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RESEARCH

The Molecular Detection of *Toxoplasma Gondii* Infection and its Impact on Leptin and Ferritin Levels in Diabetic Females

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■ Abstract

Background and Objective: Toxoplasma gondii is a widely distributed parasite infection that presents an increased risk to immunocompromised persons, particularly those with diabetes mellitus. Recent research indicates a possible link between chronic Toxoplasmosis and metabolic abnormalities in individuals with diabetes. This study sought to ascertain the incidence of Toxoplasma gondii in diabetic patients and healthy controls, evaluate serum levels of ferritin and leptin, and identify the B1 gene using real-time polymerase chain reaction. **Material and Method:** The research was carried out at General Kirkuk Hospital in Kirkuk, Iraq. A total of 180 blood samples were obtained, comprising 130 from individuals with type 2 diabetes mellitus and 50 from healthy controls, to assess the prevalence of anti-Toxoplasmosis antibodies IgM and IgG serologically using Cobas 4 e111, as well as to detect the B1 gene molecularly via real-time polymerase chain reaction. Furthermore, the detection of glycated haemoglobin, serum leptin, and ferritin is included. **Result:** The overall seropositivity for anti-Toxoplasmosis IgM and IgG was 37.80% among both diabetes patients and healthy controls, however only 13.1% tested positive for the molecular detection of the Toxoplasma gondii B1 gene. In diabetes individuals, HbA1c, serum leptin, and ferritin levels were raised compared to the healthy control group, with significant p-values for these biochemical markers being less than 0.05. **Conclusion:** It is concluded that Toxoplasma gondii infection is more prevalent in diabetes patients than in healthy individuals. Increased levels of leptin and ferritin may correlate with Toxoplasma gondii infection and diabetes. The B1 gene serves as a sensitive marker for the molecular identification of Toxoplasma gondii, especially in immunocompromised patients.

Keywords: *Toxoplasma Gondii*, T2DM, RT-PCR, *B1 Gene*, HbA1c, Serum Leptin and Ferritin.

1. Introduction

Toxoplasma gondii (T. gondii) is an obligate intracellular parasite known to infect around one-third of the global population. The life cycle of T. gondii comprises both a sexual stage, which occurs exclusively in its definitive hosts, namely felines, and an asexual stage, which develops in both definitive hosts and a diverse array of intermediate hosts, including humans and other warm-blooded species. Humans can contract Toxoplasmosis by ingesting food or vegetables contaminated with oocysts, which represent the infectious stage of the parasite. The virus may also be contracted through exposure to contaminated soil or direct contact with feline faeces [1]. In a healthy individual, Toxoplasmosis infection is frequently asymptomatic or self-limiting, typically without resulting in significant complications. Toxoplasmosis can result in severe consequences and may be deadly in immunocompromised individuals. An immunocompromised patient is defined as an individual with a weakened or impaired immune system, rendering them more susceptible to various infections. This category includes individuals with human immunodeficiency virus (HIV/AIDS), cancer patients, organ transplant recipients, those undergoing haemodialysis, and individuals with diabetes mellitus (DM). Diabetes Mellitus is a metabolic illness characterised by persistently increased blood glucose levels over an extended duration. Among the kinds of diabetes mellitus (type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus (GDM)), T2DM constitutes approximately 90% of all cases, primarily characterised by the body's ineffectiveness in responding to insulin. Diabetes mellitus patients are regarded as immunocompromised, which heightens their susceptibility to opportunistic infections from various pathogens, including viruses, bacteria, and parasites such as T. gondii, due to their often diminished quantities and compromised functionality of essential

