A Systemic Review and Meta-analysis of Human Case-Control Studies Examining the Association between Toxoplasma Gondii and Type 2 Diabetes Mellitus.

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Type 2 diabetes mellitus (T2DM) continues to be a major challenge for public health authorities worldwide. An emerging field of research is starting to examine the association of infectious and environmental pathogens with diabetes. In particular, the potential of these pathogens to cause low-grade inflammation that may facilitate the risk and development of T2DM. An understudied pathogen of potential interest is the protozoan parasite Toxoplasma gondii (T. gondii). There is some clinical evidence supporting an association between chronic T. gondii infection and the development of T2DM in both animals and humans. The objective of this review is to comprehensively assess the seroprevalence rates of T. gondii in subjects with T2DM in comparison to healthy controls to determine the risk of T2DM due to T. gondii infection. Six electronic databases (Google Scholar, Science Direct, Embase, PubMed, PLOS ONE, and Scopus) were searched using specific Medical Subject Headings terms without language or date restrictions. Fixed and random effects models were used to determine odds ratios with statistical significance being set at 5.0%. Among 1,963 studies, four studies reporting prevalence of T. gondii infection in 1,158 subjects with T2DM and 603 healthy controls met the eligibility criteria. The overall weighted prevalence of T. gondii infection in subjects with T2DM was 55.4% (range: 42.7 - 60.4%) in comparison to 32.1% (range: 21.8 - 50.6%) of healthy controls. Because of significant heterogeneity (Q = 25.529, p <0.001, I\textsuperscript{2} = 88.25%), the common odds ratio was calculated using a random effects model at 2.34 (95% CI: 1.17 - 4.64, p = 0.016). Regardless of the limited number of studies and a lack of data, T. gondii infection should continue to be regarded as a contributing factor to T2DM disease development. Further high quality studies that include inflammatory biomarker analysis are warranted to determine the specific role of this parasite in the pathogenesis of T2DM.

Keywords: Toxoplasmosis; Toxoplasma gondii; Diabetes Mellitus, Type 2; prevalence; Meta-Analysis.

Introduction

Type 2 diabetes mellitus (T2DM, ICD-10-CM Diagnosis Code: E11.9) remains one of the most challenging public health burdens of the 21\textsuperscript{st} century [1]. Accounting for roughly 90% of all
diagnosed diabetes cases [2, 3], T2DM is a chronic disorder characterised by a reduction of insulin production and an inability of body tissues to fully respond to insulin (insulin resistance) [1, 2, 3]. Accordingly, the pathogenesis of T2DM has been recognised as the continuing deterioration of the insulin secretory capability of pancreatic β cells thereby not allowing compensation for an elevated demand of peripheral insulin [4, 5].

From a worldwide perspective, the prevalence of diabetes is reaching epidemic levels with an estimated prevalence of 9% among adults aged 18 years and over [6-10, 11, 12]. When this is combined with the 5.1 million deaths caused by diabetes worldwide (one person every six seconds) [8, 9], it is clear that diabetes places an excessively high social and economic costs on all countries. It is estimated that 642 million people will be living with diabetes by 2040, which is more than a 50% increase from 415 million in 2015 [11]. While this increase can be attributed to factors such as longer life expectancy and decreased mortality of diabetic individuals, genetic predisposition, physical inactivity, dietary changes and the obesity epidemic, [1, 3, 6-8, 10-12], there may also be additional unidentified risk factors, such as subclinical inflammation caused by infectious agents, that contribute to this rising prevalence of T2DM [13]. Certainly, there is epidemiological data that link inflammatory biomarkers as important risk indicators for the development of diabetes [14]. In fact, reports suggest that insulin resistance is associated with elevated circulating levels of inflammatory cytokines such as interleukin2 (IL-2), IL-6, IL-12, interferon gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α), raising the additional possibility that metabolic irregularities in diabetes may originate from, and/or are exacerbated by the overproduction of certain cytokines [13, 14]. To this extent, an emerging field of research is beginning to investigate the potential of infectious and environmental pathogens to cause low-grade inflammation that may facilitate the risk and development of diabetes.

