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Association of seropositivity of toxoplasmosis and type 2 diabetes among adult females in Erbil city

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Abstract

Background and objective: *Toxoplasma gondii* is an important opportunistic parasite in immune-compromised individuals that causes toxoplasmosis. An intracellular pathogen could cause severe complications. Diabetes is a significant component that enhances the host's vulnerability and risk of acquiring a variety of infections.

The objectives of the current study are to reveal the relationships between toxoplasmosis and type 2 diabetes in addition to find out the most specific gene for the detection of *Toxoplasma gondii.*

Methods: In this case-control study, 201 blood samples were taken from type 2 diabetic women with 100 healthy women as control groups to detect anti-*Toxoplasma* IgG and IgM antibodies by performing latex agglutination test and Enzyme-Linked Immunosorbent Assay (ELISA). Detection of *B1* and *RE* genes of *Toxoplasma gondii* among seropositive individuals by nested PCR and evaluation of leptin hormone in both groups were performed.

Results: Among 201 type 2 diabetic cases, 64 cases (32.0%) were seropositive for anti-*Toxoplasma* antibodies, with 15 samples (15%) among 100 healthy control group via latex agglutination test. ELISA performed for only latex positive samples and the results were 64 ((32%) IgG+, (0%) IgM+) patient group (15.0% IgG+, 0.0% IgM+) healthy group with the *P*-value 0.0001 which is highly significant. The results of nested PCR were (46.88%) (IgG+, IgM-) and (1.56%) (IgG+, IgM-) for B1 and RE genes, respectively. Increased levels of leptin hormone were observed in patients in compare to healthy controls.

Conclusion: Both Diabetes and the elderly increase the chance of vulnerability to get toxoplasmosis. Diabetes, toxoplasmosis and high body mass index results in elevation of leptin hormone that leads to diabetes. B1 gene is the specific gene for detection of *T. gondii* in our area.

Keywords: Type 2 diabetes; *Toxoplasma*; ELISA; IgG; IgM; Leptin.

Introduction

Toxoplasma gondii is a unique internal parasite that reasons for human toxoplasmosis and is responsible for causing infection in warm-blooded vertebrates. many routes are being infected by *T. gondii,* like ingestion food and water contaminated by sporulated oocysts and tissue cysts in raw meat. Infants will get congenital toxoplasmosis from their mothers during pregnancy. *T. Gondii can* causes infection in approximately one-third of the world.[1]

An infection might be transmitted in many ways, through placenta, blood transfusion, or organ transplantation. Close contact transmission to human must betaken into consideration like when human deals with cat; they must carefully wash their hands with disinfectant or contact with the garden

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or dealing with soils. In a healthy person, toxoplasmosis is commonly realized to be asymptomatic. But, in an immune-deficient individual, a severe symptom is noticed, and it might be lethal.[2]

Diabetes mellitus is a long-lasting illness that happens once the pancreas does not secrete sufficient insulin or once the body cannot successfully usage the produced insulin. Hyperglycemia, or high blood sugar, is a usual outcome of the number of the body's systems, particularly the nerves and blood vessels. A strong connection was noticed between toxoplasmosis and both types of diabetes. Diabetes mellitus raises the host's sympathy and possibly getting a different disease.[3] Empathy and exposure to numerous infections could be more significant in diabetes mellitus individuals. In several circumstances, the Apicomplexan parasite, *T. gondii*, has been recommended as a probable reason for diabetes. Meanwhile, chronic toxoplasmosis has been thought of as a possible risk factor for type 2 diabetes (T2DM) recognized by a meta-analysis of investigations on the connection between chronic toxoplasmosis and diabetes mellitus.[4]

Since *T. gondii* infection in persons with T2DM has received little attention and accordingly the purpose of the current study was to determine the link between toxoplasmosis and T2DM via the investigation of seroprevalence of the anti-*Toxoplasma gondii* IgG and IgM antibodies in the blood of T2DM persons attended from the Layla Qassim Diabetic center during September to December of 2021 together with a group of non-diabetic persons to use as a control group. To find out the most specific gene for *Toxoplasma gondii* infection in molecular detection and evaluation of leptin hormone in the sera of diabetic patients.

