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Survey study on toxoplasmosis among Kirkuk university students

دراسة مسحية عن المقوسة الكوندية بين طلاب جامعة كركوك.

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Abstract

Toxoplasma the cat transmitted parasite, is a major health problem and can infect human at any ages of life time. University students are very important part of the community, therefore the aim of the study was to screen for anti-*Toxoplasma* antibody among Kirkuk university student, because latent *Toxoplasma* can led to hormonal disturbance, self-violence and schizophrenia. For this purpose, one hundred and ninety serum samples were collected from Kirkuk University students. Serum samples were examined for *Toxoplasma gondii* antibody by enzyme linked immunosorbent assay. The overall incidence of *Toxoplasma* was 21.5%, with an equal rate (11%) for both IgG and IgM antibody. The frequency of the parasite in both male and female were higher in 21-22 years with rate of 20, 32.6% for each of male and female respectively. Some behavior like blood receipting, gardening, eating under cooked meat and keeping cat at residence had positively affected the parasite prevalence. *Toxoplasma* is prevalent among Kirkuk University student, therefore attention is better to be taken to this important part of the community to grantee a healthy offspring capable of building the country.

المستخلص:

المقوسة الكوندية الطفيلي الناقل من القطط، يشكل مشكلة كبيرة و يصيب ألانسان في أي مرحلة عمرية. طلاب الجامعة يشكلون جزء مهم جدا من المجتمع، لذا كان الهدف من هذه الدراسة هو البحث عن الاجسام المضادة لطفيلي المقوسة الكوندية بين طلاب جامعة كركوك لان داء المقوسات الكامن قد يقود الى خلل هرموني، اذية النفس اومرض فقد الذاكرة.

لهذا الغرض جمع مائة وتسعين عينة مصل من طلاب جامعة كركوك. تم التحري عن الاجسام المضادة للتوكسوبلازما في مصول الطلاب باستخدام تقنية ELISA. النسبة الكلية لانتشار داء المقوسة كان 21.5%. وكانت نسبة الاجسام المضادة IgG, IgM متساوية و بنسبة 11%. الفئة العمرية الاكثر اصابة من الذكور والاناث كان (21-22 سنة) بنسبة 20، 32.5% لكل من الذكور والاناث على التوالي. بعض السلوكيات مثل (تسلم الدم، العمل في الحديقة، اكل اللحوم الغير مطبوخ جيدا، وجود القطط قرب السكن) اثر بشكل ايجابي على انتشار الطفيلي. المقوسة الكوندية منتشرة بين طلاب جامعة كركوك لذلك من الافضل الاهتمام اكثر بهذا الجزء المهم في المجتمع لضمان جيل صحي قادر على بناء الوطن.

Introcdution:

Toxoplasmosis is a disease caused by an Apicoplixan parasite, *Toxoplasma gondii*. It's a parasite of worldwide distribution present in every climates and countries. It can survive in a wide variety of vertebrate hosts. Cats and other Felidae are the final hosts, while human and wide range of animals, birds and rodents are intermediate hosts [1]. *Toxoplasma* gondii is a successful parasites. Although the burden of this parasite varies greatly from one country to another, it remain a global public health problem which effects about one billion individuals [2].

In Iraq, the occurrence of *Toxoplasma* gondii was first recorded in animals when Machattie 1938 found the parasite in smears from spleen and lungs of two street dogs in Baghdad. Najim and Al-Saffar 1963 found that the rate of infection was 40.5% among females with history of abortion and the rate of among normal children was 4.9% by skin test, while it was 11.4% in a group of mentally defective children [3]. Serological evidence indicates that human infections are common in many parts of the world, but most cases are benign in nature or are completely asymptomatic [(4)]. Among university students, 70 (21.94%) of students were positive for anti-*Toxoplasma* antibodies in different colleges of Thi-Qar university-Iraq [4] *T. gondii* IgG antibodies were detected in 66·5%

of the undergraduate female university students in Jordan and only one sample was positive for both IgG and IgM [5].