An understudied pathogen of significant interest is the protozoan parasite Toxoplasma gondii (ICD-10-CM Diagnosis Code: B58.9). First described in 1908 by Nicolle and Manceaux [15], T. gondii infects approximately one third of the world’s population and is considered one of the most successful human parasites [16-19]. Humans acquire T. gondii by the ingestion of food, water or soil contaminated by oocysts from the definitive hosts, cats. T. gondii is also transmitted in people vertically via the maternal placenta and horizontally via blood transfusion [18, 20-22]. Capable of infecting all warm-blooded animals [23], toxoplasmosis is a disease of considerable public health impact. The global prevalence rates of this parasite are phenomenal with figures ranging from 15-85% depending on dietary habits, climate condition, hygienic standards, and geographical regions [20, 21, 23, 24, 25]. Although T. gondii is distributed worldwide and has possibly the broadest host range of any parasite, only one species (gondii) exists in the genus Toxoplasma [26], and cats are the only definitive host in which T. gondii sexual development is known to take place [27, 28].

It should be noted that the term “toxoplasmosis” should be reserved to describe cases where pathological and/or clinical symptoms of the disease caused by T. gondii are present while the term “T. gondii infection” should be used to describe asymptomatic infection or the perseverance of the parasite in the host (latent or chronic) [29]. Toxoplasmosis can present with various non-specific signs and symptoms, but the majority are similar to general flu-like indicators [20, 22, 25]. In immunocompetent individuals, it is thought that the disease is asymptomatic (infection) and recovers without treatment due to an efficient immune system.
which limits the spread of the rapidly multiplying tachyzoites [22]. Therefore, people are not routinely screened for T. gondii infection unless they are immunocompromised or pregnant [5, 20, 23]. In all cases, the parasite remains detectable in the serum throughout the life of the host with dormant cysts being formed in various anatomical sites including the central nervous system, often establishing latent infection [18, 22]. T. gondii can infect and replicate in any nucleated host cells leading to the production of various detectable inflammatory markers (pro-inflammatory cytokines: TNF-α, IL-1, IL-1β, IL-6, IL-18, IL-12p40, IL-8, IL-17, IL-22, IL-15; anti-inflammatory cytokines: transforming growth factor beta, IL-4, IL-10, IL-27, nitric oxide synthase [NOS], and reactive oxygen species [ROS]) via the innate acute inflammatory responses and antigen-specific adaptive immunity [5]. This facilitates a state of chronic inflammation at various anatomical sites in the host. In recent years, much focus has been directed at finding out how early innate responses, specifically, the production of cytokines (such as IL-12 and TNF-a), induce the development of the strong host Th1 inflammatory response to T. gondii [5].

To date, chronic T. gondii infection has been linked to several autoimmune disorders including thyroid disease, systemic sclerosis, rheumatoid arthritis, and inflammatory bowel syndrome [30]. Several studies have demonstrated a positive correlation between T. gondii infection and numerous neurological disorders and cancers [5, 20, 22, 26, 30]. However, T. gondii infection in individuals with T2DM has received little recognition. This study aims to estimate the risk of T2DM due to T. gondii infection by conducting a systematic review and meta-analysis of published studies examining the seroprevalence rates of T. gondii measured in subjects with T2DM in comparison to those without T2DM (healthy controls). The association between T2DM and T. gondii, if proven, will have important and significant public health outcomes. Routine screening for T. gondii infection in individuals with T2DM would add to the risk prediction for diabetes to target individuals for early aggressive intervention. Targeting T. gondii and reducing its risk factors could be used as an Achilles heel to reduce inflammation leading to improved glucose tolerance and reduced insulin resistance in diabetic individuals. T. gondii latent infection seems to have excellent potential as a potential target for T2DM intervention and may pave a path for the newly coined term: “Toxoplasmic Type 2 Diabetes” [31].

