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Type G antibody investigation for the Kundi Toxoplasmic Parasite among the students of the faculty of basic education at the University of Mustansiriyah

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***Abstract*---**Toxoplasmosis is a transmissible disease that is common to humans and animals and is caused by the parasite *Toxoplsma gondii* from under the kingdom of protozoa, a forced intracellular parasite that intrudes on all types of nucleated cells. The current study aimed to investigate the infection rates of the Kondoni toxoplasmosis among students of the Faculty of Basic Education, Al-Mustansiriyah University, as it included the collection of 223 blood samples for the period from 1/9/2019 to 31/1/2020 and at ages ranging from 18to 24years. All information was taken when collecting samples according to the form prepared for this purpose, which included sex, age, residence, presence of cats at home, and smoking. The blood was separated for all the samples under study after being placed in sterile tubes inside the centrifuge and at a speed of 3000 cycles for 5 minutes. The serum was then withdrawn and distributed on the Ependorf tubes size of 1 ml with a volume of 250 micro liters per tube, and kept at a temperature of -20 in the Life Sciences Laboratory of the Department of the Faculty of Basic Education, Al-Mustansiriya University, until use. All samples were tested using two types of tests, namely Latex Agglutination Test (LA). The results showed that 99 samples were positive at 44%, while 124samples were positive at 55%. The second test that was adopted was the Tox IgM test and/or IgG Rapid Test-Casette, where the positive responses to IgG37 were positive at 17%, while IgM did not record any response. The results showed the distribution of the study groups and their

percentages according to gender, where the percentage of females was more than males according to the latex test, as it constituted a percentage of 66% for females and 34% for males. The results of the cassette test against IgG were 54% for females and 46% for males at the level of probability 0.05, while the largest percentage of infection with the Kondian arc of the study groups according to the residence in Baghdad was 81% according to the latex test and 84% based on the cassette test against IgG. The distribution of the study groups and their percentages according to the presence of cats at home or not, as the value of Kay square for the test of latex and cassette was 0.024 and 0.003 respectively. As for the distribution of study groups and their percentage of the smoking case in light of the results of the latex and cassette tests and the IgG, it had significant differences between smokers and non-smokers based on K-square, as it reached 2.69 for the latex test, while the cassette test reached 0.52.

***Keywords*---** Type G antibody, Toxoplasmosis, serum.

1. Introduction

Toxoplasmosis, or what is known as cat disease resulting from the infection of theparasite *Toxoplasma gondii*, is a widespread disease, as it affects about one-third of the world's population as well as a variety of fixed temperature animals such as mammals and birds. The parasite belongs to the Primary Animal Division Protozoa, which is a single-celled parasite, forced to intrude Obligate, has the potential to reproduce within the nucleated cells to proceed (Robert-Gangneux & Darde', 2012). The infection is transmitted to humans by eating foods contaminated with mature oocysts Mature bagssuch as vegetables, fruits and contaminated water that originate from the feces of infected cats or direct contact with these cats, or by eating meat that is not well cooked and contains Tissue cysts. Drinking unpasteurized milk is also one of the important sources of infection. Theinfection is also transmitted from the mother to the fetus through the transmission of rapid-breeding phases Tachyzoites through the placenta. The contribution of Blood transfusion and transplantation of organs to the transmission of infectionis addedtothe above, but in smaller proportions (2012 , Jeffer Sullivan Jr &). Immunocompetent is usually characterized by asymptomatic symptoms or limited symptoms that may interfere with symptoms of other diseases such as infiltration, such as Fever fever, headache, Myalgia, Malaise fatigue, Stiffness in the neck, Anorexia and other symptoms (Dubey, 2009). The severe pathological damage is due to the parasite's ability to invade and injure all of the body's nucleus cells and its ability to penetrate various biological barriers.

The Kundian arc parasite has the ability to migrate within the host body for long distances and cross biological barriers while entering the bloodstream and attribute various cells with nuclei, causing damage and necrosis to various tissues, and that the mechanism of the parasite's entry into the host cell depends on the possession of the parasite of the organs of the truncheons Rhopteries and micronemes in addition to the presence of the element of calcium in the host . The

process of parasite entry into the host causes various immune reactions, as it leads to the formation of antibodies, i.e. immune response incidents, as well as cellular immune response to limit its spread in the body. The tissue cysts are formed in the chronic stages of infection, which enables them to hide from the impact of the immune response (Robert-Gangneux & Darde', 2012).

