Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 3, 38-46

Molecular Detection of *B1* Gene from Blood Samples of Iraqi Diabetic Type II Patients Infected with Toxoplasmosis

[1]Sarah Ali Saeed, [2]Israa Kasim Al-Aubaidi

[1,2]Department of Biology, College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad, Baghdad, Iraq.

Received: June 6, 2024 / Accepted: July 4, 2024 / Published: November 3, 2025

Abstract: Many of warm-blooded birds and mammals, including human, are susceptible to infect with toxoplasmosis that caused by the ubiquitous intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*), which is present across the planet. The nested PCR technique has proven to have a good capacity and sensitivity for toxoplasmosis detection when compared to other PCR procedures. Furthermore, it has been demonstrated to be far more economical. Type II diabetes is primarily caused by a gradual decline in insulin production and insulin resistance. This study is objected to detect the gene *B1* of *T. gondii* in blood samples of Iraqi diabetic type II patients by using the molecular assay nested-PCR. One hundred-nine of blood samples from Iraqi type II diabetes patients and 80 blood samples from non-diabetic control volunteers were compared in the study. All of the samples were collected in March and June of 2022 from a private laboratory in Baghdad, Iraq. According to the findings of the diabetic diagnostic tests, the group of patients with diabetes had the highest mean of glycated haemoglobin 7.9. Moreover, 30/80 (15.87%) cases of non-diabetic control were sera-positive that had 32.7 ± 8.45 UI/mL for the anti-*Toxoplasma* IgG antibody in the CMIA assay, whereas 51/109 (26.98%) samples of the group of diabetic patients were sera-positive to toxoplasmosis with a titer of 34.95 ± 7.5 UI/mL for the same antibody in the same assay. While, all samples were sera-negative in CMIA for acute toxoplasmosis IgM antibody. Additionally, 11/25 (44%) of diabetic patients infected with toxoplasmosis were positive for gene *B1* detection in the nested PCR. As well as, 7/20 (35%) of toxoplasmosis asymptomatic considered control positive were also positive for the same detection. This indicate that the *B1* gene of *T. gondii* was detected by nested PCR technique in a small percentage of the studied samples.

Keywords: *Toxoplasma gondii, Diabetes mellitus II, HbA1Cand B1 gene.*

Corresponding Author: (Email: sara.Ali1102a@ihcoedu.uobaghdad.edu.iq**)**

Introduction

Within the obligate intracellular protozoa, *Toxoplasma gondii*(*T. gondii*), is one of the most prevalent parasites that infect animals with warm blood. Cats and felines family are the parasite's definitive hosts, with human acting as intermediary host. Eating raw or undercooked meat or consuming infectious oocysts from the environment are the main ways that human becomes infected postnatally. Several clinical disorders are brought on by this condition, the majority of patients are asymptomatic. On the other hand, it has the ability to vertically infect the fetus during pregnancy and result in a variety of clinical symptoms in the progeny (1-3). Toxoplasmosis infection had ability to alters behavior of chronic infection. It is a common zoonotic disease affecting one-third of the world population with

potentially serious health implications (4-6).

Diabetes mellitus is a metabolic disease that is defined by sustained hyperglycemia brought on by anomalies in the secretion, action, or both of insulin. It can lead to long-term harm, organ malfunction, and organ failure (7). The pancreas is unable to produce sufficient insulin to overcome this resistance resulting in insulin deficiency.Diabetes comes in two varieties. Type I (insulin dependent) and type II (insulin independent). Ninety-five % of instances of diabetes globally are of the second kind, whose etiology is principally linked to obesity and insufficient insulin utilization by the body (8-10).

The diagnosis of *T. gondii* can be determined by variable immunological and molecular assays. Numerous serological methods, such as the haemagglutination test, the latex agglutination test, the indirect fluorescent antibody (IFA), and the enzyme-linked immunosorbent assay (ELISA), have been developed and are used to detect anti-*Toxoplasma* antibodies (11,12). For molecular diagnosis, polymerase chain reaction (PCR)-based techniques are therefore extremely valuable. One of the most important assays in epidemiological investigations is the nested-PCR assay, which has demonstrated consistent sensitivity for detecting *T. gondii* infection (13-15). Many researches have demonstrated that the *B1* gene is extremely unique to *T. gondii*, that it is highly conserved across all strains examined thus far, and that the sensitivity of the 35-fold repeat *B1* is significantly higher than that of the ribosomal gene (16,17).

