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Is chronic toxoplasmosis a risk factor for diabetes mellitus? A systematic review and meta-analysis of case-control studiesHamidreza Majidani^a, Sahar Dalvand^b, Ahmad Daryani^c,
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ABSTRACT

Introduction: The global protozoan parasite, *Toxoplasma gondii*, infects many warm-blooded animals and humans by employing different transmission routes. There have been some recent studies on the probable relevance of infectious agents and diabetes. Therefore, we conducted a systematic review and meta-analysis to identify the possible association between chronic toxoplasmosis and diabetes mellitus.

Methods: This study was conducted following the general methodology recommended for systematic reviews and meta-analysis. Nine English literature databases (Google scholar, PubMed, Scopus, Web of science, Science Direct, Ovid, ProQuest, IngentaConnect, and Wiley Online Library) were searched, up to January 2016. Random effects model was used to determine odds ratios and their 95% confidence intervals.

Results: Our review resulted in a total of seven publications meeting the inclusion criteria. Because of significant heterogeneity, we estimated a common OR by a random effects model at 1.10 (95% CI=0.13–9.57) with $p=0.929$ and 2.39 (95% CI= 1.20–4.75) with $p=0.013$ for type 1 and type 2 diabetes mellitus, respectively.

Conclusion: Despite the limitations such as low number of studies, this meta-analysis suggests chronic toxoplasmosis as a possible risk factor for type 2 DM. However, based on random effects model no statistically significant association was observed between *T. gondii* and type 1 DM. It is highly recommended for researchers to carry out more accurate studies aiming to better understand this association.

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Introduction

The ubiquitous parasitic protozoan, *Toxoplasma gondii*, is one of the most successful microorganism on the planet owing to quite a lot of compatibility to many host species, especially mammals, various transmission pathways (e.g. food, water, congenital, blood transfusion, organs transplant, etc.) and approximately involving one-third of the human population.¹⁻⁴ Being the category B priority pathogen with zoonotic significance, *Toxoplasma* has frequently been associated with congenital complications (such as hydrocephalus, stillbirth and abortion) as well as neuropathic anomalies, predominantly in high risk populations, i.e. pregnant women and immunocompromised hosts.^{2,5-7} While unusual, there are some evidence that *T. gondii* parasites may possibly have an undiscovered role in the putative pathogenesis of autoimmune diseases.⁸

A group of chronic metabolic disorders, specified as “Diabetes”, are of medical importance with widespread distribution, especially in persons with high-calorie diets. They are distinguished by hyperglycemia elicited by the deficiencies in the insulin hormone release (type 1 diabetes) and/or failure to respond properly to insulin in target cells (type 2 diabetes), and this condition is probably overwhelmed by genetic elements, autoimmune processes, and environmental factors. According to scientific evaluations, it is anticipated that the number of diabetic patients will reach 522 million individuals by 2030.⁹ Recently, some reports have spotlighted the probable relevance of diabetes mellitus (DM) and infectious agents like *Helicobacter pylori*¹⁰ and Coxsackie B4 virus.¹¹ In this case, the Apicomplexan parasite, *T. gondii*, has been proposed as a likely cause of diabetes, and existing information nearly predicate on this issue.¹²⁻¹⁶ Hence, this review was intended to shed light on the possible association between toxoplasmosis and diabetes.

Methods

Ethical aspects

As this review did not involve any human or animal subjects, therefore ethical approval was not required.

Search strategy

A systematic review was premised on screening the literature published online in English language up to January 2016, in order to address the association between toxoplasmosis and diabetes. The search was conducted on Google scholar, PubMed, Scopus, Web of science, Science Direct, Ovid, ProQuest, IngentaConnect, and Wiley Online Library (Supplementary Fig. 1). Toward that end, the medical subject headings (MeSH) terms in PubMed and Scopus databases using the search keywords “Toxoplasmosis”, “*T. gondii*”, “*Toxoplasma gondii*”, “Diabetes mellitus” and “Diabetic patients” combined together using OR and/or AND. Also reference lists of the primary relevance records found were explored

manually. Corresponding authors of papers were contacted for more details, if deemed necessary.

