Research Article

Interferon-Gamma +874 T/A Gene Polymorphism and Susceptibility to *Toxoplasma* Infection among Children with Type 1 Diabetes: A Possible Relationship

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Abstract | Interferon-gamma (IFNγ) is associated with a number of autoimmune diseases, in addition IFNγ is a key cytokine involved mainly in the defense against intracellular. We aimed to examine the role of IFNγ gene +874 T/A polymorphism in *T. gondii* infection susceptibility among children with type 1 diabetes. This study included 107 children and adolescents with T1DM in addition to 95 healthy controls. We evaluated levels of anti-*T. gondii* Ig-G and IFNγ in the participants' sera. All participants were also genotyped for IFNγ gene polymorphism at position +874T/A. Among the type 1 diabetic patients, 51 cases were positive for anti-*Toxoplasma gondii* IgG , while in controls were 40 individuals. In addition, no significant difference was detected as regard serum levels of IFNγ between the two groups. Moreover, no statistically significant differences were detected as regard the IFNγ gene +874 T/A polymorphism genotypes frequencies among different *Toxoplasma gondii* Ig-G seropositivity subgroups (p >0.05). The serum IFNγ levels were significantly higher among diabetic patients with IFNγ +874 (A/T) genotype and T allele than controls (p=0.01). Our results do not support a role of IFN-γ gene +874T/A polymorphism in the development of T1DM or susceptibility to *Toxoplasma* infection in patients with type 1 diabetes.

Keywords | IFNγ Polymorphism, *Toxoplasma*, Type 1 Diabetes Mellitus

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite which can infect most warm-blooded vertebrates (Dubey and Beattie, 1988). This parasite is among the most prevalent chronic parasitic infection in humans estimated to be 30–50% of the world population (WHO, 2013).

Toxoplasmosis is a silent disease. During the acute phase of infection, almost 80% of infected individuals are asymptomatic. Following the acute phase, the *T. gondii* parasite enters the "dormant stage" as bradyzoites which multiply slowly within tissue cysts and persist for the life of the host without causing a host reaction. Immunocompromised individuals, especially those with deficient cellular immunity, are at risk of reactivation of the latent infection that can progress and lead to a broad spectrum of diseases (Dubey et al., 1998; Silveira et al., 2011).

Type 1 diabetes mellitus (T1DM) is a chronic, progressive immune-mediated destruction of pancreatic β-cells, leading to partial, or in most cases, absolute insulin deficiency. T1DM precipitates in genetically susceptible individuals

by environmental factors (Delovitch and Singh, 1997).

Genetic susceptibility to T1DM probably includes an inherited defect in the establishment of peripheral tolerance to β-cell autoantigens (Vyse and Todd, 1996). Proinflammatory cytokines play a fundamental role in the initial stages of T1DM and in the development of diabetes-related complications (Kevan and Sarne, 2000). Several reports demonstrate that diabetic patients have an increased susceptibility to many opportunistic infections such as toxoplasmosis (Carruthers, 2002; Gokce et al., 2008; Shirbazou et al., 2013).

Interferon-gamma (IFNγ) gene in human is located on chromosome 12, it contains four exons and intermediate introns (Trent et al., 1982**).** IFN-γ gene intron-1 polymorphisms was speculated to influence immune complex disease susceptibility which is characterized by an imbalance of various immunoregulatory systems (Cantor et al., 2005).

IFNγ is a pleiotrophic cytokine with immunomodulatory effects where, IFNγ has long been believed to play a key role in driving the autoimmune pathogenesis of T1DM (Eizirik et al., 2009; Thomas et al., 2009). On the other hand, IFNγ is the major mediator of the defense against intracellular pathogens such as *T. gondii* and is crucial for the activation of a variety of antimicrobial activities in haematopoetic and non-haematopoetic cells that limit parasite replication (Yap and Sher, 1999).

Therefore, we conducted the present study to examine the seroprevalence of *Toxoplasma gondii* and role of IFNγ gene +874 T/A polymorphism in *T. gondii* infection susceptibility among children with T1DM.

SUBJECTS AND METHODS

Study Design

The study was designed to be a case–control study conducted during the period from the February 2014 to August 2015.