In that diabetes affects innate immunity and increases vulnerability to opportunistic infections, the condition is strikingly comparable to immunodeficiency disorders such as HIV infection. Prior research has indicated that diabetic people are more susceptible to opportunistic infections like *T. gondii*, and that prompt toxoplasmosis diagnosis and detection in at-risk individuals is essential to stop the spread of the disease (18 – 21). Diabetes mellitus makes the host more sympathetic and may even lead to the development of another illness. Those with diabetes mellitus may be more susceptible to many infections and empathy. The Apicomplexan parasite *T. gondii* has been suggested as a likely cause of diabetes in a number of cases. Meanwhile, a meta-analysis of studies on the relationship between chronic toxoplasmosis and diabetes mellitus suggested that chronic toxoplasmosis may be a risk factor for type II diabetes (22,23).

This study is objected to detect the gene *B1* of *T. gondii* in blood samples of Iraqi diabetic type II patients by the molecular technique nested-PCR.

Materials and Methods

Subjects and Samples

One hundred-nine type II diabetes cases were enrolled in the periodfrom March to June 2022. However, specialized at a private laboratory in Baghdad, Iraq, recognized from 80 control samples that were taken from non-diabetic outpatient clinics. 15 to 85 years old was the range of their ages. A total of 10 ml of venous blood were drawn from each case. The blood was then separated into 5 ml and centrifuged for 10 min at 3000 rounds per min (rpm) to extract the serum for *T. gondii* antibodies diagnosis. While, the other 5 mlwere used for glycated haemoglobin meauring and for molecular detection of gene *B1*.

Diabetes Mellitus Diagnosis

The Hemoglobin A1C Architect kit (Abbott GmbH, Germany) is used to measure the glycated hemoglobin level in accordance with manufacturer's instructions.

*T. gondii*Diagnosis

Serological Diagnosis

The mean titer of anti-*Toxoplasma* IgM/IgG antibodies were detected using the chemiluminescent microparticles immunoassay (CMIA) Architect Toxo IgM/G kit (Abbott GmbH, Germany) in accordance with the manufacturer's instructions.

Molecular Diagnosis via Nested PCR Technique

The following groups were certified for molecular detection after serological test CMIA performed: a group included 25 blood samples of diabetic type II patients infected with toxoplasmosis, 20 blood samples from each group of diabetic type II patients only, control positive which include toxoplasmosis asymptomatic individuals, and control negative that included healthy individuals respectively.

Following the manufacturer's instructions, firstly, DNA was extracted from the blood samples using the gSYNCTM DNA extraction kit fast technique (Geneaid, Taiwan). Then, in accordance with the manufacturer's instructions, the *T. gondii* genomic DNA *B1* gene (AccuPower PCR PreMix, Bioneer Corporation, Korea) was used for nested PCR technique. In the first run of PCR reaction, a 287 bp fragment was amplified by using the first forward and reverse primer sets (table 1). For each 1st primer reaction, 1µl of dissolved forward or reverse primer and 5µl of extracted DNA are combined to generate the MasterMix tube. Next, 13 µl of distilled water (D.W.) was added to the MasterMix tubes until their total volume reached 20 µl. Once thoroughly combined by vortexing, the reaction mixture was put into a PCR thermocycler for the preprogrammed PCR reaction under amplification conditions mentioned in the below table (table 1). The amplified fragments were electrophoresed in 2% agarose gel and stained with ethidium bromide while being exposed to ultraviolet light. In the second PCR reaction, a194 bp fragment was amplified by using the second forward and reverse primer sets (table 1). Which for each 2nd primer reaction, 1µl of dissolved forward or reverse primer and 5µl of the first run PCR result are combined to generate the MasterMix tube. Next, 13 µl of D.W. was added to the MasterMix tubes until their total volume reached 20 µl. The reaction mix was placed into a PCR thermocycler to start the preprogrammed PCR reaction after it had been well mixed using an ultracentrifuge (exispin vortex) under ultraviolet illumination and amplification conditions that mentioned in the below table (table 1). The amplified fragments underwent ethidium bromide staining and were electrophoresed in 2% agarose gel.

Table (1): Primer squencing of *B1* gene of *T. gondii* utilizing nested PCR assay.