Study selection and data extraction

Only case-control studies on seroepidemiology of toxoplasmosis in diabetic individuals around the world were included. Other inclusion criteria were as follows: diabetes as a disease and *T. gondii* as an exposure, presence of healthy individuals as control group, and serologic diagnostic test. Records were evaluated by two independent reviewers. The selected articles were scrutinized and contradictions among studies were obviated by discussion and consensus. The data were extracted carefully from databases on the basis of title, year of publication, first author, diagnostic method, type of investigation, type of disease, aim of study, main findings, exact number of participants both in case and control groups, and details of *T. gondii* positive individuals as well. The PRISMA (preferred reporting items for systematic reviews and meta-analysis) guideline¹⁷ was followed in the reporting of current systematic review.

Meta-analysis

For each included study, the common odds ratio (OR) and respective 95% confidence interval (CI) on the association among toxoplasmosis and diabetes were calculated. The outcome of pool estimates of studies in addition to their 95% CI of independent records were depicted in a forest plot. Cochran's Q and I^2 statistics were applied to assess heterogeneity and inconsistency, respectively. Furthermore, small study effects and their publication bias were discerned by a funnel plot on the cornerstone of Egger's regression test. To comply with the results of heterogeneity test, either Der Simonian and Laird's random-effects method or Mantel-Haenszel's fixed-effects were employed to pool the approximations.

Results

Based on inclusion criteria, a total number of 7 out of 1377 studies were qualified to be ultimately included in this systematic review and meta-analysis,^{12,14,18-22} as demonstrated in Supplementary Fig. 1. The specifications of each study comprising the number of cases and controls as well as the number of IgG positive individuals, the utilized method and obtained p-value have been summarized in Table 1. Out of 2248 persons tested for toxoplasmosis infection, 717 were positive for *T. gondii* infection, 202 had type 1, and 1158 had type 2 DM; out of 888 healthy individuals 280 positive for *T. gondii* infection. The heterogeneity was statistically significant, for both type 1, $I^2 = 94.7\%$ and $Q = 38.09$ ($p < 0.001$), and type 2 DM, $I^2 = 88.1\%$ and $Q = 25.26$ ($p < 0.001$) (Table 2 and Supplementary Fig. 2). Accordingly, we calculated a common OR by random effects model at 1.10 (95% CI = 0.13–9.57) with $p = 0.929$ and 2.39 (95% CI = 1.20–4.75) with $p = 0.013$ for type 1 and type 2 DM, respectively (Table 2 and Supplementary Fig. 2). Also the calculated common OR (random effects model) for both type 1 and 2 diabetes was 1.86 (95% CI = 0.93–3.74) with $p = 0.0796$. The test of publication bias was clearly not significant in this case,

Table 1 – Prevalence of *Toxoplasma* infection among diabetic (cases) and healthy individuals (controls) included in the systematic review.

Reference	Diabetic patients (cases)		Healthy individuals (controls)		Matching	Method	OR (95% CI)	p-Value
	n	<i>Toxoplasma</i> IgG ⁺ n (%)	n	<i>Toxoplasma</i> IgG ⁺ n (%)				
Type 1 diabetes (14)	85	49 (57.6)	85	18 (21.1)	Age and gender	ELISA (EUROIMMUN)	5.066 (2.579–9.952)	<0.001
(21)	57	3 (5.2)	140	56 (40)	Unspecified	Bio-Plex 2200 kits	0.083 (0.025–0.279)	<0.001
(20)	60	27 (45)	60	14 (23.3)	Unspecified	ELISA	2.688 (1.226–5.895)	0.01
Type 2 diabetes (22)	807	457 (56.6)	250	56 (22.4)	Age and gender	ELISA (EUROIMMUN)	4.523 (3.257–6.281)	<0.001
(19)	91	55 (60.4)	93	36 (38.7)	Age and gender	ELISA (Pishtaz Teb Zaman Co.)	2.419 (1.338–4.373)	0.003
(18)	150	79 (52.6)	150	76 (50.6)	Gender	ELISA (VIRO, Germany).	1.083 (0.689–1.704)	0.72
(12)	110	47 (42.7)	110	24 (21.8)	Age and gender	ELISA (Biotech Co. England)	2.673 (1.483–4.819)	0.001

n, number of individuals; IgG⁺, anti-*Toxoplasma* antibody (immunoglobulin G); (%), percentage; ELISA, enzyme-linked immunosorbent assay; OR, odds ratio; 95% CI, 95 percent confidence interval.

Table 2 – Subgroup analysis according to type of diabetes.