2. Materials and Methods

2.1 Samples collection

The samples of the current study were collected from the beginning of September 2019 to the end of January 2020. From the students of the Faculty of Basic Education, the University of Mustansiriyah,until the age of 18-24 years, and the information was taken from all the study samples, including sex,residence, thepresence of animals and others(Annex1).
The Latex Immunological Test wasapplied to them and the study samples were distributed as follows:
1- The first group: The 99 samples of people suffering from the Kundi toxoplasmosis.
2- The second group: those who do not have the Kundi toxoplasmosis (control group), which number 124 samples.

2- 2: Blood samples

Blood samples were collected with 5 milliliters of intravenous blood for each of the infected persons and control using wine syringes, as 3 milliliters of it were placed in sterile test tubes equipped with gel and free of anticoagulant EDTA) Ethylene diamine tetra acetic acid and left for 10 minutes at room temperature 20-25 °C and then centrally ostracized with the centrifuge at speed 3000 rpm and for 5 minutes after that the serum was withdrawn using a micropipette and distributed equally to six tubes Abendorf and diagnostic immunological tests were conducted for Kundian arcuas disease for all study subjects represented by examination of latex and cassette.

2-3: Materials

Table (2-1): Laboratory devices used in the study

No.	Hardware Instrument	Company	State Country
1.	Centrifuge	Kokusan	Japan
2.	• Deep Freeze	Royal	Japan

Table (2-2): Laboratory tools used in the study

No.	Device tool	COMPANY Company	State Country
1.	Disposable syringe 5ml	Medico	UAE
2.	Gel clot tubes (without EDTA)	CE	China
3.	Medical gloves	TG-Medical	Malaysia
4.	Micropipette 0.5-10 µl, 10-100 µL, 200-1000 µl.	Biobasic	Canada

5.	Micropipette tip 100 µl, 1000 µL.	ciToTesT	China

2_4: Laboratory Kits

Table (2-3): Diagnostic data used in the study

No.	Laboratory Kits	Company	Country
1.	Latex test kit	Salucea	Holland
2.	Cassette test kit	HMG	Germany

2.5: Laboratory Methods

2.5.1 : Latex Agglutination Test (LA)

Commercial kits equipped by the Dutch private company Salucea were approved for the detection of specific antibodies against a parasite .*T.gondii*

Test kit components:

1- Latex reagent Toxo granule suspension 2.5 mm: - A suspension of latex granules made from polystyrene suspended polystyrene and coated with *T.gondii* purified dissolved antigen as a diluted antigen-bearing molecule with a saline buffer containing sodium azideNaN3 at a concentration (0.095%) .
2- Positive control detector 1 ml: - It consists of a human serum containing specialized antibodies against the *T.gondii* parasite and in an amount sufficient to achieve clear stasis and contains 0.095% increased sodium NaN3.
3- Negative control reagent 1 mL:- Composed of specialized antibody-free bovine serum against the *T.gondii* parasite where no reaction with the latex granule reagent suspensionappears, containing 0.095% NaN3.
4- Plastic slides divided into six cells .
5- Disposable wooden sticks.
6- A dropper used for latex grain suspension.
7- The kit is kept in the refrigerator at 4 °C until use.

Assay Procedure:

As instructed in the leaflet attached to the inspection kit**: -**

1- Getting the refrigerated test kit from the refrigerator and leave it until it reaches the room temperature.
2- Place 50 microlit t of the serum model to be tested on one of the plastic slide cells of the test.
3- Place 50 microliters of latex granular reagent suspension next to the serum drop after shaking the vial of the suspension well to complete the homogeneity of its components, mixing the materials with the medium of a nodal stick Stick m With the test slide moved circularly for 4minutes.
4- To infer positive screening, Agglutination with the naked eye is observed within four minutes and negative in the event that agglutination does not occur.

2-5-2 : IgG and IgM antibody cassette test of the Kundi arcuate parasite Tox IgM and/or IgG Rapid Test-Casette(Serum/ Plasma)

The IgG and IgM cassette antibody test for the Kundi arc parasite is a flow chromatography of both sides of the immunoassay for simultaneous detection and differentiation of the IgM and IgG antiparasitic to the Kondi arc parasite in the human serum.