Study Population

The current study included, 107 patients with established type 1 diabetes for at least 2 years (50 females and 57 males); their mean age was 11.7±3.1. They were selected from attendants of the out-patient clinic of Endocrinology and Diabetes unit of Mansoura University Children' Hospital (MUCH), Mansoura, Egypt. The diagnosis of diabetes was based on the World Health Organisation criteria (Craig et al., 2014**)** (fasting plasma glucose ≥ 126mg/dl, two hours postprandial plasma glucose ≥ 200mg/dl, HbA1c ≥ 6.5% The presence of one or more of diabetes-associated autoantibodies confirm the diagnosis of type 1 diabetes including: Glutamic acid decarboxylase 65 autoantibodies (GAD); tyrosine phosphatase-like insulinoma antigen 2 (IA2); insulin autoantibodies (IAA); and β-cell-specific zinc transporter 8 autoantibodies (ZnT8) (World Health Organisation, 2006).

Patients who had other immune-related disease (e.g., Addison's disease, autoimmune hepatitis, rhematoid arthritis); patients who had diabetes-related complications (e.g., nephropathy, retinopathy, neuropathy) were excluded from the study.

We included ninety-five unrelated healthy control children from the same locality. They were recruited from the General Out-patient Clinic of MUCH, matched for age, sex and socioeconomic status. All participants (patients and controls) underwent the same research protocol, which included thorough medical history and clinical examination.

Data collection included: age, sex, weight, height, and body mass index (BMI). Diabetes-related variables recorded were: duration of diabetes, mean fasting blood glucose (FBG), and HbA1c (%) levels.

The study patients were divided into 2 groups on the basis of IgG detection, into groups, those with positive *Toxoplasma* IgG and those with negative test. All study participants provided written informed consent after receiving oral and written information about the study. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Mansoura University, Egypt.

Sampling

Peripheral venous blood samples were withdrawn from all patients and control subjects in the morning after 12 hours of overnight fasting. Each blood sample was divided into 2 tubes. One tube was left for coagulation for 30 minutes and then centrifuged at 7000 rpm for 15 minutes to serum preparation, devided into aliquots and stored at -80°C until being used for *Toxoplasma* IgG detection and determination of serum levels of interferon gamma and fasting blood glucose. The tube contained 2 ethylene-diamine-tetra-acetic acid (EDTA)-K3 for whole blood collection. One part was used immediately for HB A1c determination and the rest of the EDTA anticoagulated blood samples were stored at -80°C until DNA extraction.

Genomic DNA Extraction

Genomic DNA was extracted from EDTA–anticoagulated peripheral blood leukocytes using G-spin™ Total DNA Extraction Mini Kit (Cat No. 17045, Intron Biotechnology, Sungnam-si, Kyeonggi-do, Korea) (Schur, 2001). The DNA concentration was determined from the absorbance at 260 nm (Jenway, Genova Model, UK) with average concentration at 0.138±0.017μg/μl). All samples had a 260/280 nm absorbance ratio between 1.7 and 1.9 which indicated the purity of the extracted DNA. The integrity of the DNA was checked by electrophoresis on 1.0 % agarose gel stained with ethidium bromide (0.5 mg/ml) and visualized using Light UV Transilluminator (Model TUV-20, OWI Scientific, Inc. 800 242-5560, France).

Genotyping of IFN-γ Polymorphism

The T and A polymorphism sequences were identified using a specific single stranded oligonucleotide, synthesized to cover a 24-bp region for each allele (Bazzaz et al., 2014). An amplification refractory mutation system by polymerase chain reaction (ARMS-PCR) was performed. The ARMS-PCR method was applied for genotyping of IFN-γ +874*T/A polymorphism. The sequences of designed primers were as follow: Internal control primers (Human growth hormone);

HGH-1 5′- GCCTTCCCAACCATTCCCTTA-3′,
HGH-2 5′- CAAGGATTTCTGTTGTGTTTC-3′
(PCR product size; 425 bp).
IFN-γ (+874*T/A); common forward primer: 5′-TCAACAAAGCTGATACTCCA-3′. T allele primer; 5′-TTCTTACAACACAAAATCAA
ATCT-3′ and Talleleprimer; 5′-TTCTTACAACAC A AAATCAAATCA-3′ (PCR product size; 262 bp)

For each sample, 1.5 μL of DNA was added to 15 μL of the master mix solution (i-Taq™ Mix, Cat No. 25028-purchased from Intron Biotechnology, Sungnam-si, Kyeonggi-do, Korea). Then, 5 μL of specific primer mix was aliquoted to 5 μL of master mix solution that already contains DNA template. This 10 μL reaction was then, amplified on a thermal Cycler (TECHEN TC-312, Model FTC3102D, Barloworld Scientific Ltd. Stone, Stafford Shire, st 150 SA, UK).