Type of diabetes	No. of studies	OR (95% CI)	I ² %	Heterogeneity test	
				p	Q
Type 1	3	1.10 (0.13–9.57)	94.7	38.09	<0.001
Type 2	4	2.39 (1.20–4.75)	88.1	25.26	<0.001
Overall	7	1.86 (0.93–3.74)	90.2	61.35	<0.001

Test for heterogeneity between subgroup Q: 0.703, p-value: 0.4.

$p=0.335$ (Supplementary Fig. 3). Also, the chart of forest plot has been illustrated in supplementary Fig. 2. The status of toxoplasmosis infection was based on ELISA IgG, except for one paper in which the Bio-Plex 2200 kits were employed.

Discussion

A possible association between toxoplasmosis and DM would entail significant clinical consequences, shedding light on the complicated pathogenesis of DM. Overall, the current hypothesis suggests that toxoplasmosis increases susceptibility to acquiring diabetes and, on the other hand, diabetic patients are more vulnerable to opportunistic infections such as toxoplasmosis.^{12–14,22–24} Subsequently, to elucidate this association, various empirical evidence have been appraised and proposed as plausible pathophysiological mechanisms, including: (1) infected white blood cells represent enhanced migratory feature, leading to the easier spread of *Toxoplasma* in body organs, e.g. pancreas¹³; (2) a clinically conspicuous autoimmune process could be ignited by *T. gondii* infection, trending immune machinery toward autoantibody production, for instance against β -cells of Langerhans islets¹³; (3) in light of *ex vivo* D-glucose and insulin dose-responsive experiment in 3T3-L1 cells, better replication of *Toxoplasma* occurs in insulin-producing β -cells, leading to activation of

autoimmunity pathways as well as provoking the inflammation of Langerhans islets (insulinitis) and ultimately developing diabetes²³; (4) a possibility, is that *T. gondii* itself may invade and destroy pancreatic β -cells directly, instigating pancreatitis and more importantly, diabetes²⁵; (5) production of reactive oxygen species (ROS) and nitric oxide (NO) is induced by diabetes, and these agents, as stimulant for intra-cellular pathogens, can re-activate latent, cysts of parasites, again initiating acute infection²⁶; (6) given the inability of neutrophils to correctly perform phagocytosis and intra-cellular killing in progressive phase of diabetes, there may be increased responsiveness to intra-cellular pathogens such as *Candida* and *Toxoplasma*,²⁷ and (7) the opsonization activity and leukocyte cytotoxicity of diabetic patients required for elimination of pathogens are considerably subsided, hence they would be more prone to opportunistic infections.^{12,27,28}

In the included studies (Tables 1 and 2), the OR in type 1 DM individuals was 1.10 (95% CI = 0.13–9.57) with $p=0.929$, while this ratio in type 2 DM patients was 2.39 (95% CI = 1.20–4.75) with $p=0.013$. Thus, the risk of type 1 and 2 diabetes with previous *Toxoplasma* exposure would be 1.10 and 2.39 fold higher, respectively, than for unexposed persons. However, no statistically significant association between chronic toxoplasmosis and type 1 DM was held on random effects model. In this case, the highest OR (5.066) was reported in the study by Gokce et al.¹⁴ ($p<0.001$) and the lowest OR (0.083) in the

study by Krause et al.²¹ ($p < 0.001$). According to the estimated OR by Krause et al.²¹ there is no association between toxoplasmosis and diabetes and it is hypothesized that the patients' younger ages as well as uncommon route of infection may have remarkably biased their results. Recently, some researchers have demonstrated the influence of parasite genotypes on behavior, psychomotor performance, physiology, and overall health of their host. Hence, the strain of *Toxoplasma* should be considered as an influential parameter in relation to diabetes.^{15,16,29-32}

Despite of the lack of remarkable correlation between chronic toxoplasmosis and type 1 diabetes in the current meta-analysis, *ex vivo* studies strongly demonstrate the presence of this relationship.^{13,24} Therefore, further studies are needed in the future. There were several limitations in our study, including (1) small sample sizes in included studies and no further details about disease condition and duration in cases; (2) the variable quality of reporting of studies; (3) variable methodology to define *Toxoplasma* infection; and (4) lack of representativeness of cases and controls as they were selected from restricted communities, thus limiting external validity of the results.

Implications for research

Based on currently available results, no statistically significant association was observed between chronic toxoplasmosis and type 1 DM; of course there remain many questions to be answered in future investigations.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bjid.2016.09.002>.

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