Test kit components:

1- The bag containing the cassette .
2- Konodic arcuate parasite specific buffer.

Assay Procedure: As instructed in the leaflet attached with the inspection kit**: -**

1- Open the bag with cassette, remove the kit from the bag and place horizontally on the desk.
2- Apply 1 drop (5 µl) of the sample (serum) and 1 drop (50 µl) dilution to the sample well on the cassette.
3- Read the results within 10-15 minutes.

2. Statistical Analyses

1- Percentages of Cone toxoplasmosis cases in the samples under study .
2- The Chi-squared testto determine the significancelevel of the totals under study .

3.1 Results

The Latex test was adopted as a rapid diagnostic initial test for all study groups, whose results are summarized in Table (3-1). The group of 99 individuals with Toxoplasmosis was positive for testing at 44%, while the group of 124 people without Toxoplasmosis was negative at 55% .

Table (3-1): Latex test results and percentages for study totals

Aggregates	Number and percentage	Test result		Total
		positive	negative	
Kundi toxoplasmosis	Number	99	0	99
	%	100	0	100
Non-Condylic Toxoplasmosis	Number	0	124	124
	%	0	100	100
Total	Number	99	124	223
	%	44	56	100

Tables (3-2 and 3-3) show the results of the Cassette test for IgG, IgM as a confirmatory test for all study groups, as the number of positive responses to Cundian toxoplasmosis was 37 responses and 17%, while the group of non-Condian toxoplasmosis did not record any positive response to Cundian toxoplasmosis and 83% for IgG, while IgM did not record any response to it according to the cassette test.

Table (2-3): Cassette test results for IgG and their percentages for study totals.

Aggregates	Number and percentage	Test result		Total
		positive	negative	
Kundi toxoplasmosis	Number	37	0	37
	%	100	0	100
Non-Condylic Toxoplasmosis	Number	0	186	186
	%	0	100	100
Total	Number	37	186	223
	%	17	83	100

Table (3-3): Results of the Cassette test for IgM and its percentages for the study totals

Aggregates	Number and percentage	Test result		Total
		positive	negative	
Kundi toxoplasmosis	Number	0	223	223
	%	0	100	100
Total	Number	0	223	223
	%	0	100	100

Tables 3.4 and 3.5 show the distribution of study groups and their percentages according to gender in light of the results of the latex test and the cassette test for IgG and by applying a Chi-squared test of 1.9 for the latex test, while its value was 1.2 for the cassette test for IgG at the level of 0.05 in degree of freedom 1, and this is higher than the level of 0.05, so the result is a moral function and thus indicates that the incidence of the Kundan arch parasite in females is more than males according to the latex and cassette tests for IgG .

Table (3-4): Distribution of study totals and percentages by sex in light of latex test results

Aggregates / Sex:	Kundi toxoplasmosis		Non-Condylic Toxoplasmosis	
	Number	%	Number	%
Males	34	34	54	44
Females	65	66	70	56
Total	99	100	124	100
X^2 =1.9 p=0.05				

Table (3.5): Distribution of study totals and percentages by gender in light of IgG cassette results

Aggregates / Sex:	Kundi toxoplasmosis		Non-Condylic Toxoplasmosis	
	Number	%	Number	%
Males	17	46	85	46
Females	20	54	101	54
Total	37	100	186	100
X^2 =1.2 p=0.05				

It is clear from Tables (3-6 and 3-7) the percentages of study totals according toresidence in light of the results of the latex and cassette tests against IgG that the largest percentage of infection with the Kundi arcuate parasite was in Baghdad, reaching 81% according to the latex test and 84% based on the cassette test against IgG.

Table (3.6): Distribution of study totals and percentages according toresidence in light of latex test results

Aggregates / Accommodation	Kundi toxoplasmosis		Non-Condylic Toxoplasmosis	
	Number	%	Number	%
Baghdad	80	81	79	64
Dhi Qar	7	7	18	15
Babylon	2	2	4	3
Stock	2	2	5	4
Salah Al-Din	2	2	6	5
Wassit	4	4	6	5
Al Diwaniyah	1	1	3	2
Karbala	1	1	3	2
Total	99	100	124	100

Table (3-7): Distribution of study groups and percentages according to residence in light of cassette test results for IgG.