The thermal cycling program was adjusted as follows: 1 minute at 96°C followed by 10 cycles of 15 seconds at 95°C, 50 seconds at 62°C, 40 seconds at 72°C followed by 20 cycles of 20 seconds at 95°C, 50 seconds at 59°C and 50 seconds at 72°C.

ARMS-PCR amplified products were subjected to 2.0 % agarose gel electrophoresis, stained with ethidium bromide (0.5 mg/ml) and visualized on an ultraviolet transilluminator. In gel electrophoresis, according to the presence or absence of amplified targeted sequence, the type of alleles (genotype) was identified.

Determination of Serum Interferon Gamma and *Toxpolasma* IgG

Serum IFN-γ and *Toxoplasma* IgG were measured using a sandwich enzymelinked immunosorbent assay (ELISA) (ab46025 – IFN gamma Human ELISA Kit & ab108776 – Anti-*Toxoplasma gondii* IgG Human ELISA Kit for IFN-γ and *Toxoplasma* IgG respectively). This assay was performed according to the manufacturer's instructions using a plate ELISA reader (Sunrise Rimote/Touch Screen-Tecan Austria GmbH, 5082 Grodig, Austria) for reading the absorbance of each sample at 450 nm wavelength as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength. ELISA score of 1.4-fold higher than the ELISA cut-off is diagnostic for Toxoplasmosis as described by Coutinho et al. and Uchôa et al., respectively (Coutinho et al., 1970; Uchôa et al., 1999).

Fasting blood glucose level was done according to method of (Trender, 1969). Glycosylated Hemoglobin A1c (HbA1c) was measured using Stable Glycosylated Hemoglobin A1c(HbA1c) reagent test kit by Enzymatic colorimetric method for biochemistry analyzer-Reference number;TMZ420- Ningbo,China.

Statistical Analyses

Data were analyzed using the Statistical Package of Social Sciences (SPSS) version 16 for Windows (SPSS, Inc., Chicago, IL, USA). Data were presented using mean and Standard Deviation (SD) for all quantitative values and number of cases (percentage) for qualitative values. The distribution of tested variables was examined with Kolmogorov-Smirnov test for normality. The significance of differences between continuous variables was determined with independent samples t- test. Chi- square or Fisher exact test was used for comparison between qualitative variables, as appropriate. The relationship between various interferon gamma genotypes and serum levels of interferon gamma was evaluated by analysis of variance (ANOVA). P values <.05 were considered significant.

RESULTS

One hundred and seven children and adolescents with T1DM (57 males and 50 females) with mean age 11.7 ± 3.1 years were included our study. Beside 95 apparently healthy children, (46 males and 49 females) with mean age 11.4 ± 2.9 years as controls. The diabetic patients and control groups were quite similar regarding age, sex and BMI ($P > 0.05$). Demographic and laboratory characteristics of the study groups were presented in Table 1.

As regard *T. gondii* seroprevalence results among the study groups, 51/107 (47.7%) diabetic patients were seropositive compared to 40/95 (42.1%) seropositive controls with no statistical significance between both group ($P>0.05$) (Table 1).

In addition, no significant difference in serum IFNγ levels between different groups was detected (P=0.11) (Table 1).

Table 1: Demographic and laboratory Characteristics of the study groups

Parameters	Controls (n=95)	Diabetics (n=107)	P
Age (years)(mean±SD)	11.4± 2.9	11.7±3.1	0.43
Gender (male/female)	(46/49)	(57/50)	0.57
BMI (Kg/m^2)	20.5±2.9	21.03±2.6	0.2
Duration of diabetes (years)	-	3.3±2.4	-
HbA1c (%)	-	8.38 ±1.02	-
FBG (mg/dl)		134±23	-
Toxoplasma gonii IgG antibodies			
seropositive (n%)	40 (42.1%)	51 (47.7%)	0.48
Seronegative (n%)	55 (57.9%)	56 (52.3%)	0.9
Serum IFNγ (pg/ml)	12.38±4.1	13.21±3.2	0.11

N=number; **BMI**= body mass index; **FBG**= fasting plasma glucose mg/dl; **IFNγ**=interferon gamma

Genotype frequency analysis of IFNγ gene at the +874 position showed that, among patients with T1DM, 22 (20.6%) showed A/A homozygocity, 48 (44.9%) were A/T heterozygous and 37 (34.5%) were T/T homozygous where among controls the results of genotyping showed that, 11 (11.5%) showed A/A homozygocity, 52 (54.7%) were A/T heterozygous and 32 (33.8%) were T/T homozygous.