MATERIALS AND METHODS

Strategy for literature search
The present study was conducted according to the recommendations of the PRISMA Statement [32]. To identify published studies on the relationship between T. gondii infection and T2DM, we conducted a systematic search of published literature with no language or date restrictions (from inception until June 2017) from six electronic databases (Google Scholar, Science Direct, Embase, PubMed, PLOS ONE, and Scopus). The Medical Subject Headings terms used in the search were: “Toxoplasma” OR “Toxoplasma gondii” OR “toxoplasmosis” OR “T. gondii” combined with (AND) “type 2 diabetes mellitus” OR “type 2 diabetes” OR “T2DM”.

Selection of studies
Potentially relevant articles were initially selected based on title content followed by abstract content. The retained articles were read in full and screened for eligibility using a checklist of
inclusion-exclusion criteria. All selected studies had to meet the following inclusion criteria: (i) cross-sectional observational studies using a case-control design; (ii) T2DM must be the disease state and *T. gondii* infection as the exposure; (iii) participants of the T2DM group must be subjects diagnosed with T2DM with details of techniques used to diagnose T2DM; (iv) participants of the control group must be subjects without T2DM selected from the normal population; (v) the sample sizes must be suitably estimated; (vi) diagnosis of *T. gondii* infection must be based on the following standard laboratory detection methods: serological examination of *T. gondii* IgG and/or IgM antibodies, indirect fluorescent antibody test (IFAT), immunohistochemical (IHC) staining, or molecular methods detecting *T. gondii* DNA; where positive results were characterised by the presence of IgG and/or IgM; or a positive IFAT test; or a positive IHC stain; or the detection of *T. gondii* DNA; and negative results were defined as a lack of IgG or IgM antibodies; a negative IFAT test; a negative IHC stain; or no detection of *T. gondii* DNA. Likewise, studies were excluded if they were: (i) animal studies; (ii) repeated studies; (iii) abstracts; and (iv) studies which only included T2DM subjects without a control group. Any discrepancies with the final selection of studies were resolved by discussion and consensus with another author.

**Data collection**

The following information was extracted from each study: title, primary author, year of publication, location of the study, aims, methods, results, characteristics of the study population including collection criteria, numbers of case and control subjects, and diagnostic methods used in the diagnosis of T2DM and detection of *T. gondii* infection. Additionally, we also examined the reference lists of full text publications and text books to identify any additional studies not retrieved by the initial database search.

**Data analysis**

The meta-analysis was performed using Comprehensive Meta-Analysis (CMA) software (Biostat, Inc., USA) version 3.3.070. The common odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated for each individual study, and used as the measure of association between T2DM and *T. gondii* infection. Likewise, the ORs and respective 95% CIs were calculated for the overall (pooled) estimates employing both random and fixed effect models. Consequently, a forest plot summarising the study statistics including the ORs and relative weights (% for both fixed and random effect models) was generated. The implication of the pooled ORs was determined by the Z test. Heterogeneity among the studies was examined using the Cochrane Q, I^2, and Tau squared tests (fixed effect model). In addition, another forest plot was generated from the sensitivity analysis data (random effects model) to illustrate the effect of each individual study on the overall OR. Furthermore, publication bias was assessed using the Egger’s regression test that is illustrated by a funnel plot of standard error by log odds ratio of the selected studies using the random effect model.

**RESULTS**

The results of the literature search are presented in Fig. 1. From the six databases searched (Google Scholar, Science Direct, Embase, PubMed, PLOS ONE, and Scopus), a total of 1,963 published papers were eligible under the pre-defined search terms. From these, 1,240 were cited more than once. Of the remaining 453 papers, 440 were eliminated as they were not epidemiological studies. The remaining 13 papers were read as abstracts with eight being
rejected due to being literature reviews or animal studies. The residual five papers were read in full and one study [33] was excluded as it lacked a control group and hence did not meet our inclusion criteria. The references of the review articles did not add any new studies. Consequently, four studies were retained [34-37]. Three of the included studies were from Iran published in the last few years and one was from Turkey published in 2008.