low rate(4.6%) *Toxoplasma* seropositivity was indicated among female students in Ahwaz Joundishapoor University of Medical Sciences, Iran [6]. Also Questionnaire form was conducted in Ferdowsi University of Mashhad Iran, only eighty six (15.7%) students had heard about toxoplasmosis out of 549 male and female [7], in São Paulo State University, Brazilian students seroprevalence was 22.3% by indirect fluorescent antibody test (IFAT) and that was similar to the result obtained by ELISA (24.1%) [8]. Also among the 80 blood samples analyzed, 27 female students were positive for IgG, whereas none of them were positive for IgM antibodies in the Presidente Prudente region, State of São Paulo [9]. A serum prevalence of 21.8% was observed by indirect immunofluorescence (IFA) and ELISA in public universities in Rio De Janeiro state, Brazil [10]. Serum samples from 336 students (252 veterinary students and 84 undergraduate students) at Virginia Polytechnic Institute and State University and the Virginia-Maryland Regional College of Veterinary Medicine, United States were examined for *Toxoplasma* IgG antibodies. The prevalence of *T. gondii* in these subjects was 5.6% in veterinary school students and 2.4% in undergraduates [11]. Among 174 volunteer students, prevalence of *Toxoplasma gondii*-specific IgG and IgM antibodies were 17.8% and 4.6%, respectively in Guadalajara, Jalisco, Mexico [12].

The overall estimation for prevalence of anti-*Toxoplasma* total antibody was 39.9%, The prevalence of anti-*Toxoplasma* IgG antibody using IFTA serological method was 34.5% and was 37.6% using ELISA in Iranian childbearing age women [13]. The seroprevalence of *T. gondii* infection in women of child-bearing age in Central Ethiopia was high. Anti- *T. gondii* IgG antibodies were detected in 81.4% of the samples [14]. In a descriptive study a total of 549 sera were randomly collected from high school girls aged between 13 to 19 years in Ajabshir from East Azarbaijan province Iran, The highest frequency of IgG and IgM *Toxoplasma* antibodies was observed in 18 years old (30.8%), while infection less than 14 years old had the lowest frequency (6.3%) [15]. The choice of university students was though appropriate because of the great value of this important part of the community, therefore the aim of the current study was to screen for anti-*Toxoplasma* antibody among Kirkuk university student.

Materials and Methods:

Population study:

From October 2014 to February 2015, a total of 190 serum samples (94 male, 96 female) were collected from Kirkuk university's student. A questionnaire form was given to each student which includes: age, having cat at residence, gardening, eating undercooked or raw meat and blood receipting.

Samples collection:

About 5 ml of venous blood was withdrawn carefully and transferred into disposable tube, the specimen was left to clot then centrifuged to separate clear serum. The sera were kept at (-20˚C) till used [6].

Enzyme immunoassay for the detection of IgG and IgM antibodies to *Toxoplasma gondii* in human serum:

Toxoplasma screening:

In order to investigate for serum *Toxoplasma* antibody, kits (Bio Check, Inc. USA) of *Toxoplasma* IgG and IgM Enzyme Immnoassay test were used for this purpose. The steps for detection were performed according to the kit instructions. Diluted serum was added to purified *Toxoplasma* antigen coated wells (12 x 8 wells). All unbound materials were washed away by ELISA washer. HRP-conjugate was added. Excess HRP-conjugate was washed off and a solution of TMB Reagent was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG or IgM-specific antibody in the sample. Optical density (OD) was read at 450 nm within 15 minutes by a microwell reader compared in a parallel manner with calibrator and controls (a micro well reader Toxo- antibody index of 1.00 or greater is positive and indicates the probability of current or recent toxoplasmosis) [9].

Statistical analysis: The association among the positive results and variables related to risk factors were determined using chi-square analysis, with the help of the Epi Info version 6.04 statistics software. P value < 0.05 was considered statistically significant.

Results

As shown in table 1 , 190 serum samples were examined by ELISA, 41 samples were positive with an overall prevalence rate of 21.5 % , 149 samples were negative with a rate of 78.4 %, significant differences had appeared between male and female.

Table (1): Number of samples examined by ELISA

No. of samples examined		+ve	%	-ve	%
Male	94	15	15.9	79	84
Female	96	26	27.08	70	72.9
Total	190	41	21.5	149	78.4
p value=0.062 significant differences					

Regarding the age group in female as indicated in table 2 no significant differences had appeared between the female ages, but the most female age group which was infected with the parasite was 21-22 with a rate of 32.6 % . In male as indicated in table 3, the most age group which was infected with parasite was 21-22 with a rate of 20 %.