Methods

Selection of patients and sampling

In this case-control work, blood samples were taken from 201 T2DM females who were already diagnosed by a physician that visited Layla Qassim Diabetic center for diabetes from Erbil city, north of Iraq, and 100 control groups that visited health care centers from 12 of September to 18 of December of 2021. All T2DM women were approached, and their ages ranged from26 to 75. They were asked about the history of the disease, family history, age, residency, occupation, contact with soil, contact with cats, and eating undercooked meat or not. To confirm the diagnosis of diabetes like HbA1C test and diabetic rates were evaluated in the laboratory of Layla Qassim center for diabetes.

Inclusion criteria: All age groups of females ranging between 26-75 years old with T2DM and non-diabetic were included in the current study.

Exclusion criteria: All males cases, women with T2DM who are below 26 years and above 75 years old, cases with other types of diabetic and diabetic cases of control group were excluded in the present study.

Ethical statement

The ethics committee of the College of Health Sciences at Hawler Medical University approved the current research, and official permission was taken from Layla Qassim Centre for diabetes in Erbil city for sample collection. The included subjects were given details about the aim of the study, and following their agreements and permission were enrolled, and they were ensured that their identities would be kept anonymous. All the subjects were included voluntarily, and they were given the right to decline participation in the study.

Latex agglutination test

To reduce the cost of the project latex agglutination test was performed on all the 201 samples and the100 healthy control group according to the available kit (TOX/012, REF, UK). Briefly, after allowing each component to room temperature and shaking the reagents, a drop of undiluted serum is placed on the circle of the test slide, then 25 µL of the latex reagent is

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placed next to the serum and mixed well all over the given area of the circle by using separate stirrer for each sample, after that the test slide tilted gently backward and forwards for four minutes. For interpretation of results, the test slides were microscopically examined for the detection of agglutination, which indicates a positive result.

Rheumatoid Factor test

The rheumatoid factor test was the second test that was performed for only the latex positive tests to avoid false-positive results since sometimes false-positive results occur between Toxoplasmosis and Rheumatoid arthritis. The test is done according to the available kit (RA-MA-050, REF, Egypt). The same latex test steps were performed for the rheumatoid factor test, except that instead of a drop of serum, 0.05 mL of serum was placed on the test slide and rotated gently for two minutes instead of four minutes as performed in the latex test.

Enzyme-Linked Immunosorbent Assay

In the current research, samples were examined to detect anti-*Toxoplasma* antibodies IgG and IgM by available kits (TOXM02 and TOXG01, Bioactiva Diagnostic a GmbH, Germany). First, specimens and reagents reached room temperature, and then microtitration strips were marked and used. Serum samples were diluted with 10 µL of serum to 1 mL of sample diluent; after that, 100 µL of diluted serum and ready to use control were pipetted to the appropriate wells and incubated for 30 min. at 37°C. Following the incubation, each well is aspirated and washed four times for 30 seconds with washing solution manually by performing a dispenser, then 100 µL of TOXO-HRP-conjugate is introduced to each well and incubated for 30 minutes at 37°C, aspirated, and washed again for four times. A 100 µL of TMB chromogen solution was introduced to each well and incubated for 15 minutes at room temperature to avoid exposure to direct sunlight. For the last step, 100 µL of stopping solution is added to each well, and the absorbance of the wavelength read at 450-620 nm within 30 minutes; the absorbance was taken from the ELISA reader and then changed to concentration (pg/mL) with the using of GraphPad Prism 9.0 program.

Leptin Sandwich ELISA

This test is used to evaluate serum levels of leptin hormone for three groups (diabetic patients with positive anti-toxoplasma antibody, diabetic patients with negative anti-toxoplasma antibody, and a control group) by the available kit (DRG Instruments GmbH, Germany). First, 15 µL of each standard, control, and sample were dispensed into appropriate wells. Then 100 µL of *Assay Buffer* was added to each well and carefully mixed for 10 seconds, then incubated for 120 minutes at room temperature. After that step, contents of the wells are smartly shaken out and washed three times. Following that, 100 µL of Antiserum is dispensed to each well and incubated for 30 minutes at room temperature; the contents of the well are shaken out and rewashed three times.