immune cells, including neutrophils, monocytes, and macrophages. Furthermore, numerous investigations have emphasised a substantial correlation between Toxoplasmosis and the onset of both types of diabetes mellitus [2]. Once T. gondii infection is established, the parasite possesses the capability to enter and proliferate within any nucleated cell, remaining in a latent form within the host for life. This enduring infection prompts the secretion of inflammatory mediators via both innate and adaptive immunological responses, leading to immune activation that fosters chronic inflammation in various bodily tissues. In diabetes mellitus patients, infection with T. gondii stimulates cytokine pathways that subsequently create low-grade chronic inflammation, resulting in insulin resistance and hyperglycemia. Leptin is a hormone that regulates human hunger and fullness, hence influencing insulin levels in the blood. Consequently, the measurement of leptin levels is regarded as a crucial inflammatory marker in the diagnosis of diabetes mellitus and explores the correlation between T. gondii and diabetes mellitus with respect to inflammatory biomarkers [3]. In the setting of Toxoplasmosis, infection with T. gondii has been demonstrated to augment leptin production by contact with its unique cell surface receptor. Consequently, it participates in regulating the inflammatory response by enhancing macrophage activity and facilitating cytokine release [4]. Ferritin is regarded as the principal protein responsible for iron storage in nearly all body cells [5]. Ferritin plays a crucial role in defending against infectious agents by facilitating immune system development and influencing pathogen virulence [6]. Infection with T. gondii can affect the ferritin levels in affected individuals. In immunocompromised patients, including those with diabetes mellitus, infection with T. gondii, particularly the reactivation of latent infection, induces a systemic inflammatory response that results in elevated blood ferritin levels, which are regarded as indicators of severe disease progression and immunological dysfunction. Various methodologies exist for the identification and diagnosis of T. gondii, encompassing serological assays, bioassays, microscopic analysis, conventional polymerase chain reaction (PCR), nested PCR, and real-time PCR (RT-PCR) [7]. The fundamental objective of serological testing is the identification of antibodies (Abs). This approach may yield false positive results, primarily due to antibodies that cross-react with the microbial antigens utilised in the assay or due to interfering chemicals that interact with assay components. This study aimed to ascertain the prevalence of T. gondii both serologically and molecularly in women with type 2 diabetes mellitus (T2DM) and to evaluate the serum concentrations of ferritin and leptin in diabetic women infected with T. gondii, in order to investigate potential associations between T. gondii infection and these parameters.

2. Material and Method

2.1. Study Population

This research was conducted in Kirkuk General Hospital in Kirkuk, Iraq. A total of 180 females, comprising 130 with Type 2 Diabetes Mellitus (T2DM) and 50 healthy controls (HC), aged between 15 and 75 years, were studied from September 2024 to March 17, 2025.

2.2. Sample Collection

Approximately 5 mL of venous blood was extracted from the study groups (both T2DM and HC) using a sterile syringe. Three millilitres of blood sample were placed into sterile gel tubes and allowed to stand at room temperature for 20 to 30 minutes to facilitate clotting, followed by centrifugation at 3000 rpm for 5 to 10 minutes. The serum was thereafter placed in Eppendorf tubes and stored in a deep freezer at -20°C until use. Two millilitres of collected blood were placed in EDTA tubes and transported to the laboratory for HbA1c analysis, while the remainder was stored in a deep freezer at -20°C for subsequent DNA extraction for use in RT-PCR detection methods (see Table 2).

2.3. Enzyme- linked Immunosorbent Assay (ELISA)

This test was conducted to quantify leptin hormone levels using the sandwich ELISA method using the Quick Step Human Leptin ELISA Kit, following the manufacturer's protocol.

2.4. Cobas e 411 and Cobas c 111 Immunoassay Analyzer for Detection of T. gondii Abs and Measurement of Serum Ferritin and HbA1c

The Elecsys *T. gondii* Abs (IgM, IgG), Elecsys ferritin and Tina quant HbA1c Gen 3. Kits respectively were used to detect and measure *T. gondii* Abs, serum ferritin and HbA1c following the protocol provided by manufacture company. Cobas e 411 is automated immunoassay method and Cobas c 111 is clinical chemistry analyzers by Roche/Germany used in laboratories for fast and accurate diagnostic testing.

2.5. DNA extraction and the molecular detection of T. gondii B1 gene by Real-time PCR techniques

The all positive samples for *T. gondii* (68) abs (IgM and IgG) from both patients and healthy group were subjected to *T. gondii* B1 gene molecular detection. DNA extraction were performed on 68 samples (61 DM and 7 HC). DNA extraction were carried out using FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit were used according to manufacture company instructions then extracted DNA were kept in -20 C°.

In order to carry out PCR reaction test, the PCR mixture were prepared by adding all the essential components according to GoTaq® 1-Step RT-qPCR system by following all instructions of manufacture company as listed in Table 1.

Table 1: The Components of the Real-time PCR Mixture.

Material	Volume for each Sample
GoTaq® qPCR Master Mix	13µl
Forward Primer (5' CGCTGTCTGTCTAGGGCACC 3')	1 µl
Reverse Primer(5' GCATCTCATTTTCTTGCCCG 3')	1 µl
Nuclease-Free Water	5 µl
DNA template	5 µl
Total	25 µl

2.6. The Primers were Designed in this Study and Synthesized by Macrogenes Ltd, Korea

Table 2: Thermal Cycling Condition that used in this Study.