Primers Sequences (5'— 3').	Amplicon size bp	PCR round	PCR amplification conditions
F1: TCA-AGC-AGC-GTA-TTG-TCG-AG	287	1st	Initial denaturation 95 C◦ for 5 min
			Denaturation 95 C◦ for 30 sec.
			Annealing 55 C◦ for 30 sec.
R1: CCG-CAG-CGA-CTT-CTA-TCT-CT			Extension 72C◦ for 1 min.
			Final extension 72 C◦ for 5 min.
F2:GGA-ACT-GCA-TCC-GTT-CAT-GAG	194	2nd	Initial denaturation 95 C◦ for 5 min
			Denaturation 95 C◦ for 30 sec.
			Annealing 55 C◦ for 30 sec.
R2: TCT-TTA-AAG-CGT-TCG-TGG-TC			Extension 72 C◦ for 1 min.
			Final extension 72 C◦ for 5 min.

Statistical Analysis

The Statistical Analysis System-SAS (24) application was used to examine the effects of several research parameter components. The examination of Variation (ANOVA) Least Significant Difference (LSD) test was used to provide a thorough examination of the mean titers. The Chisquare test wasutilized in order to compare the probability (0.05 and 0.01) in a meaningful way.

Results and Discussion

Studies on diabetes patients have already highlighted some of the immunological abnormalities associated with the disease, such as humoral disorders, decreased T-cell response, decreased neutrophil function, and dysregulation of innate immunity. As a result, opportunistic infections like *T. gondii* infection may become more common in these people. As a result, early identification of *T. gondii* in individuals who are at risk can stop the disease's progression and its aftereffects (25 – 27).

Figure (1) demonstrated that the group of diabetic type II patients have the mean of glycated haemoglobin 7.9 while the group of non diabetic individuals have 4.98. According to the reference range of HbA1C, the last group have normal mean of HbA1C in contract to the 1st group.

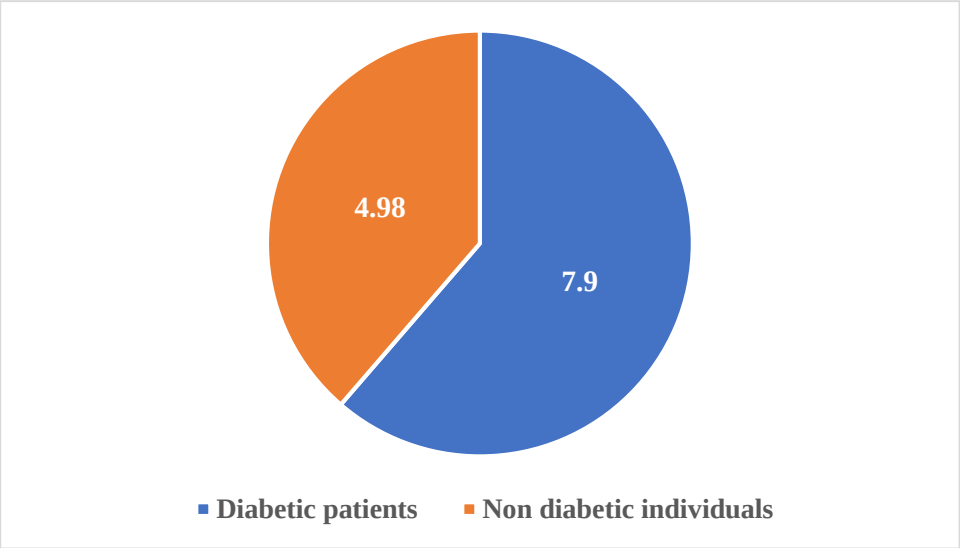

Figure (1): Mean values of glycated haemoglobin HbA1C in the studied groups.

The results of the study are in line with those of Waheed *et al.* (28) which showed that, the group of Iraqi diabetic type 2 patients (30/50) had the highest levels of glucose (7.993±0.646) in comparison to the Iraqi non-diabetic group (20/50) that had (4.68±0.484) of glucose in the glycated haemoglobin test. The recommended test is HbA1C since it is more useful over an extended period of time and more time-varying. The HbAlc test has become one of the best tools for confirming that diabetes is under control in recent times. Their benchmarks are unchanged from the previous few years Saudek*et al.*(29), Taher *et al.* (30) and Hammad *et al.*(31).

However, the study cohorts were divided into four groups according to

the mean anti-*Toxoplasma* IgG titer: diabetic patients who also had toxoplasmosis, diabetic patients only, non-diabetic toxoplasmosis asymptomatic people as a positive control, and healthy people as a negative control. Moreover, significant differences in the negative response to anti-*Toxoplasma* IgM were found in all samples from diabetes patients when compared to controls without the disease.