This time, 100 µL *Enzyme Complex* was added to each well, incubated for 30 minutes at room temperature, and rewashed three times. After that, 100 µL of Substrate solution was dispensed to each well and incubated for 15 minutes at room temperature. The last step was stopping the reaction by adding 50 µL of Stopping solution to each well and reading at 450±10nm with a microtiter plate reader (BioTek 800TS, USA).

Molecular detection of *B1* and *RE* genes via nested PCR

The blood samples of ELISA seropositive cases were used for molecular detection, and the DNA was extracted according to kit instructions (MDE101, BETA BAYERN genomic DNA extraction Kit, Germany). After the extraction, the concentration of the extracted DNA is detected by measuring absorbance at 260 nm using an ultra-violet spectrophotometer. Following the detection of *B1* and *RE* genes via nested polymerase chain reaction (PCR)

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by two pairs of oligonucleotide sequence-specific primers, forward and reverse primers, as shown in (Table 1), the primers were designed by using sequences from the GenBank database as a guide.[5]

The initial PCR amplifications were conducted with a final volume of 20 µL. The PCR technique required 10 µL master mix (AMPLIQON Taq 2× master mix DENMARK), 10 pmol/mL primer forward and reverse (each 0.7 µL), 2 µL template DNA (80-100 ng), and 6.6 µL free-nuclease water. Following the second PCR run, the PCR output was diluted to a concentration of 1:20. For the PCR experiment, a 10 µL master mix was prepared using 2 µL of dilution DNA, 1 µL of ten pmol/mL primer forward and reverse, and 6 µL of free-nuclease water (Table 1).

Table 1 Primer B1 and RE gene sequences using Nested-PCR technique for molecular detection of *T. gondii*.[5]

Nested PCR	Primer sequence	Amplicon size (bp)	Round	PCR program
B1 gene	F1: 5′-TCAAGCAGCGTATTGTCGAG R1: 5′-CCGCAGCGACTTCTATCTCT	287	First	95°C for 5 min. 95°C for 1 min. 57°C for 1 min. 72°C for 1 min. 30 cycles 72°C for 5 min-.
	F2: 5′-GGAACTGCATCCGTTCATGAG R2: 5′-TCTTTAAAGCGTTCGTGGTC	194	Second	95 °C for 5 min. 95 °C for 30 sec. 56 °C for 1 min-. 72 °C for 30 sec. 35 cycles 72 °C for 5 min.
RE gene	F1: 5′ CTGCAGGGAGGAAGACGAAAGTTG R1: 5′-CAGTGCATCTGGATTCCTCTCC	520	First	95° C for 5 min. 95 ° C for 60 sec. 60 ° C for 90 sec. 72°C for 60 sec. 35 cycles 72 °C for 5 min.
	F2: 5′ GTGCTTGGAGCCACAGAAGGGAC R2: 5′ GAGGAAAGCGTCGTCTCGTCTGG	243	Second	95° C for 5 min. 95°C for 60 sec. 58°C for 50 sec. 72°C for 60 sec. 30 cycles 72 °C for 5 min.

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Data analysis

The GraphPad Prism software (San Diego, CA, USA version 9.0) was performed for statistical analysis. The data are compared using Fisher's exact, Chi-square tests, unpaired t-test, t-test and KruskalWallis statistic, which also demonstrated the association between parameters and *T. gondii* infection. All tests considered statistically significant if the *P*-value was less than 0.05.

Results

A total of 201 patients with diabetes and 100 control individuals included in the present study (Table 2).Thereisa significant distribution of toxoplasmosis among diabetic and non-diabetic individuals (*P* = 0.001).

The serum of both diabetic and non-diabetic patient samples was investigated for anti-*T. gondii* antibodies by latex agglutination test found that among 201 diabetic cases, only 72 sera were seropositive (32.0%), and in 100 non-diabetic cases, 19 sera were seropositive for *T. gondii* (Table 3). The rheumatoid factor (RF) test was performed, and the results revealed that 8 cases among diabetic and 4 cases among non-diabetic individuals became false positives ignored eventually.