Figure 1. Flowchart summarising the search strategy for epidemiological studies demonstrating a correlation between type 2 diabetes mellitus and *Toxoplasma gondii* infection.
As outlined in Table 1, two studies [35, 37] recruited cases and controls from hospitals with only one [37] matching for age and sex. The remaining two studies [34, 36] did not specify the sources of the case and control subjects but did match for age and sex. All studies employed commercially available ELISA IgG techniques to determine *T. gondii* infection status of their case and control subjects. In addition to the IgG ELISA technique, some studies also utilised the following additional diagnostic methods to further aid in the diagnosis of *T. gondii* infection status, as is often recommended [29]: commercial ELISA IgM assay [34, 35, 37] and IFAT [34, 37]. Table 2 summarises the main findings of each individual study in addition to the ORs and respective 95% CIs, p-, and Z-values calculated in the present study. Only one study [36] recruited subjects of all ages. The total number of subjects in all included studies was 1761, of whom 65.6% (n = 1158) had T2DM. The frequency of diabetic subjects with *T. gondii* infection ranged from 42.7% and 60.4% in comparison to 21.8% to 50.6% for subjects without T2DM. The overall weighted prevalence of *T. gondii* infection in subjects with T2DM was 55.4% (n = 641) in comparison to 32.1% (n = 194) of healthy controls. The seroprevalence of *T. gondii* is therefore higher in subjects who had T2DM than those who did not. Hence, all but one study [35] reported a relationship between *T. gondii* infection and T2DM. The pooled ORs of all included studies ranged from 1.08 (95% CI: 0.69-1.70) to 4.52 (95% CI: 3.26 - 6.28) with three of these results being significant (Table 2). A forest plot of the individual study and pooled ORs are presented in Fig. 2. The common fixed effects model OR was calculated at 2.66 (95% CI: 2.13 - 3.33, p < 0.001). The heterogeneity was significant (Q = 25.529, p <0.001, I² = 88.25%, Table 3) therefore the common OR using a random effects model was calculated at 2.34 (95% CI: 1.17 - 4.66, p = 0.016). Accordingly, subjects with *T. gondii* infection exposure have a 2.3 fold higher risk of having T2DM when compared to unexposed subjects.

**Table 1.** Description of studies included in the meta-analysis searching for an association between *Toxoplasma gondii* and type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Publication year</th>
<th>Country</th>
<th>Study</th>
<th>Source 1</th>
<th>Definition</th>
<th>Source 2</th>
<th>Matching*</th>
<th>Measure of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saki et al. (2016)</td>
<td>2016</td>
<td>Iran</td>
<td>CC</td>
<td>Hospital</td>
<td>Unspecified</td>
<td>Hospital</td>
<td>No</td>
<td>IgG/IgM ELISA kits (Trinity Biotech, USA) and IFAT kit (Euroimmun, England)</td>
</tr>
<tr>
<td>Siyatapatnah et al. (2013)</td>
<td>2013</td>
<td>Iran</td>
<td>CC</td>
<td>Hospital</td>
<td>Unspecified</td>
<td>Hospital</td>
<td>Yes</td>
<td>IgG/IgM ELISA kits (VIRO, Germany)</td>
</tr>
<tr>
<td>Shirbazou et al. (2013)</td>
<td>2013</td>
<td>Iran</td>
<td>CC</td>
<td>Unspecified</td>
<td>Unspecified</td>
<td>Unspecified</td>
<td>Yes</td>
<td>IgG ELISA kit (Pishtaz Teb Zaman Diagnostics, Iran)</td>
</tr>
<tr>
<td>Gokce et al. (2008)</td>
<td>2008</td>
<td>Turkey</td>
<td>CC</td>
<td>Unspecified</td>
<td>ADA</td>
<td>Unspecified</td>
<td>Yes</td>
<td>IgG/IgM ELISA and IFAT kits (Euroimmun)</td>
</tr>
</tbody>
</table>