Steps	Cycles	Temperature	Time
Initial Denaturation	1	95 C°	4 mins
Denaturation	40*	95 C°	15 secs
Annealing		64 C°	25 secs
Extension and data collection		72 C°	55 secs

2.7. Ethical Approval

Prior to the initiation of the investigation, ethical approval was secured from the Kirkuk Directorate of Health, Ministry of Health, Iraq. The objectives and methodologies employed in this study were elucidated to participants, after which data were collected from them during their hospital visits, as per document number 694 dated September 10, 2024.

2.8. Statistical Analysis

The data acquired from this study were analysed using the Statistical Package for the Social Sciences (SPSS) application, employing one-way ANOVA and the Chi-square test. The p-value was deemed significant when it is less than 0.05.

3. Result

The serum of 180 patients in this study was analysed for the presence of T. gondii antibodies (IgM and IgG), revealing a total seropositivity for Toxoplasmosis of 37.80% among both T2DM and HC, as illustrated in Table 3. The results indicate a markedly elevated seroprevalence of T. gondii in patients with T2DM relative to healthy control people. Statistical analysis indicated a p-value < 0.05, signifying that the disparity between the two groups is very significant.

Table 3: Seroprevalence of T. *gondii* among T2DM Patients and HC Group.

Groups	Sample No.	Seropositive		Seronegative	
		Number	Percentage	Number	Percentage
T2DM	130 (100%)	61	46.92%	69	53.08%
HC	50 (100%)	7	14%	43	86%
Total	180 (100%)	68	37.80%	112	62.20%
p-value: < 0.05					

Table 4 indicates that the prevalence of IgM and IgG in T2DM patients was 1.54% and 45.38%, respectively. The prevalence of IgM and IgG in HC was 0% and 14%, respectively. The results clearly indicated a strong correlation between Type 2 Diabetes Mellitus (T2DM) and Toxoplasmosis infection, suggesting that T2DM patients exhibit increased susceptibility to this parasite (P-value: <0.05).

Table 4: Number and Percentage of Seropositivity of T. *gondii* Abs.

Study Group	T. *gondii* IgM		T. *gondii* IgG		Total
	Number	Percentage	Number	Percentage	
T2DM	2	1.54%	59	45.38%	61 (46.92%)
HC	0	0.00%	7	14.00%	7 (14.00%)
p-value: <0.05					

The correlation between Toxoplasmosis seropositivity and age groups is illustrated in the Table 5 below. The findings indicate that the majority of T2DM patients infected with T. gondii IgG antibodies belong to the age groups over 40 and over 15, with percentages of 55.74% and 34.43%, respectively. The highest seropositivity to IgG in the HC group was observed in the age group over 40, with a percentage of 57.14%.

Table 6 displays the mean ± SD levels of HbA1c, serum ferritin, and leptin. The mean HbA1c level in T2DM patients with positive T. gondii was 9.08±2.52, whereas in T2DM patients with negative T. gondii and the healthy control group, it was 186.1±160.5 and 4.91±0.66, respectively. There is no significant

correlation between HbA1c levels in T2DM patients and Toxoplasmosis infection.

Table 5: Distribution of Toxoplasmosis among Age Group.

Groups	Age Group	IgM	IgG	Total
T2DM	>15 (n:44)	0	21	**21 (34.43%)**
	>40 (n:69)	2	32	**34 (55.74%)**
	>65 (n:17)	0	6	**6 (9.83%)**
Total	130	2	59	**61 (100%)**
HC	>15 (n:21)	0	1	**1(14.29%)**
	>40 (n:22)	0	4	**4 (57.14%)**
	>65 (n:7)	0	2	**2 (28.57%)**
Total	50	0	7	**7 (100%)**
P-value 0.291				

In T2DM patients with positive T. gondii, the mean blood leptin level was 281.24±309.50, compared to 162.23±240.24 in T2DM patients with negative T. gondii and 98.32±48.63 in healthy controls. The results indicated a potential correlation between leptin hormone levels in T2DM patients and T. gondii infection, with a p-value of less than 0.05, as presented in Table 6.

Conversely, the mean ± SD serum ferritin level in T2DM patients with positive T. gondii was 207.2 ± 180.9, while in T2DM patients with negative T. gondii and healthy controls, the levels were 186.1 ± 160.5 and 124.9 ± 115.7, respectively. This finding indicates a link between ferritin levels and Toxoplasmosis in T2DM patients, as the p-value is less than 0.05, as shown in Table 6.

Table 6: The Mean Levels of HbA1c, Serum Leptin and Ferritin in T2DM and HC Group.

