders, no smokers and having a habit of eating away from home).

The accepted nourishment and ecological hygiene of a society is a vital component that facilitates the transmission of toxoplasmosis; as well, the awareness introduction about the infection using health extension employees will play a role in preventing the infection.

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Conflicts of interest
There are no conflicts of interest.

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TABLES

Table 1: Combination of IgG and IgM anti-*T. gondii* antibodies prevalence among students using TRT-C (screening test) and *Toxoplasma* ELISA (confirmatory test)

Anti-*T. gondii* antibodies	TRT-C Positive (%)	ELISA (%)	Positive
Positive for IgG only	22 (19.64)	20 (17.86)	
Positive for IgM only	0 (0.0)	0 (0.0)	
Positive for IgG and IgM	0 (0.0)	1 (0.89)	
Negative for IgG and IgM	90 (80.36)	91 (81.25)	
Overall Positive for either IgG and IgM	22 (19.64)	21 (18.75)	

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Table 2: Categorical variables (Sociodemographic traits) of the students in CPUM and prevalence of *T. gondii* infection by using TRT-C

Variable	Tested (%)	Positive (%)	OR(95%CI)	P-value
Gender				
Female	56 (50)	14 (25)	1.047(0.209–5.241)	0.956
Male	56 (50)	8 (14.29)	Reference	
Residence area				
Urban	78 (69.64)	19 (25)	0.235 (0.049 –1.118)	0.069
Rural	34 (30.36)	3 (8.82)	Reference	
Socioeconomic level				
High	13 (11.60)	2 (15.38)	3.243 (0.117 –90.226)	0.488
Medium	90 (80.36)	19 (21.11)	0.449 (0.028 –7.177)	0.572
Low	9 (8.04)	1 (11.11)	Reference	
Cat holders				
No	84 (75)	18 (21.43)	0.603 (0.139–2.625)	0.501
Yes	28 (25)	4 (14.29)	Reference	
Smokers				
No	84 (75)	21 (25)	0.848 (0.168–4.274)	0.842
Yes	28 (25)	1 (3.57)	Reference	
Eating away from home				
Yes	69 (61.60)	18 (26.09)	0.414 (0.112 –1.535)	0.187
No	43 (38.40)	4 (9.30)	Reference	
Marital status				
Married	5 (4.46)	3 (60)	0.081 (0.008–0.803)	0.032*
Single	107 (95.54)	19 (17.76)	Reference	
Visual impairment				
Yes	28 (25)	8 (28.57)	0.272 (0.064 –1.163)	0.079
No	84 (75)	14 (16.67)	Reference	

χ^2 = 18.461;DF= 9;* = Statistically significant at P < 0.05

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Table 3: Categorical variables (Sociodemographic traits) of the students in CPUM and prevalence of *T. gondii* infection by using *Toxoplasma* ELISA

Variable	Tested (%)	Positive (%)	OR (95%CI)	P-value
Gender				
Female	56 (50)	13 (23.21)	1.343 (0.286 – 6.305)	0.709
Male	56 (50)	8 (14.29)	Reference	
Residence area				
Urban	78 (69.64)	18 (23.08)	0.116 (0.022 –0.619)	0.012 *
Rural	34 (30.36)	3 (8.82)	Reference	
Socioeconomic level				
High	13 (11.60)	1 (7.69)	1.517 (0.03 –76.13)	0.835
Medium	90 (80.36)	19 (21.11)	0.204 (0.007 –6.051)	0.358
Low	9 (8.04)	1 (11.11)	Reference	
Cat holders				
No	84 (75)	17 (20.24)	0.127 (0.020–0.831)	0.031 *
Yes	28 (25)	4 (14.29)	Reference	
Smokers				
No	84 (75)	20 (23.81)	0.074 (0.007–0.788)	0.031 *
Yes	28 (25)	1 (3.57)	Reference	
Eating away from home				
Yes	69 (61.60)	17 (24.64)	0.171 (0.036 –0.816)	0.027 *
No	43 (38.40)	4 (9.30)	Reference	
Marital status				
Married	5 (4.46)	3 (60)	0.017 (0.001–0.342)	0.008 *
Single	107 (95.54)	18 (16.82)	Reference	
Visual impairment				
Yes	28 (25)	7 (25)	0.602 (0.105 – 3.439)	0.568
No	84 (75)	14 (16.67)	Reference	

χ^2 = 33.908; DF= 9;* = Statistically significant at P < 0.05

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Table 4: The ABO blood group phenotypes in students of CPUM with positive cases for IgG and IgM anti-*T. gondii* antibodies by using TRT-C

Blood samples	ABO blood group phenotypes								Total (%)
	O+ (%)	A+ (%)	B+ (%)	AB+ (%)	O- (%)	A- (%)	B- (%)	AB- (%)	
No. examined (%)	40 (35.7)	31 (27.7)	28 (25)	11 (9.82)	1 (0.89)	0 (0.0)	0 (0.0)	1 (0.89)	112 (100)
No. Positive for IgG only (%)	11 (9.82)	3 (2.68)	7 (6.25)	1 (0.89)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22 (19.64)
No. Positive for IgM only (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Positive for IgG and IgM (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

$\chi^2 = 3.951$; DF= 5;P = 0.557 (P > 0.05)

Table 5: The ABO blood group phenotypes in students of CPUM with positive cases for IgG and IgM anti-*T. gondii* antibodies by using *Toxoplasma* ELISA

Blood samples	ABO blood group phenotypes								Total (%)
	O+ (%)	A+ (%)	B+ (%)	AB+ (%)	O- (%)	A- (%)	B- (%)	AB- (%)	
No. examined (%)	40 (35.7)	31 (27.7)	28 (25)	11 (9.82)	1 (0.89)	0 (0.0)	0 (0.0)	1 (0.89)	112 (100)
No. Positive for IgG only (%)	10 (8.93)	3 (2.68)	6 (5.36)	1 (0.89)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (17.86)
No. Positive for IgM only (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Positive for IgG and IgM (%)	0 (0.0)	0 (0.0)	1 (0.89)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.89)

$\chi^2 = 5.206$; DF= 5;P = 0.391 (P > 0.05)