CC, case-control; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; IFAT, immunofluorescence antibody test; *matching is by age and gender alone; source 1, source of type 2 diabetes mellitus cases; source 2, source of non-type 2 diabetes mellitus controls; definition, definition of type 2 diabetes mellitus cases.
Table 2. Description of data extracted from the included studies in the meta-analysis searching for an association between *Toxoplasma gondii* and type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Age</th>
<th>T2DM+ (n)</th>
<th>T2DM- (n)</th>
<th>T2DM &amp; TG+ n (%)</th>
<th>Non-T2DM &amp; TG+ n (%)</th>
<th>OR (95% CI)</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saki et al. (2016)</td>
<td>22</td>
<td>Adults</td>
<td>110</td>
<td>110</td>
<td>47 (42.7%)</td>
<td>24 (21.8%)</td>
<td>2.67 (1.48 to 4.82)</td>
<td>3.2 70</td>
<td>0.001</td>
</tr>
<tr>
<td>Siyadatpanah et al. (2013)</td>
<td>30</td>
<td>Adults</td>
<td>150</td>
<td>150</td>
<td>79 (52.7%)</td>
<td>76 (50.6%)</td>
<td>1.08 (0.69 to 1.70)</td>
<td>0.3 47</td>
<td>0.729</td>
</tr>
<tr>
<td>Shirbazou et al. (2013)</td>
<td>18</td>
<td>Adults</td>
<td>91</td>
<td>93</td>
<td>55 (60.4%)</td>
<td>38 (40.9%)</td>
<td>2.21 (1.23 to 3.99)</td>
<td>2.6 39</td>
<td>0.008</td>
</tr>
<tr>
<td>Gokce et al. (2008)</td>
<td>10</td>
<td>15-88 years</td>
<td>807</td>
<td>250</td>
<td>457 (56.6%)</td>
<td>56 (22.4%)</td>
<td>4.52 (3.26 to 6.28)</td>
<td>9.0 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total (fixed effects)</td>
<td>17</td>
<td>-</td>
<td>1158</td>
<td>603</td>
<td>641 (55.4%)</td>
<td>194 (32.1%)</td>
<td>2.66 (2.13 to 3.33)</td>
<td>8.5 69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total (random effects)</td>
<td>17</td>
<td>-</td>
<td>1158</td>
<td>603</td>
<td>641 (55.4%)</td>
<td>194 (32.1%)</td>
<td>2.34 (1.17 to 4.66)</td>
<td>2.4 10</td>
<td>0.014</td>
</tr>
</tbody>
</table>

n, number of subjects; age is in years; CI, confidence interval; T2DM+, subjects with type 2 diabetes mellitus; T2DM-, subjects without diagnosed type 2 diabetes mellitus; T2DM & TG+, subjects with type 2 diabetes mellitus and *Toxoplasma gondii* positive; non-T2DM & TG+, subjects without type 2 diabetes mellitus and *Toxoplasma gondii* positive; OR, odds ratio.

Table 3. Calculated data extracted from the included studies to assess heterogeneity in the meta-analysis searching for an association between *Toxoplasma gondii* and type 2 diabetes mellitus using both fixed and random effect models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Number of studies</th>
<th>Point estimate</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-value</th>
<th>p-value</th>
<th>Q-value</th>
<th>df (Q)</th>
<th>P-value</th>
<th>I² (%)</th>
<th>Tau²</th>
<th>Standard error</th>
<th>Variance</th>
<th>Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed</td>
<td>4</td>
<td>2.66</td>
<td>2.13</td>
<td>3.33</td>
<td>8.5</td>
<td>&lt;0.001</td>
<td>25.529</td>
<td>3</td>
<td>&lt;0.001</td>
<td>88.25</td>
<td>0.43</td>
<td>0.42</td>
<td>0.17</td>
<td>0.65</td>
</tr>
<tr>
<td>Random</td>
<td>4</td>
<td>2.34</td>
<td>1.17</td>
<td>4.66</td>
<td>2.4</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 2. Forest plot of odds ratios for individual studies describing epidemiological correlations between *Toxoplasma gondii* and type 2 diabetes mellitus. Heterogeneity: $Q = 25.529$, df = 3, $p = <0.001$, $I^2 = 88.25\%$, Overall effect size (fixed): $Z = 8.569$, $p = <0.001$, Overall effect size (random): $Z = 2.410$, $p = 0.016$. Relative weights are reported as %.