Totals / Accommodation	Kundi toxoplasmosis		Non-Condylic Toxoplasmosis	
	Number	%	Number	%
Baghdad	31	84	128	69
Dhi Qar	2	5	23	12
Babylon	0	0	6	3
Stock	1	3	6	3
Salah Al-Din	1	3	7	4
Wassit	2	5	8	5
Al Diwaniyah	0	0	4	2
Karbala	0	0	4	2
Total	37	100	186	100

Tables (3-8 and 3-9) show that the distribution of the study groups and their percentages according to the presence of cats at home or not in light of the results of the latex and cassette tests against IgG were immaterial in terms of 0.05, as the value of a square as any of the latex and cassette tests was 0.024 and 0.003 respectively.

Table (3.8): Distribution of study groups and percentages according to the presence of cats at home in light of latex test results

The presence of cats / Aggregates	Having cats in the house.		Lack of cats in the house		Total
	Number	%	Number	%	
Kundi toxoplasmosis	20	20	79	80	99
Non-Condylic Toxoplasmosis	24	19	100	81	124
Grandtotal	44	20	179	80	223
X^2 =0.024 p=0.05					

Table (3.9): Distribution of study groups and percentages according to the presence of cats at home in light of the results Cassette test for IgG antibody

The presence of cats / Aggregates	Having cats in the house.		Lack of cats in the house		Total
	Number	%	Number	%	
Kundi toxoplasmosis	10	27	27	73	37
Non-Condylic Toxoplasmosis	50	27	136	73	186
Grandtotal	60	27	163	73	223
X^2 =0.003 p=0.05					

The results of the distribution of the study groups and their percentages according to the smoking status in light of the results of the latex and cassette tests against IgG tables (3-10 and 3-11) formed a significant difference between smokers and smokers in terms of 0.005 based on the value of K square, as it reached 2.69 for the cassette test, reaching 0.52

Table(3-10): Distribution of study groups and percentages according to smoking status in light of latex test results.

Smoking Status / Aggregates	- Smoking!		- Nonsmoking.		Total
	Number	%	Number	%	
Kundi toxoplasmosis	25	25	74	75	99
Non-Condylic Toxoplasmosis	44	35	80	65	124
Grandtotal	69	31	154	69	223
X^2 =2.69 p=0.05					

Table (3-11): Distribution of study groups and percentages according to smoking status in light of cassette test resultsfor IgG

Smoking Status / Aggregates	- Smoking!		- Nonsmoking.		Total
	Number	%	Number	%	
Kundi toxoplasmosis	6	16	31	84	37
Non-Condylic Toxoplasmosis	40	22	146	78	186
Grandtotal	46	21	177	79	223
X^2 =0.52 p=0.05					

4.1 Discussion

The results of Table 3.1 and 3.2 showed through the examination of 223 samples that 99 of the samples were infected with T-gondii parasite, 44%, while 124 samples were not infected with T-gondiii parasite, 55% using latex test. The results of the cassette test as shown in Table 3.2 and 3.3, where 37 samples gave a positive response of 17% for IgG, while the group of people with Cundi toxoplasmosis did not record any positive response to to toxoplasmosis and 83% for IgG. No response was recorded for IgM according to the cassette test. A study (2012, Solaiman) on the spread of asymptomatic toxin disease and using latex test in a sample of students of the University of Egypt reached 44.3%. (2015, Al-Hakim) indicated in a study on toxoplasmosis in Ibn Al-Haytham Eye Hospital that there are such type of injuries under clinical control in healthy individuals (control) and by 21.9%.Subclinical morbidity also varied in various studies: 8% in Korea,9% in Colombia,10.2% in Taiwan, 45% in Indiaand 55.3% in Malaysia. (de-la-Torre *et al.,* 2009) giving this test some support for clinical diagnosis.

The results of the cassete test showed that (37) samples gave a positive response of 17% to the IgG antibody, while the group of non-Condylococcosis patients did not record any positive response to to toxoplasmosis and 83% to the IgG antibody. No response was recorded for IgM according to the cassette test, with significant differences according to the type of antibodies, and this variance may be due to many factors that play an important role in causing the injury.