Parameters	Mean±SD		HC Mean±SD	p-value
	T2DM T. *gondii* (+ve)	T2DM T. *gondii* (-ve)		
HbA1c	9.08±2.5[2] a	9.01±2.51 [a]	4.91±0.6[6] b	<0.05
Serum leptin	281.24±309.50 a	162.23±240.24 b	98.32±48.63 c	<0.05
Serum Fe	207.2±180.9 [a]	186.1±160.5 [b]	124.9±115.7 [c]	<0.05

Same letter (e.g., both have "a") → No significant difference between those groups.
Different letters (e.g., "a" vs. "b" or "c") → Statistically significant difference between those groups
SD: standard deviation

Concerning RT-PCR results, out of a total of 68 positive samples for T. gondii (61 from diabetic mellitus patients and 7 from healthy controls), only 8 samples (13.1%) exhibited the presence of the T. gondii B1 gene, comprising 2 IgM and 6 IgG from diabetic patients. Samples containing the B1 gene of T. gondii displayed a sigmoid amplification curve, indicating a positive result for T. gondii. All other samples tested negative for this gene, exhibiting no amplification and presenting a flat baseline in the curve, indicative of negative results for T. gondii (Figure 1).

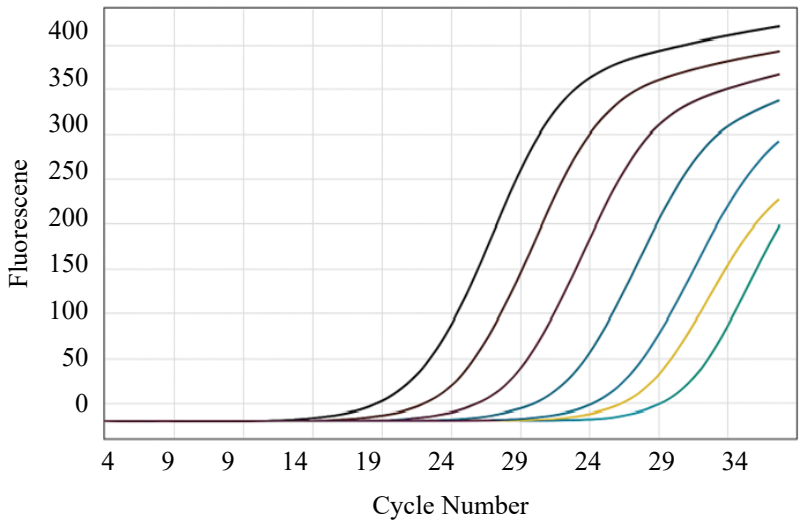

Figure 1: The Amplification Curve of RT-PCR using FAM Channel to Detect B1 Gene of T. *gondii* in T2DM Patients and HC.

4. Discussion

Toxoplasmosis is a parasite illness caused by T. gondii, prevalent globally and affecting roughly 25–30% of the human population, including immunocompromised individuals with type 2 diabetes mellitus (T2DM) [8]. The relationship between diabetes risk and T. gondii infection continues to be a subject of active discussion. Numerous prior research have investigated the relationship between the severity and consequences of T. gondii infection in patients with diabetes [9]. The current investigation found the seroprevalence of Toxoplasmosis in the sera of 130 T2DM patients and 50 healthy controls. The seropositivity rates of anti-Toxoplasmosis IgM and IgG antibodies in individuals

with Type 2 Diabetes Mellitus were 1.54% and 45.38%, respectively. Additionally, the seroprevalence of anti-Toxoplasmosis IgM and IgG in healthcare workers was 0% and 14%, respectively.

Toxoplasmosis manifests as an acute illness and subsequently evolves into a chronic infection if left untreated. When IgM antibodies, indicative of an acute or recent infection, commence to manifest within one month of infection. Conversely, IgG antibodies, indicative of chronic infection, emerge two months post-infection and remain detectable for years [4, 10]. One probable cause is that T. gondii in diabetic individuals may induce inflammation by destroying pancreatic β-cells, leading to a reduction in β-cell mass and impairing their capacity to make sufficient insulin.

The findings of this investigation were consistent with the research conducted by Kuba *et al.* [11] and Younis *et al.* [12] on diabetic patients afflicted with T. gondii infection, which reported anti-Toxoplasmosis IgG antibody prevalence rates of 40.00% and 41.5%, respectively. Conversely, the research conducted by Shamsinia *et al.* [13] and Shams *et al.* [14] on diabetic patients infected with Toxoplasmosis revealed that the prevalence of anti-Toxoplasmosis IgM antibodies was 2.04% and 0.8%, respectively.