The dissemination and seropositivity of *T. gondii* among age groups and the relationship with each cat's contacts, soil contact, HbA1c, and BMI of diabetic patients revealed a significant difference in infection with *T. gondii* among age groups taken from this research (*P* = 0.001).

Table 2 Occurrence of anti-*Toxoplasma* antibodies among diabetic cases and control individuals.

Types of samples	Diabetic patient (n=201)	Control (n=100)	*P*-value
Seropositive *T. gondii*	64 (31.84%)	15 (15.0%)	0.001
Seronegative *T. gondii*	137 (68.16%)	85 (85.0%)	
Total	201 (100)	100 (100)	

Table 3 Detection of latex agglutination, ELISA IgG and IgM and rheumatoid factor tests among *Toxoplasma* seropositive individuals.

Parameters	Diabetes (n=201)	Control (n=100)	Total	*P*-value*
Latex	72	19	91	0.003
IgG	64	15	79	0.002
IgM	0	0	0	0.999
RF	8	4	12	0.999

*: Using Fisher's exact test.

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In Table 4, all variables had extraordinary significance with seropositivity to *Toxoplasma gondii*, especially with the apparent significant difference in age group (56-60), which are the most vulnerable age to seropositivity with *T. gondii.*

According to body mass index (BMI), the leptin hormones in the serum of diabetic patients and control individuals were shown significant differences, as mentioned in Table 5. The average of leptin hormone in the serum of diabetic patients was increased with rising BMI of patients; in normal-weight person, the average of leptin hormone was 7.024±1.419, while the mean of serum leptin hormone in overweight person was increased and became 13.176±1.068. When we were evaluated the concentration of serum leptin hormone in an obese person, they revealed elevation, and the mean became 24.837±1.935. On the other hand, the leptin hormone levels in the serum among control individuals also have significant differences (P = 0.002) with their BMI. The hormone levels increased with the increase in the BMI of individuals. Based on the consequences of the present study, leptin hormone levels are associated directly with BMI. Despite that there is a significant increase in leptin hormone in diabetic patients compared to the healthy group which shows normal rates of the hormone.

Table 4 Seropositivity with anti-*Toxoplasma* IgG antibodies among age groups with cat contact, soil contact, HbA1c, and BMI.

Age	IgG positive	Cat contact	Soil contact	*P*-value*	HbA1c	BMI	*P*-value**
26-30	1	3	1	0.169	8.8	29.28	0.001
31-35	2	8	0		8.335	35.223	
36-40	5	12	4		7.87	32.024	
41-45	6	15	5		9.029	31.559	
46-50	10	31	12		8.463	30.899	
51-55	10	4	10		10.15	29.956	
56-60	16	21	11		9.993	32.009	
61-65	7	23	12		8.5	28.266	
66-70	6	15	7		8.795	28.584	
71-75	0	2	0		7.675	27.11	

*: Using Chi-square test; **: Using unpaired T. test.

Table 5 Association among body max index (BMI) with leptin hormone among diabetes cases and control.

Individual cases (n.)	Leptin (Mean±SEM)			*P*-value
	18.5-24.99*	**25-30**	**>30**	
Diabetes case (201)	7.024±1.419	13.176±1.068	24.837±1.935	0.009
Control (100)	3.445±0.928	8.77±0.419	12.76±0.687	0.002

*: BMI of both diabetes cases and control.

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Leptin hormones have been associated with glucose levels in diabetic patients (24.768±3.434) when compared with control individuals (10.698±0.708) and have a direct connection with infection of toxoplasma (14.867±1.063), while the significant differences were not found among the diabetic patients with seropositive and seronegative anti *Toxoplasma* IgG antibodies with control individuals (*P* = 0.312) as clarified in the (Table 6).

According to our findings, the *B1* gene is more specific than the *RE* gene for molecular detection of *T. gondii*. Among 64 diabetic cases infected by *T. gondii*, 30 cases (46.88%) were positive for the *B1* gene, while only one case (1.56%) was positive for the RE gene (Table 7).

