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Association of Toxoplasma gondii Seropositivity and TNF-? -308G/A Polymorphism with Type 2 Diabetes Mellitus in Women

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Abstract

General Background: Type 2 Diabetes Mellitus (T2DM) is a multifactorial metabolic disorder involving genetic and environmental components with chronic low-grade inflammation. **Specific Background:** Infectious agents such as *Toxoplasma gondii* and inflammatory cytokine gene variations, including TNF-α -308G/A polymorphism, have been investigated for their association with metabolic disturbances. **Knowledge Gap:** The combined relationship between *T. gondii* seropositivity and TNF-α genetic variation in women with T2DM remains insufficiently characterized. **Aims:** This study aimed to evaluate the association of *T. gondii* infection and TNF-α -308G/A polymorphism with T2DM in women. **Results:** In a case-control design involving 480 women, T2DM cases showed higher *T. gondii* IgG seropositivity (68.3% vs. 45.8%) and elevated IgG titers (47.3 ± 28.6 vs. 32.1 ± 24.8 IU/mL). Additionally, the A allele frequency of TNF-α -308G/A was higher in cases than controls (0.256 vs. 0.208; OR = 1.31, P = 0.047). **Novelty:** This study integrates parasitic infection status with inflammatory genetic polymorphism in a single analytical framework among women. **Implications:** Findings suggest that chronic infection and genetic susceptibility are associated with T2DM, supporting further investigation into inflammatory and infectious pathways in metabolic disorders.

Highlights:
- Higher IgG seroprevalence observed among diabetic participants
- Elevated antibody titers indicate persistent parasitic exposure patterns
- Allelic variation linked with increased disease susceptibility

Keywords: Toxoplasma Gondii, Type 2 Diabetes Mellitus, TNF Alpha Polymorphism, Seropositivity, Case Control Study

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1- Introduction

Metabolic disorders represent a growing global health challenge, and grow in both developed and developing countries with their spread [1]. The pathogenesis of these conditions involves a complex difference in genetic sensitivity, environmental factors and lifestyle effects, which contains inflammation of the chronic low -grain that appears as a central integrated mechanism [2]. Recent research has quickly recognized the possible role of infectious agents in modifying metabolic health results, challenging traditional paradigms that mainly focus on genetic and lifestyle factors [3]. Among the contagious agents of potential metabolic relevance, toxoplasm gondi, which is an intracellular parasite, has attracted significant attention due to its high global spread and ability to establish old infections in immunocomatent hosts [4]. Current estimates suggest that about a third of the global population's chronic T. Gondi interferes with the infection, which varies greatly in geographical areas, which ranges from 10% to 80% depending on climate, cultural practice and socio -economic factors [5]. The unique ability of the parasite makes sleeping tissue ulcers that persists throughout the host, especially in the nerve and muscle tissue, provides a biological basis for long health results [6]. Evidence of emerging epidemiology has provided T. Gondi is linked to various metabolic disorders and neurocycatric conditions. Several studies have reported a relationship between chronic toxoplasmosis and converted glucose metabolism, insulin resistance and dyslipidimia [7;8] A large -scale cross -sectional study compared to Serongetive controls. Performed a significant frequency of metabolic syndrome components among gondi seropoctive individuals. Similarly, recently meta-analysis of T. Gondi infection and potential relationships between increased body mass index, although the directions and causes of these contexts remain subject to the ongoing study [9; 10]. Especially TNF-A plays an important role in both antitoxoplasma immunity and metabolic regulation. This playotropic cytokine t. Gondi is necessary to control replica and cyst formation, yet chronic overproduction is involved in insulin resistance and metabolic syndrome development [11]. The TNF -α gene consists of many functional polymorphism, with a variant -308G/a variant between the largest scale. This simple nucleotide polymorphism (SNP) affects TNF-A transcript, allelic associated with increased cytokin production in inflammatory conditions [12]. Recent genome-wide association studies have identified the TNF-α track variants as significant contributors to sensitivity to metabolic disease, although the effect the size is usually modest [13].

2- Materials and Methods

2.1 Study Design and Population

This case-control study was conducted between September 2024 and May 2025 at the University Medical Center and affiliated clinics. The study protocol was approved by the Institutional Review Board (IRB approval number: 2021-0847), and all participants provided written informed consent before enrollment.

2.2 Participants

A total of 480 women aged 25-65 years were recruited for this study. The case group consisted of 240 women with confirmed type 2 diabetes mellitus, while the control group included 240 women without diabetes or prediabetes. Participants were recruited through endocrinology clinics, primary care practices, and community health screening programs.

2.3 Toxoplasma gondii Serological Testing

Venous blood samples (10 mL) were collected from all participants after overnight fasting. Serum was separated within 2 hours of collection and stored at -80°C until analysis. T. gondii infection status was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits for both IgG antibodies (Euroimmun AG, Lübeck, Germany).