Sensitivity analysis was performed to estimate the effect of each individual study on the overall OR (Fig. 3). No adverse effect was observed in the absence of any particular study (point range: $1.81 – 2.37$). However, the exclusion of Siyadatpanah et al. [35] skews the OR significantly in favour of a stronger relationship between *T. gondii* infection and T2DM (3.16, 95% CI: $1.97 – 5.06$, $p <0.001$). No publication bias was observed among the included studies, as displayed in the symmetry of the funnel plot (Fig. 4). However, due to the low number of studies included in this analysis (less than 10), the funnel plot may be a less reliable method of investigating publication bias.

<table>
<thead>
<tr>
<th>Model</th>
<th>Study name</th>
<th>Odds ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>p-Value</th>
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<tr>
<td></td>
<td>Saki (2016)</td>
<td>2.67</td>
<td>1.48</td>
<td>4.82</td>
<td>0.001</td>
</tr>
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<td></td>
<td>Siyadatpanah (2013)</td>
<td>1.08</td>
<td>0.69</td>
<td>1.70</td>
<td>0.729</td>
</tr>
<tr>
<td></td>
<td>Shirbazou (2013)</td>
<td>2.21</td>
<td>1.23</td>
<td>3.99</td>
<td>0.008</td>
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<tr>
<td></td>
<td>Gokce (2008)</td>
<td>4.52</td>
<td>3.26</td>
<td>6.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Fixed</td>
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<td>2.66</td>
<td>2.13</td>
<td>3.33</td>
<td>0.000</td>
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<tr>
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<td>0.016</td>
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<td>14.46</td>
<td>23.74</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>46.61</td>
<td>26.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fixed Random

Figure 3. Forest plot describing the data generated from the sensitivity analysis calculated using the random effects model. The plot illustrates the effect of removing individual studies on the overall OR.
DISCUSSION
In the present study, we performed a systemic review and meta-analysis of the literature to evaluate the relationship between *T. gondii* infection and T2DM. Six different databases were searched without date and language restrictions making the literature search comprehensive with minimal publication bias. However, it is possible that our search may have missed significant studies available exclusively in other languages.

One of the most striking findings to come out of this meta-analysis was that very little human data exists on the relationship between *T. gondii* and T2DM despite the fact that *T. gondii* infects approximately one third of the world’s population. Our results demonstrate that *T. gondii* infection is associated with an increased prevalence of T2DM warranting further research to determine whether *T. gondii* increases the risk of developing T2DM and possible mechanisms underlying this association.

The degree of risk estimated in the present study (OR: 2.34, 95% CI: 1.17 - 4.66, p = 0.016) matches a recently published meta-analysis by Majidiani et al. [38] (OR: 2.39, 95% CI: 1.20 - 4.75, p = 0.013). While our inclusion/exclusion criteria were more stringent than the comparative meta-analysis, the authors also included the four studies extracted in our literature search. However, there was a difference in the data extraction between the earlier meta-analysis [38] and ours. For example, in the study conducted by Shirbazou et al. [36], the number of healthy controls with *T. gondii* infection was 38 in comparison to the erroneously stated 36 subjects in the comparative meta-analysis. This would give an OR of 2.42 which is higher than the 2.21
reported by the authors of the original paper [36] and the figure calculated in the present meta-analysis (2.21). The common OR reported by Majidiani et al. [38] was therefore higher than ours (2.39 and 2.34, respectively).