Table 6 The discrepancy of leptin hormone in both diabetic patients and seropositivity to anti *Toxoplasma* IgG antibodies with control cases.

Parameters (Mean±SEM)	**Diabetic cases with seropositivity to anti *Toxoplasma* IgG antibodies**			***P*-value***
	Diabetes (+) and Toxoplasma (+)	**Diabetes (+) and Toxoplasma (-)**	**Control**	
BMI	29.505±0.792	34.01±1.974	30.563±0.775	0.312
Leptin	14.867±1.063	24.768±3.434	10.698±0.708	0.001

*: Kruskal-Walli's statistic.

Table 7 Comparative outcomes of Nested-PCR methods focusing on the *B1* and *RE* genes on seropositivity to anti *Toxoplasma* IgG and IgM antibodies in diabetic patients.

Examine	**IgG+, IgM+**	**IgG-, IgM-**	**IgG-, IgM+**	**IgG+, IgM-**
B1-nested-PCR + (30)	0 (0.00)	0 (0.00)	0 (0.00)	30 (46.88)
B1-nested-PCR - (171)	0 (0.00)	137 (100)	0 (0.00)	34 (53.12)
RE-nested-PCR + (1)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.56)
RE-nested-PCR - (201)	0 (0.00)	137 (100)	0 (0.00)	63 (98.44)
Total	0	137 (100)	0	64 (100)

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Discussion

The occurrence of *T. gondii* among diabetic patients is high in Iraq, in the neighboring countries, and around the world, according to the studies that have been done on the same problem.[4, 6-8] Making an allowance for this and the significance of the complications and impairment of the cellular and humoral immunity resulting from diabetes, this experiment was intended to find out the relationship between *Toxoplasma* seropositivity and T2DM women that attended Layla Qassim Center for diabetes for the first time in Erbil city, north of Iraq.

The consequences of the current research revealed that 32.0% of cases were seropositive for anti-toxoplasma IgG antibodies among 201 diabetic patients with no any (0.0%) patients were seropositive for IgM antibodies and (15.0%) control subjects were seropositive for anti-Toxoplasma IgG with no positive subject (0.0%) for IgM from with the *P*-value of 0.0014. In diabetic individuals, the risk factor for *T. gondii* infection was approximately twice that of healthy controls. Furthermore, *T. gondii*-infected individuals might be at a greater risk of acquiring diabetes than uninfected persons.[6] These outcomes imply that individuals with Toxoplasmosis are more likely to develop diabetes than those without the infection.

The results of the current study were close to the studies of Yong-Xin and colleagues[4] and Nihad with Hamad[8], which showed 23% seropositive cases for IgG antibodies among 400 type two diabetic patients and 11.75% seropositive cases among 400 control groups. The results of the current study also were close to that done in Kirkuk province by Nihad and Hamad,[8] in which the results showed that 21% 0f cases were positive for IgG antibodies and 10% of the case were positive for IgM antibodies. In comparison, 11.11% of healthy patients were positive for IgG with no case for IgM.

In contrast, this study showed lower results in comparison with the investigations of Mollan and Ismail,[7] with Modrek and colleagues[9] showing higher results. In the study of Mollan and Ismail,[7] the outcomes revealed that 66.6% of the diabetic patients were found seropositive for IgG among 300 T2DM patients, while 33.4% among 150 of the seemingly control group were detected seropositive for the same antibody. In the study of Modrek and colleagues,[9] the results showed that among 205 diabetic patients, 36.6% were acute phase (IgG,IgM+) and 49.6% were chronic phase (IgM-, IgG+).The seroprevalence rate of *T. gondii* IgM and IgG antibodies was found to be higher in older women. This is comparable with the findings of our investigation as well as those of earlier research conducted in a variety of geographic locations and environmental conditions.[9-11] This could be an indication of high infection risk in early adolescents; additionally, older adults have a greater propensity to be subjected to a wide variety of risk factors for T. gondii infection during their lives than younger persons; for instance, this could be connected to the fact that older women participating heavily in the housework, particularly trying to deal with meats, preparing salads, cooking, and sanitizing and subsequently be more exposed to the risk factors of toxoplasmosis and other diseases. Because of this, a link between age and toxoplasmosis that was statistically significant was discovered. These findings aligned perfectly with the results of the Sarkari study.[12]