2.4 Genetic Analysis

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer, with A260/A280 ratios between 1.8-2.0 considered acceptable.

2.5 Target Polymorphisms:

2.5.1 *(Cytokine Gene Polymorphisms):*

TNF-α -308G/A (rs1800629) (Primers: Forward Primer: 5'-AGGCAATAGGTTTTGAGGGCCAT-3'; Reverse Primer: 5'-TCCTCCCTGCTCCGATTCCG-3', Amplicon Size: ~107 bp).

2.6 Genotyping Methods:

Polymorphisms were analyzed using TaqMan allelic discrimination assays on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Each 25 µL reaction contained 12.5 µL TaqMan Universal PCR Master Mix, 1.25 µL 20× TaqMan Genotyping Assay, 50 ng genomic DNA, and nuclease-free water to volume.

2.7 PCR Conditions:

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- Initial denaturation: 95°C for 10 minutes
- 40 cycles of: 95°C for 15 seconds, 60°C for 1 minute
- Post-PCR read at 60°C for 30 seconds

Genotype calling was performed using ABI 7500 Software v2.3, with manual inspection of all clustering patterns. Approximately 10% of samples were re-genotyped for quality control, showing >99% concordance.

2.8 Statistical Analysis

Statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY) and R version 4.3.0. Descriptive statistics included means ± standard deviations for continuous variables and frequencies with percentages for categorical variables.

3. Results

3.1 Participant Characteristics

As shown in Table 1, the present study demonstrated that participants in the case group were significantly older than those in the control group (mean age 52.1 ± 11.8 vs. 45.3 ± 12.1 years; P < 0.001). When stratified by age, a higher proportion of cases fell into the 50–59 year (37.1% vs. 24.2%) and 60–65 year (17.1% vs. 7.1%) brackets, whereas controls predominated in the younger 25–39 year category (37.1% vs. 17.5%) (P < 0.001).vSimilarly, body mass index differed markedly between groups: cases exhibited a mean BMI of 28.9 ± 4.7 kg/m² compared to 23.4 ± 3.2 kg/m² in controls (P < 0.001). In line with this, overweight and obesity were far more prevalent among cases (46.7% and 34.6%, respectively) than controls (26.3% and 4.2%), while the normal-weight category predominated in the control group (69.6% vs. 18.8%) (P < 0.001).vThese findings indicate that both advancing age and higher BMI are significantly associated with case status in the study population.

Table 1. Demographic and Anthropometric Characteristics of Study Participants

Characteristic	Cases (n=240)	Controls (n=240)	P-value
Age (years), mean ± SD	52.1 ± 11.8	45.3 ± 12.1	<0.001
Age groups, n (%)			<0.001
25-39 years	42 (17.5)	89 (37.1)	
40-49 years	68 (28.3)	76 (31.7)	
50-59 years	89 (37.1)	58 (24.2)	
60-65 years	41 (17.1)	17 (7.1)	
BMI (kg/m²), mean ± SD	28.9 ± 4.7	23.4 ± 3.2	<0.001
BMI categories, n (%)			<0.001
Normal (18.5-24.9)	45 (18.8)	167 (69.6)	
Overweight (25.0-29.9)	112 (46.7)	63 (26.3)	
Obese (≥30.0)	83 (34.6)	10 (4.2)	

3.2 Toxoplasma gondii Seroprevalence

Table 2 Serological features of cases (n = 240) compared to controls (n = 240). A much larger percentage of cases were positive for IgG than controls (68.3% vs 45.8%, P 50 IU/mL) than the controls (31.3% vs 13.3%). These results imply there is a clear trend in the association of IgG seropositivity/titer with case.

Table 2: Serological Characteristics of Cases and Controls

Parameter	Cases (n=240)	Controls (n=240)	Total (n=480)	P-value
IgG positive, n (%)	164 (68.3)	110 (45.8)	274 (57.1)	<0.0013
IgG titer (IU/mL), mean ± SD	47.3 ± 28.6	32.1 ± 24.8	39.7 ± 27.2	<0.0015
IgG titer categories, n (%)				<0.0025
Negative (<8)	76 (31.7)	130 (54.2)	206 (42.9)	
Low positive (8-50)	89 (37.1)	78 (32.5)	167 (34.8)	
High positive (>50)	75 (31.3)	32 (13.3)	107 (22.3)	

3.3 Genetic Polymorphism Distribution

Table Table 3Distributions of genotype frequencies and allele frequency of TNF-α -308G/A polymorphism in cases and controls. The distribution of genotypes was at Hardy-Weinberg equilibrium (P = 0.543). Although individual genotype odds ratios were not statistically significant (GA: OR = 1.33, 95% CI: 0.92-1.92, P = 0.128; AA: OR = 1.61, 95% CI: 0.75-3.44, P = 0.222), a significant difference of A allele frequency between cases and controls was found (0.256 vs.208, OR =1.31,95 %CI :1.00 -171,P=047).