The findings of serological test are displayed in figure (2), the mean titers of anti-*Toxoplasma* IgG antibodies varied greatly; the group of patients with diabetes (51/109) had the highest mean titer, at 34.95 ± 7.5 UI/mL, while the group of non-diabetic(30/80)(asymptomatic) people came in second, at 32.7 ± 8.45 UI/mL.

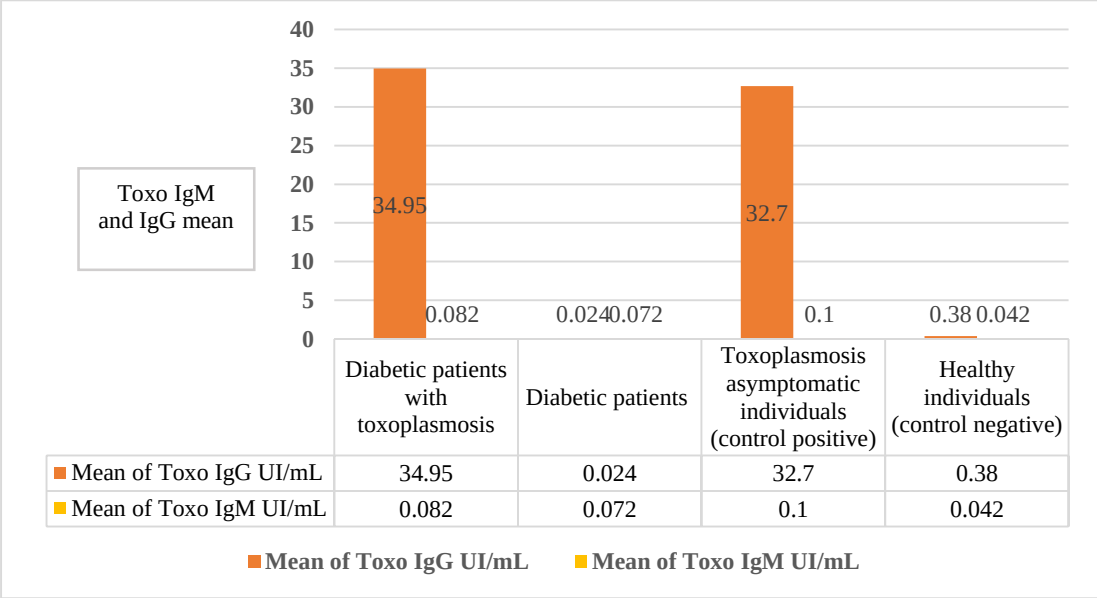

Figure (2): Mean titers of anti-*Toxoplasma* IgG and IgM antibodies via CMIAin thestudiedgroups.

Tenter *et al*. (32) state that the reason for the elevated IgG antibody levels is that the immune humoral system response plays a vital role in limiting the parasite's ability to spread. However, it should be emphasized that anti-*Toxoplasma* IgG antibodies are often found 1-2 weeks after infection, peak in concentration in the next 6-8 weeks, and then gradually drop over the course of a year or two, with low levels perhaps lasting a lifetime. Moreover, because it may substitute large molecules in the absence of antibody makers, the half-life of the IgG antibody is longer than that of the IgM antibody (33).The current results are similar to those of Al-Khafajii (34), who revealed that diabetic patients only accounted for 22/45 (48.88%) of the seropositive cases, whereas 28/55 (50.9%) of the non-diabetic control group tested positive for the same antibody. Moreover, the most recent results were consistent with those of Al-Aubaidi *et al.* (35) who showed that 47/100 diabetic patients were seropositive for *Toxoplasma* IgG antibody, whereas healthy controls showed highly significant variations in seronegative status for the same antibody in the same test.

The aforementioned information suggests that diabetes patients were more susceptible than healthy

individuals to infect with toxoplasmosis. As a result, toxoplasmosis patients may be more likely to acquire diabetes than those who are not affected. Diagnosis and treatment of toxoplasmosis have advanced dramatically with the use ofPCRto directly observe the parasite in biological samples (36,37).

In diabetes type II patients infected with toxoplasmosis, as shown in table (2), the results of the nested PCR technique demonstrated that 11/25 (44%) were positive for the detection of the gene *B1* in contrast 14/25 (56%) of the same group were negative. In addition, 7/20 (35%) of the group of control positive for toxoplasmosis who did not exhibit any symptoms tested positive for gene *B1*. On the other hand, 13/20 (65%) tested negative for the same detection. Additionally, there was no *B1* gene found in the groups of healthy people and diabetic patients.

Table (2): Gene *B1* detection via using nested PCR technique.