No statistically significant differences were observed as regard frequencies of IFNγ +874 (A/T) genotype and allelic polymorphisms among patients with T1DM compared to control subjects (*P*> 0.05) (Table 2).

Table 2: Interferon gamma gene +874*T/A polymorphism genotype and allelic frequencies among children with T1DM compared to control subjects

	Control subjects (n= 95)	Diabetic patients (n= 107)	Odd s (95% CI)	P
Genotypes				
A/A	11 (11.5%)	22 (20.6%)	1.98 (0.90-4.33)	0.06*
A/T	52 (54.7%)	48 (44.9%)	0.67 (0.39-1.17)	0.10*
T/T	32 (33.8%)	37 (34.5%)	1.04 (0.58-1.86)	0.51*
Alleles				
A	74 (38.9%)	92 (43%)	0.96 (0.54-1.72)	0.51*
T	116(61.1%)	122 (57%)	0.51 (0.23-1.11)	0.06*

CI = confidence interval; *= Fishers exact test

Moreover, no statistical significant differences were detected as regard the IFNγ gene +874*T/A polymorphism genotypes frequencies among different *Toxoplasma* Ig-G seropositivity subgroups (p >0.05) (Table 3).

The serum levels IFNγ were significantly higher among T/T genotype and T allele when compared with other genotypes and alleles in both diabetics and controls (p<0.001). In addition; the serum IFNγ levels were significantly higher among diabetic patients with IFNγ +874 (A/T) genotype and T allele than controls (p=0.01) (Table 4).

DISCUSION

Cytokine gene polymorphisms have been shown to be involved in the resistance or susceptibility, the severity and clinical outcomes of several diseases, including immunoinflammatory and infectious diseases (Spriewald et al., 2005; El-Shabrawi et al., 2006; Martinez-Pomar Net al., 2006; Karimi et al., 2014).

The polymorphism in IFN-γ at the +874 T/A position has been previously described to associate with several diseases. The A/A genotype has been shown to be associated with hepatitis B in China (Yu et al., 2006), Helicobacter pylori gastritis in Italy (Zambon et al., 2005), tuberculosis in Spain (López-Maderuelo et al., 2003), type 2 diabetes mellitus in Greece (Tsiavou et al., 2005) and Wegener's granulomatosis in Germany (Spriewald et al., 2005). The A/T and T/T genotypes have been shown to associate with breast cancer in Iran (Kamali-Sarvestani et al., 2005), hepatitis C in Taiwan (Dai et al., 2006) and Hashimoto's disease in Japan (Ito et al., 2006).

Interferon gamma (IFN-γ) is involved in the immunological response against intracellular parasites as *T. gondii* (Gazzinelli et al., 1994) and leishmaniasis (Kamali-Sarvestani et al., 2006). IFN-gamma also directly induces enterocyte resistance against *Cryptosporidum parvum* infection; this observation may have important consequences for our understanding of the mucosal immune response to invasive pathogens (Pollok et al., 2001).

To the best of our knowledge, this is the first study to investigate the role of IFN-γ +874 T/A gene polymorphism in the susceptibility of patients with T1DM to *Toxoplasma* infection. High susceptibility to opportunistic infections such as toxoplasmosis has been described in diabetic patients, which may be caused by several defects of the immunological defense system (Shirbazou et al., 2013).

In our study, type 1 diabetic patients with seropositive *Toxoplasma* Ig G, were 47.7 % compared to controls 42.1%, with no statistically significant difference between both groups (P>0.05), this results are similar to Siyadatpanah et al. (3013).

IFN-γ +874 T/A polymorphism frequencies among seropositive toxoplasmosis among controls showed that A/T was the predominant genotype followed by T/T, A/A (22 (55%), 11(27.5%) and 7 (17.5%) respectively). These results were similar to that reported by Albuquerque et al. (2009).