These include location, age, residence, climatic conditions and healthand living conditions (Terazawa et al., 2003). Tables 3-4 and 3-5 showed the distribution of study totals and their percentage that the percentage of infections of the Kundan toxin parasite in females is higher than that of males according to the latex and cassette test against IgG.The reason for this variation is due to many worlds that play a role in the occurrence and increase of infection in females more than males, including direct and indirect contact with animals, the method of cooking and cutting meat, and the abundance of eating vegetables that are not well washed (1996, Buffolano etal). These studies showed the presence of the highest rate of appearance of IgG, which reached 17% compared to IgM, which did not record any response, and this is in line with the study (2014, Al-Abudi) and (2014, Al Mousawi) in the governorate of Dhi Qar, where the percentage of the holders of IgG was 27.5% and 11.91% respectively, while the percentage of the holders of IgM was 3.44% and 5.33%, respectively. The reason for the increase in IgG is that it remains for long periods after the injury and increases it indicates the presence of a rudimentary injury or re-activation or continuous immune response to the ambush (2001) Remalton eting

Tables 3-6and3-7 also showed that the highest percentage of infection with a parasite of conidoid toxoids was in Baghdad, which reached 81% according to the latex test and 84% based on the cassette test against IgG, while the rest of the governorates recorded a varying percentage of tests and study totals, and this may be due to geographical diversity and climatic variation of these areas as well as different social activities

Economic and food preparation methods and the type of meat consumed (Hershey, 1987) and(Rajkowa, 2005) The results of the table 3-8and 3-9 showed the distribution of study groups and their percentage according to the presence of cats at home was 20% for people with toxoplasmosis and 24% for those who do not have toxoplasmosis, while the distribution of results according to the cassette test against IgG was 27% a positive response when cats are at home, while the percentage of IgG was 73% when there are no cats at home. The results showed that the high incidence of people who are inside their homes, cats, or in a garden or planted area, such areas constitute an appropriate shelter for the final host represented by cats and the availability of moisture necessary for egg bags (1999 Garcia J). The residence in agricultural areas also increases the risk of exposure to direct dealing with animals and their products (Baril) . The results of Table3.10and3.11 showed the distribution of study groups and percentages according to the smoking status in light of the results of the latex and cassette tests against IgG , as it is likely that the *T. gondii* parasiteis infected by carrying the parasite from hands to mouth during smoking. In principle, hands are not washed before smoking commonly; therefore, handling cigarettes and touching lips with unwashed hands while smoking may contribute to *T. gondii* infection. Sharing cigarettes between people may increase the risk of infection as cigarettes touch more hands for more people. In fact, further research is needed to determine the role of smoking in the transition of *T. gondi*ï. On the other hand, *T.gondii* infection may lead to changes in behavior, and it is not known whether *T.* gondiii infection may lead to a desire to smoke. It should be noted that both *T.gondii* infection and smoking are associated with the release of dopamine. The experimental infection of the cat disease parasite in mice led to increased

dopamine secretion in the brain. Similarly, nicotine may cause the release of dopamine, and it is unclear whether the behavioral changes caused by nicotine may result in increased exposure to the *T.gondii* parasite. (McConkey et al., 2013;et al., 2013 ; Gatkowska et al., 2015 Cosgrove).

Conclusions

The current study reached the following conclusions:

1- This study is one of the few studies in Iraq to show the incidence of the Kundi toxoplasmosis among college students in Iraqi universities.
2- Efficiency of latex screening in the initial diagnosis of cosmic toxoplasmosis that contributed to the division of study groups.
3- The sensitivity of cassette selection to IgM and IgG specific antibodies in confirming the diagnosis of Konodic toxoplasmosis.
4- Describe the role and impact of some factors associated with the injury, such as gender , age, raising cats at home, and the smoking situation in increasing infection rates.

Recommendations

The current study recommends the following:

1- Conducting further studies on Kondu toxoplasmosis among Iraqi university students in all governorates of Iraq.
2- *Measuring T. gondii* IgM parasite specific antibody levels, IgG (IgG and IgM Enzyme-associated Immunosorbent Assay IgG& IgM (ELISA)
3- Conducting genetic studies on the genotypes of the Kundi arc parasite, investigating the strains of the parasite and identifying the most prevalent patterns that affect humans and animals in Iraq.
4- Spreading health and cultural awareness among people for the purpose of educating about the truth of how to acquire the disease and its appearance as an acquired disease and as a disease that appears as a setback for a congenital or acquired disease that cannot be cured.

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