Groups	Samples No.	+ ve No. (%)	- ve No. (%)	P-value
Diabetic patients with toxoplasmosis	25	11 (44.00%)	14 (56.00%)	0.548 NS
Diabetic patients	20	0 (0.00%)	20 (100%)	0.0001 **
Toxoplasmosis asymptomatic (control positive)	20	7 (35.00%)	13 (65.00%)	0.179 NS
Healthy individuals (control negative)	20	0 (0.00%)	20 (100%)	0.0001 **
P-value		0.041 *	0.0035 **	0.0392 *
Significant: * (P≤0.05), Highly Significant: ** (P≤0.01).				

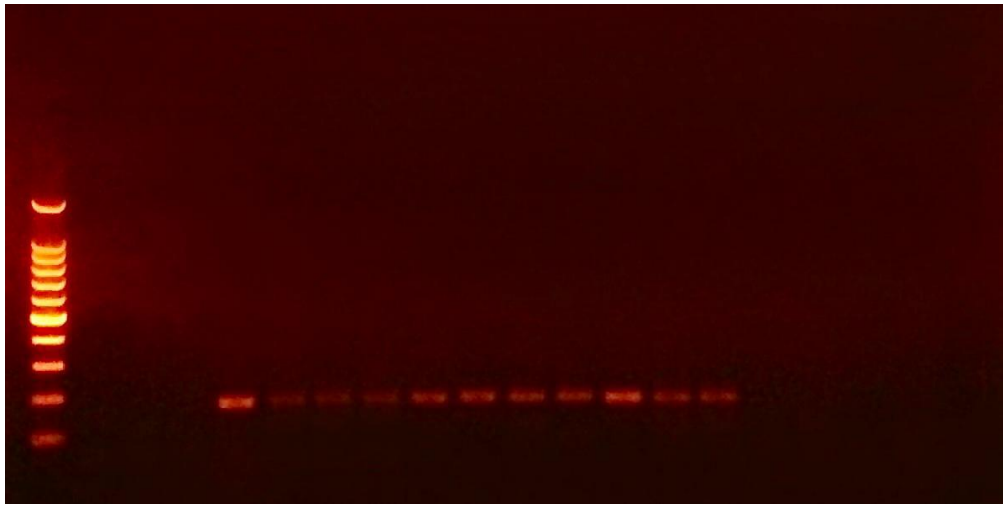

Figure (3): Gel electrophoresis bands of gene *B1* detection in the group of diabetic patients infected with toxoplasmosis via nested PCR technique.

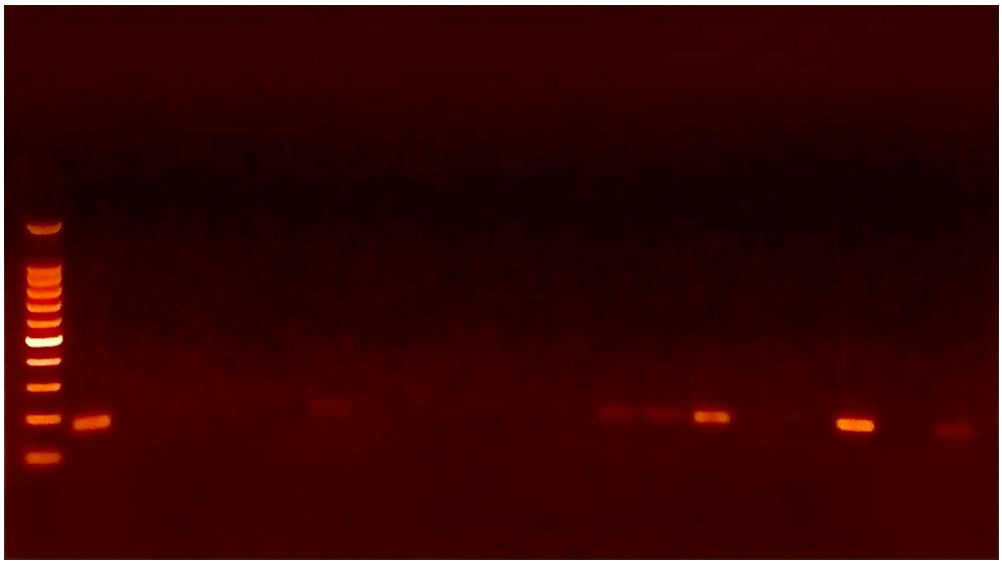

Figure (4): Detection of gene *B1* in the group of toxoplasmosis asymptomatic (control positive) in the nested PCR technique.