Table 3: Interferon gamma gene +874*T/A polymorphism genotype and allelic frequencies among children with T1DM compared to control subjects in relation to *Toxoplasma* Ig-G seropositivity rates

Parameter	Controls (n=95)			Diabetics (n=107)		p
	IgG-positive (n=40)	IgG-negative (n=55)	P	IgG-positive (n=51)	IgG-negative (n=56)	
Genotype						
A/A (n%)	7 (17.5%)	4 (7.3%)	0.19*	13(25.5%)	9 (16.1%)	0.24
A/T (n%)	22 (55%)	30 (54.5%)	0.57*	20 (39.2%)	28 (50%)	0.33*
T/T (n%)	11(27.5%)	21 (38.2%)	0.38*	18 (35.3%)	19 (33.9%)	0.95*
Alleles						
A	36 (45%)	38 (34.5%)	0.38*	46 (45.1%)	46 (41.1%)	1*
T	44 (55%)	72 (65.5%)	0.19*	56 (54.9%)	66 (58.9%)	0.24*

N=number; *=Fisher's exact test

Table 4: Serum Levels of interferon gamma in patients with type 1 diabetes and controls in relation to different IFNγ gene +874*T/A polymorphism genotypes and alleles

	Controls Serum IFNγ level (mean±SD)	Diabetics Serum IFNγ level (mean±SD)	P
IFNγ +874 Genotypes			
A/A	7.82 ± 2.56	9.18±3.28	0.24
A/T	11.83 ± 3.19	13.23±1.96	0.01
T/T	14.84 ± 4.17	15.54±1.94	0.37
IFNγ +874 alleles			
A	11.13±3.4	11.96±3.1	0.14
T	12.98±3.9	14.2±2.3	0.01

An interesting finding in the current study is that, there was no significant difference regarding the IFN-γ +874 T/A polymorphism frequencies of genotype or alleles among seropositive toxoplasmosis among type 1 diabetic patients and controls. However, IFNγ gene +874 T/A polymorphism genotype AA, TT frequencies were higher among type 1 diabetes with seropositive *Toxoplasma* Ig-G than seropositive controls (25.5%, 35.3% versus 17.5%, 27,5 respectively), with no statistical significant differences (p >0.05).

In this study, the IFN-γ +874 T/A polymorphism genotype A/T was the predominant genotype among type 1 diabetes and controls (54.7% and 44.9% respectively). These data are similar to that observed in Bazzaz et al., (2014) study.

We observed also that, 11.5% of the control populations possessed the A/A genotype, 54.7% possessed the A/T genotype and 33.3% with the T/T genotype; and among type 1 diabetes were 20.6% A/A, 44.9% A/T, and 34.5% T/T genotype distribution. However, Laguila et al. (2005) observed 30.3%, 55% and 14.7% respectively. In addition; Matos et al. (2007) reported a 38.4%, 45% and 16.6% genotype distribution and Visentainer et al. (2008) identified a profile of 31.8%, 54% and 14.2% of each genotype. Interestingly, Amim et al. (2008) described a different profile of genotype distribution in their population (45%, 32% and 23%). This difference may be related to different race of the studied population.

Polymorphisms in several cytokine genes have been described and shown to influence gene transcription, leading to inter-individual variations in cytokine production (Doffinger et al., 2004).

In this study we found that the serum levels of IFNγ were significantly higher among T/T genotype and T allele when compared with other genotypes and alleles in both diabetics and controls (p <0.001). Our results were similar to those previously reported by (Pravica et al., 2000; López-Maderuelo et al., 2003; Dai et al., 2006; Henaoet al., 2006). They reported that IFN-γ gene polymorphism at position 874 T/A in intron-1 affects its gene expression and therefore plays a fundamental role in IFN-γ production; genotypes A/A, A/T and T/T are respectively linked to low-, medium- and high-IFNγ producers.

In addition, the serum IFNγ levels were significantly higher among diabetic patients with IFNγ A/T genotype and T allele than controls (p=0.01).

Our study showed no significant differences between IFN-γ gene polymorphism at position +874 genotypes and alleles and T1DM and we observed that IFN-γ +874 AT genotype was the most frequent genotype in both diabetic patients and control subjects, (44.9% and 54.7%, respectively) but with no statistically significant difference. In addition, children with T1DM in our study did not display statistically significant difference as regard serum levels of IFN-γ compared to controls.

On the contrary, the first intron polymorphism of IFN-γ

gene was speculated to influence immune complex disease susceptibility which is characterized by an imbalance of various immunoregulatory systems. IFN-γ supports the immune system to perform cytolysis of target cells (Cantor et al., 2005).