Of interest, none of the studies found during our search were from Europe, USA, South America, Asia Pacific, and Africa. In addition, most of Asia and the Middle East also remains unaddressed albeit three papers from Iran and one from Turkey. The majority of the studies selected for analysis in the present study had moderate adult sample sizes with the exception of Gocke et al. [34] who had a large sample size (n=1057) of age and sex matched subjects aged 18-55 years. Three [34, 36, 37] of the four studies found that subjects with T2DM had significantly higher prevalence rates of *T. gondii* when compared to healthy controls therefore concluding that a significant relationship exists between *T. gondii* infection and T2DM. The fourth [35] study did not find a statistically significant difference in prevalence of the parasite in diabetic and non-diabetic individuals. However, this study had a major limitation in its design with regards to the lack of information on the parameters and tests used to diagnose case (diabetic) and control (non-diabetic) subjects.

The other three studies clearly defined the diagnostic procedures used to confirm the presence or absence of diabetes in their subjects. In addition, Gocke et al. [34] employed standard ADA criteria in the diagnosis of diabetic subjects. All four studies shared common fundamental limitations which also may account for the large observed heterogeneity in the present study. Firstly, there is a lack of standardisation in regards to the criteria used to define various parameters, especially the diagnosis of T2DM (methodical heterogeneity). This includes defining inclusion and exclusion criteria consistent with the latest definitions of diabetes by the WHO. Secondly, there is no mention of having excluded individuals with psychiatric conditions, including personality disorders, from these studies (clinical heterogeneity). This feature is important because an association between positive *T. gondii* serology and various forms of serious mental illness has been previously reported [18, 21, 39]. Thirdly, the studies were not comparable in their methods of measuring *T. gondii* exposure, a fundamental factor that also increases methodical heterogeneity. As a result, statistical heterogeneity is born as a consequence of clinical and/or methodical heterogeneity.

Inflammation not only participates in host defences against environmental and infectious agents such as *T. gondii*, it also contributes to the pathophysiology of several chronic diseases [40]. Increased circulatory pro-inflammatory cytokines and acute phase response markers in subjects with T2DM were first reported in 1997 [40]. Since then, many reports describe changes to immune cell function in T2DM [5, 39, 41-48], suggesting that inflammation participates in the pathogenesis of T2DM and that T2DM is a chronic inflammatory disease. In addition, there is also experimental data that provides evidence for a direct link between inflammation and T2DM which adds evidence that this disorder is, at least in some part, an inflammatory condition [13].

Analysis of serum-based inflammatory biomarkers as a measurement of subclinical inflammation has been implicated in the development of T2DM with several cross-sectional and prospective studies describing elevated circulating levels of acute phase proteins as well as chemokines and cytokines [45-47]. In addition, pro-inflammatory cytokines are increasingly thought to contribute to the dysfunction and death of β cells during the progression of T2DM [49]. More specifically, it has been recognised that pancreatic β cells, in addition to neural cells, can be destroyed by noxious stimuli and several toxic agents such as ROS, NO, and various cytokines (TNF-α, IL1-β, and IFN-γ) (5). The subsequent pro-inflammatory cytokine balance has been directly linked to T2DM.
by several in vivo studies showing that the inhibition of key inflammatory cytokines protects rats from insulin resistance [34, 36, 50-53]. These reports suggest that increased cytokine production not only precedes, but also maintains insulin resistance in animals, supporting inflammatory mechanisms in T2DM pathogenesis.