The above results were similar to those of Mala and Bakre (38), who showed that in the nested PCR, gene *B1* was detected in 30/64 (46.88%) of diabetic type II patients infected with chronic toxoplasmosis, whereas no detection were found in the group of healthy individuals. The findings of this investigation, however, is dissmiliar to those of Mousavi *et al.* (39), who demonstrated that 41/60 (68.3%) of healthy individuals and 51/72 (69.8%) of diabetic patients with chronic toxoplasmosis infection had gene *B1* detection in the same assay. Moreover, the current results disagree to the results of Saki *et al.* (40) that demonstrated 36/167 (21.55%) of diabetic type II patients which have sero-positive to *T. gondii* infection have gene *B1* detection as well as 5/38 (13.15%) of healthy individuals were positive detection to the same gene in the same assay.

According to the previous and current results, there are two probable explanations: the first is that the patient's blood had an acute infection that was probably clinically significant based on the clearance period for *Toxoplasma* DNA (5.5–13) weeks (41). On the other hand, accurate techniques like PCR are the only ways to identify the presence of a tiny number of parasites that may have been liberated from tissue into the blood at a subclinical level (42,43).

Conclusion

In conclusion, the *B1* gene of *T. gondii* was detected by the nested PCR technique in a small percentage of the studied samples.

References

1. Hade, B. F. (2014). Molecular detection to *Toxoplasma gondii* in serum sheep samples. Iraqi Journal of Biotechnology, 13(2): 58 – 65.
2. Abood, M.A. and Saheb, E.J. (2023). The variable levels of IL-6 and nitric oxide in hemodialysis patients upon exposure to toxoplasmosis. Iraqi Journal of Biotechnology, 21(2): 161 – 171.
3. Abdullah, R.S. and Al-Aubaidi, I.K. (2021). Seroprevalence of toxoplasmosis in Iraqi patients with liver diseases. International Journal of Pharmaceutical Quality Assurance and Pharmaceutical Analysis, 12(3): 173 – 178.
4. Shaker, M.M.; Rahman, S.A.H. and AL-Abbasi, H. M. (2019). Assessment level of TSH, T4, T3 and testosterone in Iraqi depressed women with chronic toxoplasmosis infection. Biochemical and Cellular Archives, 19(1): 2721 – 2724.
5. Alkubaisi, S.A. and Al-Zubaidy, I.A. (2023). Toxoplasmosis in females from Al-Anbar,