Table 3. Polymorphisms of TNF-α -308G/A in Cases and Controls

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4. Discussion

The current analysis elucidated pronounced demographic and lifestyle differences that are consistent with recognised risk factor patterns between cases and controls. The mean age was markedly older when the case group and control group were compared (52.1 vs 45.3 years), that reflects an alleged aging-related inductance of metabolic disorders [14]. The striking difference in BMI achieved for cases and controls (28.9 vs. 23.4 kg/m^2) highlights the established association of metabolic physiology with obesity as typically reported in recent epidemiologic research [15]. The high family history association seen in this study (68.3% vs 12.5% in controls) is indicative of the strong genetic background of the disease studied, as has been suggested by large genome-wide association studies that have implicated multiple susceptibility loci [16]. Our results show a significantly greater seroprevalence of T. gondii in cases compared to controls (68.3% vs 45.8%), which contributes to the already accumulating evidence that links this parasite with multiple metabolic and neurological disorders. The overall seroprevalence of 57.1% observed in our study participants is also similar to the rates documented in geographic areas such as ours [17]. More importantly, the higher mean IgG titers in cases (47.3 vs. 32.1 IU/mL) suggest not only increased exposure but potentially more robust or persistent immune responses to the parasite. The distribution pattern of IgG viewers is particularly remarkable, with cases shown in cases shown a high ratio of high positive results (> 50 IU/ml: 31.3% against 13.3%). This discovery may indicate chronic infection with high parasitic stresses or increased immune activation in the case group. An analysis of TNF -α -308G/A -polymorphism discovered an allele frequency (0.256 against 0.208) in cases, despite the fact that he did not reach statistical significance of individual genotype associations. This discovery corresponds to recent meta-analysis, which performs the role of TNF-A genetic variants of inflammatory diseases and metabolic disorders

[18; 19]. The TNF-α-308A allele has been paired with an increase in TNF-A production, possibly contributing to the characteristics of inflammation of the chronic lower grain of metabolic syndrome and related conditions [20]. The minor effects observed for individual genotypes (OR = 1.33 for GA = 1.33 and AA = 1.61) are typical of normal genetic variants of complex diseases, where many genes of small effects contribute to general disease sensitivity [20], [21].The convergence of T. gondii infection and inflammatory genetic variants in our study population raises intriguing questions about potential gene-pathogen interactions. T. gondii infection is known to induce robust Th1 immune responses, with TNF-α playing a crucial role in host defense [22]. People harboring high-producer TNF-α alleles may elicit an enhanced inflammatory response to chronic T. gondii infection and this prolonged inflammation could participate in the metabolic alteration by eliciting long-term inflammatory activity. The “inflammasome” and other inflammatory pathways have been implicated in the pathogenesis of metabolic diseases [23]. Chronic T. gondii infection and genetic susceptibility to inflammation could induce a pro-inflammatory status, which may contribute to metabolic disturbance, as a connection has been found between parasitic infections and altered glucose metabolism/ insulin resistance [24]. The cross-sectional design constrains the possibility of causal inference and longitudinal studies should be conducted to clarify these temporal relationships. It was also not evaluated T. gondii genotypes that may be related to pathogenicity and immune response [25]. Further studies are needed to explore the functional consequences of these genetic polymorphisms on cytokine production in T. gondii infection. Additional mechanistic studies of inflammatory biomarkers, metabolic profiles, and parasitic burden are required to elucidate connections between infection, genetics and disease states. Intervention studies against T. gondii or inflammatory pathways may provide therapeutic implications. These results do indicate that T. gondii seropositivity and specific genotypes markers could be used as risk classifiers when considering those metabolite disorders [26]. High seroprevalence (...) underscores the importance of enhanced preventive measures, mainly targeting at-risk populations." T. gondii parasite is preventable because it can only be transmitted by eating contaminated food or water, or handling cat poo. Although not near- term for clinical actionability, genetic discoveries enhance the knowledge of susceptibility and risk to disease if pharmacogenomic research progresses this would be the driver for future precision medicine being able to provide personalized dietary and lifestyle as well as treatment advice [27].

5. Conclusion

Through a case-control study, significant associations were observed between T. gondii seropositivity with TNF-α -308G/A A allele and female gender was significantly associated with Type 2 Diabetes Mellitus (T2DM). Patients with T2DM were older and more obese. The A allele was significantly increased in T2DM cases, and indicated that persistent parasite infection and inflammatory genetic susceptibility were involved. Potential areas of future inquiry include causality, functional and clinical implications, and pathways through which infection, genetics, and metabolic dysfunction interact.

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