IFN-γ has repeatedly been implicated in the susceptibility, pathogenesis and progression of diabetes mellitus (Stalenhoef et al., 2008). It has been observed that the knocking out of the IFN-γ gene, IFN-γ neutralisation, IFN-γ blockade, or deletion of IFN-γ receptor (IFNγR) positive cells in NOD mice and BB rats all led to delayed or decreased incidence of T1DM (Rabinovitch, 1998). Moreover, the associations of IFN-γ gene polymorphisms within intron-1 and T1DM have been reported in two independent studies (Awata et al., 1994; Jahromi et al., 2000).

In another report, Rafinejad et al. (2004) examined the relation between IFN-γ gene polymorphisms at position 5'UTR +5644 and T1DM in Iranian patients and found a negative association between IFN-γ gene and T1DM pointing to T allele as a protective factor against T1DM .

This apparent discrepancy may be due to the lack of linking this polymorphism with other important polymorphisms like that TNF gene.

Although, the selected polymorphism had advantageous criteria (functionality, high frequency of both alleles), the number of patients were reasonably and well-defined diagnostic criteria were applied during patients recruitment, may suggest that this polymorphism is not functional in the context of diabetes and other polymorphisms may be of importance in β-cell destruction as it has been found that IFN-γ alone has no effect on β-cell death. Notably, β cells deficient in STAT-1, a key signaling molecule activated in β-cells by IFN-γ, are resistant to apoptosis induced in vitro by IFN-γ with either IL-1β or TNF-α (Gysemans et al., 2005; Kim et al., 2007).

In addition, absence of significant difference in serum levels of IFN-γ between children with T1DM in our study compared to control subjects can be explained by the fact that proinflammatory cytokines including IFN-γ play a fundemental role in the initial stages of T1DM and our patients had established (long-standing) T1DM. This is in accordance with Hussain et al. (1996) who found that recently diagnosed T1DM patients had significantly higher level of IFN-γ beside other Th-1 and macrophage-derived cytokines than patients with long-standing T1DM or control subjects.

Moreover, IFN-γ level was examined in the supernatants of peripheral blood mononuclear cells (PBMC) cultures from patients with T1DM at different durations of the disease. Karlsson et al. (2004) found increased levels of IFN-γ in patients with T1DM during the first month after the diagnosis while Foss-Freitas et al. (2007) observed increased levels of IFN-γ in type 1 diabetes patients in comparison to type 2 diabetic patients several years after diagnosis which suggested that the immunological characteristics observed in early stages of T1DM may further persist.

Furthermore, it cannot be excluded that plasma concentrations of cytokines is affected by many non-immunological factors such as level of metabolic control in diabetic patients. Poor metabolic control is progressively induced change in gene expression resulting in impairment of cellular immune activity (Lorenzi, 1992).

Immunocompromised individuals are at risk of reactivation of the latent infection that can progress and lead to a broad spectrum of diseases (Dubey et al., 1998; Silveira et al., 2011).

The results of our study showed that the level of INF-γ is higher in type 1 diabetic patients than controls. However, this difference was not statistically significant (13.21±3.2 vs. 12.38±4.1; p=0.11).

Foss-Freitas et al. (2007) observed that an increase in level of INF-γ released from PBMC of type 1 diabetic patient with adequate metabolic control, suggest that diabetic control improves the capacity of activation and maintenance of the immune response, reducing the susceptibility to infections.

Regarding the absence of similar studies on IFN-γ gene polymorphism in type 1 diabetes with toxoplasmosis, further studies involving gene polymorphisms in other cytokines and polymorphisms covering the entire length of the interferon gamma gene should be performed to understand the role of the immune system in the course of *T. gondii* infection among patients with type 1 diabetes

Our results do not support a role for IFNγ gene polymorphism +874 in the susceptibility to T1DM and or increase susceptibility to *Toxoplasma* infection among T1DM.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR'S CONTRIBUTION

Doaa salem presented the idea of the research, contributed in sample collection and processing, writing and correction of the manuscript. Nanees Salem helped in building idea of the research, contributed in clinical examination and selection of the cases, writing the manuscript, Abdel Hameed Fureeh contributed in statistical analysis of the data and asisted in selection of cases and revised the manuscript. Ayman Elsamanoudy contributed in sample processing, geno typing and helped shared in writing the manuscript.

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