In order to find out which genes are more specific in the molecular detection of *T. gondii* that can be trusted, molecular detection for the most particular genes of human *T. gondii* is performed for *B1* and *RE* genes. The results of the Nested PCR that was performed for the detection of *B1* and *RE* genes show that the *B1* gene is in great abundance in comparison to the *RE* gene. The results indicate that out of 64 cases of *T. gondii*; thirty cases were positive for *B1* and only one case positive

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for *RE* genes. Our outcomes are comparable to the consequences of Cardona et al.,[13] Rahimi et al. ,[14] Wahab et al.,[15] and Mousavi et al..[5] They informed that the targeting of the *B1* gene was more competent than the *RE* genomic repeated element. They also resolved that, in the strains of the parasite, part or all of the 529 bp *RE* has been removed or absent in 4.8% of human *T. gondii*-positive samples tested. Our numbers were contradictory the results of Saglam et al.[16] and Fallahi et al.,[17] as they reveal that the *RE* gene may be the desired diagnostic target over the *B1* gene for the recognition of *T. gondii*.

Regarding the results of leptinhormone, a significant increase in the serum leptin of first group (T2DM) is observed in comparison with the second control group. At the same time, there was no significant difference between the serum leptin levels of diabetic seropositive for anti-toxoplasma antibodies, diabetic seronegative for anti-toxoplasma antibodies. It means that *T. gondii* infection has no significant effect on the elevation of serum leptin hormone. Instead of that diabetes and BMI have the role of increasing the hormone. Our results are similar to most of the studies of Hassan et al.,[18] Radia et al.,[19] and Anandhan et al.[20] that performed on the same issue.

Leptin amounts were increased proportionally with both BMI and body fat, is similar to the study of Facey et al.[21] These have been discovered that people who are obese are more likely to put on weight when they take the hormone leptin.

Conclusion

Toxoplasmosis is more common in those who have diabetes or are old. As a result, it is recommended that research on blood levels of antibodies towards toxoplasmosis in diabetic patients be conducted and continued regularly. Obesity, Toxoplasmosis and high body mass index results in elevation of leptin hormone that leads to diabetes. Molecular detection of the *B1* gene is more specific than the *RE* gene.

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Competing interests

The authors declare that they have no competing interests.

References

1. Khalili M, Mahami-Oskouei M, Shahbazi A, Safaiyan A, Mohammadzadeh-Gheshlaghi N and Mahami-Oskouei L. The correlation between serum levels of anti-*Toxoplasma gondii* antibodies and the risk of diabetes. Iran J Parasit 2018; 13(4):637–42.
2. Ozcelik S, Alim M and Ozpinar N. Detection of *Toxoplasma gondii* infection among diabetic patients in Turkey. Clin Epidem Glob Health 2020; 8(3):899–902. https://doi.org/10.1016/j.cegh.2020.02.020
3. Hemida MH, Shahat SR, Bayoumy AS and Mohammad KAH. Prevalence of toxoplasmosis among diabetic patients. Europ J Pharmaceut Med Res 2017; 4(11):137–40.
4. Li Y-X, Xin H, Zhang X-Y, Wei C-Y, Duan Y-H, Wang H-F, et al. *Toxoplasma gondii* infection in diabetes mellitus patients in China: seroprevalence, risk factors, and case-control studies. Bio Med Res Intern 2018; 2018:1–9. DOI: 10.1155/2018/4723739
5. Mousavi M, Saravani R, Modrek MJ, Shahrakipour M and Sekandarpour S. Detection of *Toxoplasma gondii* in diabetic patients using the nested PCR assay via RE and B1 genes. Jundishapur J Microbiol 2016; 9(2):1–6. DOI: 10.5812/jjm.29493
6. Helieh SO. Toxoplasmosis, pancreatitis, obesity and drug discovery. Panc Diso Therap 2014; 4(2):1–5.
7. Molan A-L and Ismail MH. Study the possible association between toxoplasmosis and diabetes mellitus in Iraq. World J Pharmaceut Sci 2017; 6:85–96.
8. Nihad SF and Hamad SS. Seroprevalence of Toxoplasma gondii infection in diabetic patients at Kirkuk province. IJSBAR 2017; 36:157–65.
9. Modrek MJ, Saravani R, Mousavi M, Khorashad AS and Piri M. Investigation of IgG and IgM antibodies against *Toxoplasma gondii* among diabetic patients. Int J Infect 2015; 2(3):1–5. DOI:10.17795/iji-27595
10. Han K, Shin D-W, Lee T-Y and Lee Y-H. Seroprevalence of *Toxoplasma gondii* infection and risk factors associated with seropositivity of pregnant women in Korea. J Parasit 2008; 94(4):963–5. DOI: 10.1645/GE-1435.1
11. Zemene E, Yewhalaw D, Abera S, Belay T, Samuel A and Zeynudin A. Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town,