Table (2): *Toxoplasma* prevalence according to age group in female

Age group	Total No. examined	%	+ve	%	-ve	%
18-20	29	30.2	4	13.7	25	86.2
21-22	52	54.1	17	32.6	35	67.3
23-25	15	15.6	4	26.6	11	73.3
Total	96	100	25	26	71	73.9
p value=0.177 no significant differences						

Table (3): *Toxoplasma* prevalence according to age group in male

Age group	Total No. examined	%	+ve	%	-ve	%
18-20	32	34	4	12.5	28	87.5
21-22	35	37.2	7	20	28	80
23-25	27	28.7	4	14.8	23	85.18
Total	94	100	15	15.9	79	84
p value=0.69 significant differences						

The result in table 4 indicate that *Toxoplasma* infection may be transmitted during blood transfusion, *Toxoplasma* prevalence in blood receipting students was significantly higher (47 %) comparing with 19 % in none blood receipting students.

Table (4): *Toxoplasma* prevalence in relation to blood receipting

Blood receipting	Total No. examined	%	+ve	%	-ve	%
Yes	17	9	8	47	9	53
No	173	91	33	19	140	81
Total	190	100	41	21.5	149	78.4
p value=0.0074 significant difference						

30 % of *Toxoplasma* positive individuals were clean their own garden or work in gardens which was significantly higher comparing with 17.6 % for those samples who were not cleaning or working in gardens (table 5). A significantly high rate (30%) of the students who had *Toxoplasma* infection had cat at residence, whereas 17.6% of the students had no cat at residence table 6.

Table (5): Relationship between gardening and *Toxoplasma* prevalence

Gardening	Total No. examined	%	+ve	%	-ve	%
Yes	60	31.5	18	30	42	70
No	130	68.4	23	17.6	107	82.3
Total	190	100	41	21.5	149	78.4
p value= 0.055 significant differences						

Table (6): Relationship between cat at residence and *Toxoplasma* prevalence

Cat at residence	Total no. examined	%	+ve	%	-ve	%
Yes	60	31.5	18	30	42	70
No	130	68.4	23	17.6	107	82.3
Total	190	100	41	21.5	149	78.4
p value = 0.055 significant differences						

Table 7 reveals the relationship between consumption under cooked or raw meat and *Toxoplasma* infection, 24.5 % of *Toxoplasma* positive samples had a habit of consumption of undercooked or raw meat comparing with 6.4 % who had no habit of eating undercooked or raw meat with significant differences between the two cohort.

Table (7): Relationship between eating undercooked or raw meat and *Toxoplasma* prevalence

Eating undercooked or raw meat	Total No. examined	%	+ve	%	-ve	%
Yes	159	83.6	39	24.5	120	75.4
No	31	16.3	2	6.4	29	93.5
Total	190	100	41	21.5	149	78.4
p value=0.0252 / significant differences						

The result in table (8) indicate an equal overall rate (11%) for each of IgG and IgM antibody (equal rate for chronic and acute infections), but low rate (0.5) for sub acute cases were noted. The antibodies rates were significantly higher in female than those of male.

Table (8): Seroprevalence of IgG and IgM *Toxoplasma* antibody in males and females

Sex	Total No. examined	IgG+ve %	IgG-ve %	IgM+ ve %	IgM-ve %	IgG+IgM +ve %	IgG+IgM-ve %
Male	94	8 8.5	86 91.5	7 7.4	87 92.5	0 0	94 100
Female	96	12 12.5	84 87.5	14 14.5	82 85.4	1 1.04	95 98.9
Total	190	20 11	170 89.4	21 11	16 88.9	1 0.5	189 99.4
P value = 0.514 / no significant differences							

IgM (immunoglobulin M) , IgG (immuneglobulin G) , +ve (positive) , -ve (negative) % (percentage) , No. (number).

The IgG antibody concentrations are shown in table (9), fig. 1. Most of IgG positive samples (12 (60%)) had frequency concentration rate of 10-100 IU\ml followed by a concentration of 300-500 IU \ ml with a rate of 20 % table (9).

Table (9): IgG antibody concentration in positive sample.