Iraq. Journal of the Faculty of Medicine Baghdad, 65(1): 74 – 78.
6. Halonen, S.K. and Weiss, L.M. (2013). Toxoplasmosis. Handbook of Clinical Neurology, 114 (1):125 – 145.
7. Al-Khdhairi, Z. M. A. and Ali, B. H. (2018). Comparison study of the effect of erlotinib as a tyrosine kinase inhibitor on electrolyte levels in type 2 diabetic and diabetic nephropathy. Ibn Al-Haitham Journal for Pure & Applied Science, 31(3): 63 – 69.
8. Abd, H.A. and Al-Jumaili, E.F. (2022). The relationship between some biochemical parameters and type 2 diabetes mellitus among Iraqi patients. Iraqi Journal of Biotechnology, 21(2): 268 – 275.
9. World Health Organization. (2013). Global strategy on diet, physical activity and health: 1-18.
10. Asgari, Q.; Motazedian, M.H.; Khazanchin, A.; Mehrabani, D. and Naderi Shahabadi S. (2021). High prevalence of *Toxoplasma gondii* infection in type I diabetic patients. Journal of Parasiitology Research, 2021(1): 1 – 6.
11. AL-Mossawei, M.T.; AL-Mossawei, H.M. and AL-Dujaily, K.Y. (2016). Serological study of toxoplasmosis spread among unmarried female university students using LAT, ELISA and IgG avidity. Baghdad Science Journal, 13(4): 714 – 720.
12. Casartelli-Alves, L.; Boechat, V.C.; Macedo-Couto, R.; Ferreira, L.C.; Nicolau, J.L.; Neves, L.B.;*et al.*, (2014). Sensitivity and specificity of serological tests, histopathology and immunohistochemistry for detection of *Toxoplasma gondii* infection in domestic chickens. Veterinary Parasitology204(3–4): 346–351.
13. Alanad, A.I.; Abdullah, B. H. (2023). Molecular and serological detection of *Toxoplasma gondii* in three species of wild birds of Babylon province, middle Iraq. Iraqi Journal of Veterinary Sciences, 37(1): 39 – 44.
14. Khademvatan, S.; Rahim, F.; Tavalla, M.; Abdizadeh, R. and Hashemitabar, M. (2013). PCR-based molecular characterization of *Toxocara* spp. using feces of stray cats: a study from Southwest Iran. PloS One8(6): 1 – 6.
15. Hosseini, S.A.; Sharif, M.; Sarvi, Sh.; Abediankenari, S.; Hashemi-Soteh, M.B.; Amouei, A. *et al.*, (2020). Genetic characterization of *Toxoplasma gondii* iin iranian HIV positive patients using multilocus nested-PCR-RFLP method. Parasitology, 147(1): 322 – 328.
16. Catchpole, A.; Zabriskie, B.N.; Bassett, P.; Embley, B.; White, D.; Gale, Sh. D. and Hedges, D. (2023). Association between *Toxoplasma gondii* infection and type 1 diabetes mellitus: A systematic review and meta-analysis. Inetrnational Journal of Enviromental Research and Public Health, 20(5): 1 – 10.
17. Jones, C.D.; Okhravi, N.; Adamson, P.; Tasker, S. and Lightman, S. (2000). Comparison of PCR detection methods for *B1*, P30, and 18S rDNA genes of *T. gondii* inaqueous humor. Investigative Ophthalmology & Visual Science, 41(3):634 – 644.
18. Shirbazou, S.; Delpisheh, A.; Mokhetari, R.;and Tavakoli, G. (2013). Serologic detection of anti-*Toxoplasma gondii* infection in diabetic patients. Iranian Red Crescent Medical Journal,15(8): 701–703.
19. Gokce, C.; Yazar, S.; Bayram, F.; Gundogan, K.; Yaman, O. and Sahin, I. (2008). Anti-*Toxoplasma gondii* antibodies in type 2 diabetes. TheNational Medical Journal of India,21(1): 51 pp.
20. Gokce, C.; Yazar, S.; Bayram, F. and Gundogan, K. (2008). *Toxoplasma gondii* antibodies in type 1 diabetes mellitus. Turkiye Klinikleri Journal of Medical Sciences,28(5): 619–622.
21. Majidiani, H.; Dalvand, S.; Daryani, A.; Galvan-Ramirez, M.L. and Foroutan-Rad, M. (2016). Is chronic toxoplasmosis a risk factor for diabetes mellitus? A systematic review and meta-analysis of case-control studies. Brazilian Journal of Infectious Diseases20(6): 605–609.
22. Hemida, M.H.; Shahat, S.R.; Bayoumy, A.S. and Mohammad, K.A.H. (2017). Prevalence of toxoplasmosis among diabetic patients. Europian Journal of Pharmaceutical and Medical Research, 4(11):137– 140.
23. Li, Y.X.; Xin, H.; Zhang, X.Y.; Wei, C.Y.; Duan, Y.H.; Wang, H.F. and Niu, H.T. (2018). *Toxoplasma gondii* infection in diabetes mellitus patients in China: Seroprevalence, risk factors, and case-Control studies. Biomed Research International, 2018(1). 1 – 9.
24. SAS. (2018**).** Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Institute Incorporation Cary. N.C. USA.
25. Nakashima, F.; Pardo, V.S.; Miola, M.P.; Murata, F.H.A.; Paduan, N.; Lonngo, S.M.; De Mattos, C.C.B.; Pereira-Chioccola, V.L.;

Ricci, O. and De Mattos, L.C. (2019). Serum IgG anti-*Toxoplasma gondii* antibody concentrations do not correlate nested PCR results in blood donors. Frontiers in Cellular and Infection Microbiology, 9(461): 1 – 6.