Southwestern Ethiopia. BMC Infect Dis 2012; 12(1):1–6. DOI: 10.1186/1471-2334-12-337
12. Sarkari B, Shafiei R, Zare M, Sohrabpour S and Kasraian L. Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran. J Infect Devel Count 2014; 8(4):543–7. DOI: 10.3855/jidc.3831
13. Cardona N, Basto N, Parra B, Zea AF, Pardo CA, Bonelo A, et al. Detection of *Toxoplasma* DNA in the peripheral blood of HIV-positive patients with neuro-opportunistic infections by a real-time PCR assay. J Neuroinfect Dis 2011; 2(2011):1–6. DOI: 10.4303/jnp/N110402
14. Rahimi Esboei B, Kazemi B, Zarei M, Mohebali M, Keshavarz Valian H, Shojaee S, et al. Evaluation of RE and B1 genes as targets for detection of *Toxoplasma gondii* by nested PCR in blood samples of patients with ocular toxoplasmosis. Acta Parasitologica 2019; 64(2):384–389. DOI: 10.2478/s11686-019-00056-6
15. Wahab T, Edvinsson B, Palm D and Lindh J. Comparison of the AF146527 and B1 repeated elements, two real-time PCR targets used for detection of *Toxoplasma gondii*. J Clinic Microbiol 2010; 48(2):591–2. DOI: 10.1128/JCM.01113-09
16. Sağlam T, Düşen S, Mete E and Karaman Ü. Comparative Evaluation of Re 529-Bp Sequence and B1 Gene in the Detection of *Toxoplasma gondii* Through PCR in Water Samples of Denizli, Turkey. Acta Parasitologica 2022; 67(1):555–9. DOI: 10.1007/s11686-021-00494-1
17. Fallahi S, Kazemi B, Bandehpour M, Lasjerdi Z, Taghipour N, Zebardast N, et al. Comparison of the RE and B1 gene for detection of *Toxoplasma gondii* infection in children with cancer. Parasit Intern 2014; 63(1):37–41. DOI: 10.1016/j.parint.2013.08.005.
18. Hassan AJ, Mohammed NS, Shweash M and Hadeed HM. Impact of Anti-*Toxoplasma gondii* and adipose hormones with Insulin Resistant on obese aborted women. Internat J Pharmaceut Qual Assuran 2020; 10(1):114–8. DOI: 10.25258/ijddt.v10i1.25
19. Yahya R, Awad S, Hussein D, Hamed E, Raida S, Yahya, et al. Leptin and Leptin Receptor Polymorphism in Egyptian Diabetic Children with Latent Toxoplasmosis. Intern J Scie Eng Res 2019; 10:296–311.
20. Anandhan P. The Effect Of Exposure To *Toxoplasma gondii* Profilin On Leptin Level In Rattus Norvegicus Wistar Strain Rats Given Normal Diet And Hypercaloric Diet [Bacelore]: Universitas Brawijaya; 2018.
21. Facey A, Dilworth L and Irving R. A review of the leptin hormone and the association with obesity and diabetes mellitus. J of Diabet Metab 2017; 8(3):1–13. DOI: 10.4172/2155-6156.1000727