Antibody concentration	Number of positive samples	%
10-100 IU\ml	12	60%
100-200 IU\ml	2	10%
200-300 IU\ml	2	10%
300-500 IU\ml	4	20%

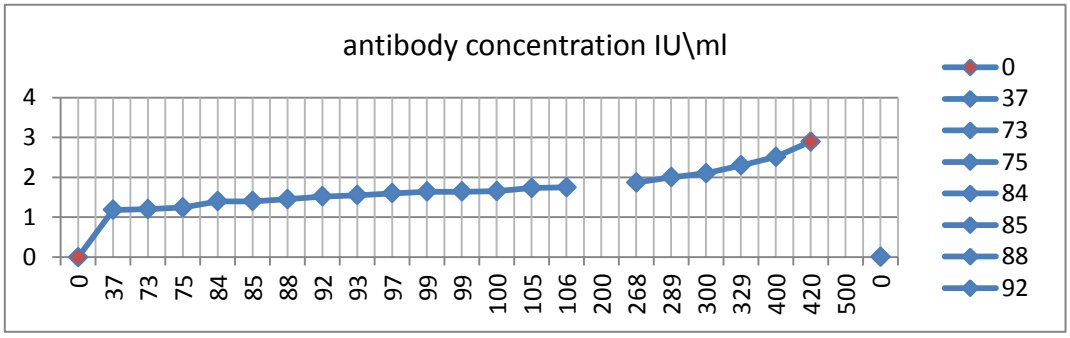

Figure (1): IgG antibody concentration curve

Discussion:

The present study evaluated the prevalence of toxoplasmosis in apparently healthy Kirkuk University students. The overall prevalence of *Toxoplasma gondii* in students whom were examined was 21.5%, compared with other studies, 21.94 % of students were positive for anti-*Toxoplasma* antibodies in different colleges of Thi-Qar university-Iraq [4]. A serum prevalence of 21.8% was observed by indirect immunofluorescence (IFA) and ELISA in Iran [10]. Our seroprevalence was also similar to the seroprevalence reported in Rio De Janeiro state, Brazil, and in East Azarbaijan Province, Iran [8, 15], Qazvin, Islamic Republic of Iran [16]. This similarity may be due to the similarities of environmental conditions and climatic factors.

This prevalence is much lower than that reported in Jordan and Presidente Prudente region, State of São Paulo and Central Ethiopia [5, 9, 14], where researchers found that 66.5% and 33.8, 81.4% of students age were positive for anti-*T. gondii* antibodies, respectively. It is possible that differences in the characteristics of the student age and differences in the environments might contribute to explain the lower prevalence of *T. gondii* infection found in our student age. But lower than our result was that reported in student age from countries including; Brazil (Lowa state), Syria, Venezuela, Saudi Arabia and Kuwait [17-20]. In Guadalajara, Jalisco, Mexico [12] Ahwaz Joundishapoor University, Iran [6] and USA [11] the seroprevalences were varied from 2.4% to 17.2%. This low prevalence is probably may be due to the eating habit [15] or hygienic education of the population in these cities.

The prevalence of IgG and IgM antibodies specific to *T. gondii* according to the sex of the student was 15.9% for males and 27.08% for females, it's not possible to detect a significant difference on the incidence of toxoplasmosis between sexes, but there are also studies pointing either to higher incidence for males or for females [14, 15, 20]. Our results showed a seropositive perceptual for female higher than for male and this in agreement with [4] whom recorded 24 (7.52%) and 46 (14.42%) respectively for male and female, also its identical with that found by [21]. This is undoubtedly due to many factors. First, women traditionally take more care of pet animals including cats at home, and secondly, women handle raw meat more frequently than men due to the fact that they spend more time cooking at home. Seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes [20]. Our results indicate that *T. gondii* infection may occurs during gardening process in both males and females or in those having

cat at residence or eating undercooked meat, and that the seroprevalence is higher in women more the man, this may be due to the women who are household clean home garden more than men. Students who had consumed undercooked meat had greater risk of acquiring *Toxoplasma gondii* infection. that agree with that found in Mexico [12]. Age, contact with cats, consumption of undercooked or raw meat, contact with campus soil and ignorance of prophylactic measures for toxoplasmosis were positively associated with prevalence of infection by *T.gondii* [6, 8, 10, 14, 22].

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