The study results indicated that the prevalence of anti-Toxoplasmosis IgG antibodies in T2DM patients was greater in the age groups over 40 and over 15 years, with percentages of 55.74% and 34.43%, respectively, compared to the healthy control group, which was higher in the age group over 40. This may result from the immune system's deterioration due to ageing, which encompasses a reduction in B and T cell production, rendering older diabetic individuals more susceptible to Toxoplasmosis infections. These findings concur with Mihu *et al.* [15] and Kuba *et al.* [11], who observed a greater frequency of Toxoplasmosis in age groups over 40 and over 15.

HbA1c is a major biomarker for normal blood glucose levels in humans, and an elevated level indicates that individuals may have uncontrolled blood sugar due to pancreatic beta-cell failure resulting from prolonged diabetes duration. The HbA1c levels were measured in T2DM patients, revealing no significant changes between those infected with Toxoplasmosis and those without, indicating that acute or chronic Toxoplasmosis does not affect blood sugar levels in T2DM persons. The results obtained by Mohamed *et al.* [16] were consistent with those of Asgari *et al.* [17], who examined the impact of Toxoplasmosis on diabetic patients.

It is important to consider that leptin production increases due to its capacity to modulate the immune response and stimulate the inflammatory process via Th1 cells and Th1-associated cytokines such as IL-2 and TNF-α, with leptin secretion being regulated by TNF-α, a proinflammatory mediator that leads to elevated leptin secretion in adipose tissue. The findings of this study align with previous research by Hassan *et al.* [18] and Ansari-Lari *et al.* [3], which supports the elevation of leptin levels in patients infected with Toxoplasmosis.

This study's results indicate that serum ferritin levels were elevated in T2DM patients infected with Toxoplasmosis compared to other groups. Several investigations identified a correlation between T. gondii infection and increased blood ferritin levels in patients with Type 2 Diabetes Mellitus (T2DM). The culprit may be the inflammation induced by T. gondii infection, which affects ferritin levels during both acute and chronic phases [6, 19]. Infection with T. gondii may increase serum ferritin levels directly due to virulence factors produced by T. gondii, such as chitinase. The chitinase enzyme facilitates macrophage induction via activation, resulting in the production of pro-inflammatory cytokines such as IL-12, IL-6, and TNF-α. These cytokines activate the host's immune response and stimulate the synthesis of ferritin, an acute-phase reactant.

The ferritin findings in this investigation aligned with those reported by Bahadory *et al.* [20] and Lindell *et al.* [19], which indicated elevated ferritin levels in immunocompromised patients infected with Toxoplasmosis.

In immunocompromised patients, the identification of Toxoplasmosis necessitated a mix of serological and molecular methodologies [21]. This outcome arises from the fact that serological detection methods possess various limitations that result in false positive results, including antibodies that induce autoimmune diseases (such as rheumatoid factor and antinuclear antibodies), viral infections, and non-specific binding in vitro. Consequently, dependence on serological techniques such as ELISA and Cobas for the precise diagnosis of T. gondii is inadequate for correct identification [22]. Various types of molecular diagnostic methods include conventional PCR, nested PCR, loop-mediated isothermal amplification (LAMP), multiplex PCR, and RT-PCR. The RT-PCR technology has good specificity, reproducibility, and efficiency, along with a low contamination rate, eliminating the necessity for gel electrophoresis to visualise data [23, 24]. Furthermore, RT-PCR can detect minuscule quantities of DNA, reaching as low as 0.05µl per reaction, rendering it a very sensitive technique [25]. Furthermore, molecular diagnostics remains unaffected by the patient's immunologic status, rendering it the optimal choice for immunocompromised individuals. Molecular diagnostic approaches can identify Toxoplasma in various specimens (fluid or tissue) even at low concentrations. This study utilised the B1 gene as a target for detection, as it is present in 35 copies within the T. gondii genome and is highly conserved. Out of a total of 68 samples positive for IgM and IgG by Cobas, only 8 (13.1%) exhibited the presence of the T. gondii B1 gene. All eight positive samples for the B1 gene were from patients with type 2 diabetes mellitus, with two testing positive for IgM and six for IgG. The findings of this study concur with those of a prior investigation

conducted by Amiri *et al.* [26], which identified the T. gondii B1 gene in 13.69% of individuals afflicted with diabetes mellitus. Furthermore, the B1 gene was identified in 9% of the subjects in the investigation conducted by Fadel *et al.* [21].

5. Conclusion

The molecular and serological detection of Toxoplasmosis in T2DM patients is crucial to mitigate the detrimental effects of this parasitic infection on individuals who are immunocompromised. Furthermore, infection with Toxoplasmosis results in increased levels of leptin hormone and serum ferritin in individuals with Type 2 Diabetes Mellitus, indicating the detrimental impact of this parasite on their health.

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