26. Abbaszadeh, S.; Teimouri, A.; Mahmoudi, M.R.; Roushan, Z.A.; Hajipour, N.; Majidii-Shad, B. and Sharifdini, M. (2022). Molecular detection of *Toxoplasma gondii* in chicken hearts from markets and retail stores in northern Iraq. Food and Waterborne Parasitology, 27(1): 26 – 32.
27. Ekawasti, F.; Azmi, Z.; Desem, M.I.; Subekti, D.T.; Nugraha, A.B.; Sa'diah, S. and Cahyaningsih, U. (2023). Evaluation of *B1* gene to detect *Toxoplasma gondii*: comparison of three sets of nested PCR primer. Journal of Kedokteran Hewan, 17(2): 62 – 67.
28. Waheed, H. J.; Khalil, M. and Fawzi, Sh. (2015). Evaluation of monocytes chemoattractant protein-1 (MCP-1) in type 2 diabetes mellitus. International Journal of Scientific Engineering and Research, 6(5): 791 – 797.
29. Saudek, C.D.; Herman, W.H.; Sacks, D.B.; Bergenstal, R.M.; Edelman, D. and Davidson, M.B. (2008). A new look at screening and diagnosing diabetes mellitus. Journal of Clinical Endocrinology and Metabolism., 93(7): 2447 – 2453.
30. Taher, M.A.; Mostafa, M.M. and Mahmood, A.S. (2011). Measurements of HbA1c for patients with diabetes mellitus and foot ulceration. Iraqi Journal of Pharmaceutical Sciences, 20(1): 19 – 24.
31. Hammad, I.K.; Abed, B.A. and Rashid, N.F. (2012). Glycated haemoglobin as a dual biomarker association between HbA1c and dyslipidemia in type 2 diabetic patients. Iraqi JournalofFacultyof Medicine Baghdad, 54(1): 88 – 92.
32. Tenter, A. M.; Heckeroth, A. R. and Weiss, L. M. (2000).*Toxoplasma gondii* : from animals to human. International Journalof Parasitology, 30(1): 1217 – 1258.
33. Al-Quraishi, M. A. and Jawad, Z. N. (2020). Study of biochemical parameters (HbA1C, C-Peptide, Fibrinogen) with toxoplasmosis in women. Biochemical and Cellular Archives 20(1): 2121 – 2128.
34. Al-Khafajii, Gh. S.; Al-Warid, H. S. and Al-Abuddi, F. A. (2021). The association between *Toxoplasma gondii* seropositive status and diabetes mellitus in obese and non-obese subjects in Baghdad. Iraqi Journal of Sciences, 62(6): 1793 – 1803.
35. Al-Aubaidi, I.K.; Saeed, S.A. and Jaaffar, A.I. (2020). Blood lymphocytes detection in Iraqi diabetic type 2 patients infected with chronic toxoplasmosis by using flow cytometry *Indian* Journal of Medical Toxicology and Forensic Medicine., 14(4): 2298 – 2303.
36. Fazel, R.; Rezanezhad, H.; Solhjoo, K.; Kalantari, M; Erfanian, S.; Armand, B. and Jahromi, M.E. (2021). PCR-based detection of *Toxoplasma gondii* from cattle in southern Iran. Comapartive Immunology Microbiology and infectious Diseases, 77(1): 1 – 7.
37. Ji, M.; Cho, H.; Park, Y.; Jang, D.; Park, J. and Choi, K. (2023). Molecular detection of *Toxoplasma gondii* in blood samples of domestic livestock in the republic of Korea. Pathogens, 12(4): 1 – 9.
38. Mala, Sh. F. and Bakre, H. M. (2023). Association of seropositivity of toxoplasmosis and type 2 diabetes among adult females in Erbil city. Zanco Journal of Medicine Sciences, 27(3): 234 – 243.
39. Mousavi, M.; Saravani, R.; Modrek, M. J.; Shahrakipour, M. and Sekandarpour, S. (2016). Detection of *Toxoplasma gondii* in diabetic patients using the nested PCR assay via RE and *B1* Genes. Jundishapur Journal of Microbiology, 9(2): 1 – 6.
40. Saki, J.; Khodkar, I.; Shadnoosh, F.; Safi, M.; Ghadiri, A. and Shafieenia, S. (2022). Study of toxoplasmosis in type 2 diabetic patients using ELISA and *B1* nested-PCR methods. Annals of Parasitology, 68(2):367 – 373.
41. Alizadeh-Sarabi, Z.; Pasandideh, Z.; Shokrani, H. and Dezfoulian, O. (2020). Bioassay-based detection of *Toxoplasma gondii* in free-range chickenns from Khoraamabad, Iran: comparison of direct microscopy and semi-nested PCR. Molecular Biology Report, 47(1): 4969 – 4974.
42. Aziz Anah, S. and Aziz Anah, S. (2023). New Recording of *Toxoplasma gondii* in Wild *Tortoise Testudo* graeca using nested PCR method. Archives of Razi Institute, 78(3): 1029 – 1034.
43. Khanaliha, K.; Bokharaei-Salim, F.; Hedayatfar, A.; Esteghamatu, A.; Alemzadeh, S.A.; Asgari, Q.; Garshasbi, S. and Salemi, B. (2021). Comparison of real-time PCR and nested PCR for toxoplasmosis diagnosis in toxoplasmic retinochoroiditis patients. BMC Infectious Diseases, 21(1): 1– 7.