The role of *T. gondii* in the pathogenesis of T2DM remains unknown. One possible mechanism could be the inflammatory-mediated destruction of pancreatic β cells which leads to the reduction in β cell mass that ultimately contributes to the failure of the β cell to produce enough insulin. This in turn would increase the risk of developing acute and chronic pancreatitis as well as diabetes [4, 54-56]. Additionally, it is known that T2DM is linked with an increased risk of developing acute pancreatitis [57] and that *T. gondii* infection can cause pancreatic tissue necrosis [58]. In fact, IL-18 and IL-1β are partly responsible for the development of pancreatic inflammation, and the neutralisation of either of these cytokines decreases inflammation [59]. Accordingly, individuals infected with *T. gondii* may be at increased risk of developing diabetes than uninfected individuals. Certainly, insulin has been shown to have a stimulatory effect on the in-vitro replication of *T. gondii* [60]. While insulin and D-glucose have been shown to have a synergistic dose-responsive stimulating effect on the replication of *T. gondii* tachyzoites in vitro [61]. Animal studies have also shown positive correlations between both the *T. gondii* parasite load and IgM antibody titre, and blood glucose levels in diabetic rats infected with *T. gondii* monitored at 15-day intervals for 60/105 days post diabetes/*T. gondii* infection [33].

During this period, Modrek et al. [33] observed a significant increase in the number of *T. gondii* cysts (in the brain) and blood glucose levels in the infected diabetic group when compared to the uninfected-diabetic group. In addition, the elevated brain parasite load and IgM titer corresponded to the elevated glucose levels in infected diabetic group which led to the view that *T. gondii* infection can increase the risk of developing diabetes [33]. Hassanian et al. [61] reported a significantly high *T. gondii* seroprevalence rate of 70.7% among diabetic patients in Iran (n = 205; age range, 13 - 60 years). In addition, the difference between the presence of toxoplasma antibodies and fasting blood sugar was statistically significant (p < 0.05). While this study lacked a control group, a meta-analysis conducted on 35 reports to determine the seroprevalence rate of *T. gondii* infection in the Iranian general population reported an overall seroprevalence rate of 39.3% (95% CI: 33.0% - 45.7%) [62]. This supports a higher seroprevalence rate of *T. gondii* infection in T2DM between *T. gondii* infection and diabetes in humans.

Furthermore, it has also been proposed that infection with *T. gondii* may be associated with obesity due to the ability of the organism to alter inflammatory fat distribution as it resides in fatty tissues [63]. As obesity is an established T2DM risk factor which has been previously referred to as “a state of chronic inflammation” [64], it is plausible that *T. gondii* could be involved in the obesity-T2DM paradigm. Certainly, excessive gestational weight gain has been previously reported from a study comparing pregnant woman infected with *T. gondii* with uninfected pregnant woman [53]. In this study, 979 mothers (mean age 30 years, range 19-44 years) were examined, of whom 194 (19.2%) were tested positive for *T. gondii* infection and 758 (80.2%) were tested negative for infection with *T. gondii* during the 16th week of pregnancy. The authors collected data on maternal weight and other parameters before pregnancy and at the 16th, 20th, 30th and 36th week of pregnancy. One of the main findings of this study was that the mean maternal weight of the *T. gondii* infected mothers before pregnancy was much higher (63.6 Kg) than that of the uninfected mothers (61.5 Kg). In addition, the effect of *T. gondii* infection on weight gain was significant in the 16th (p = 0.006) and 20th (p = 0.049) weeks of pregnancy and nearly significant in the 30th
week (p = 0.073). Moreover, the extra weight remained until the end of the pregnancies. In another study, the authors utilised a murine pregnancy model to mimic toxoplasmosis complications in humans and found that dams infected with high doses of *T. gondii* tachyzoites had significant excess body weight gain that led to foetal abortion or mummified embryos [50].

Since obesity in humans is characterised by hyper-leptinemia and a body weight-suppressing response to exogenous leptin [51], it is of note that the hormone leptin has also been implicated in the *T. gondii* host inflammatory response [63]. Using a rat model, Baltaci and Mogulkoc [52] found that plasma leptin concentrations significantly increased (p < 0.01) with no change in body weight during infection with *T. gondii*. The authors concluded that this biomarker, similar in structure to that of IL-2, exerts a pro-inflammatory action in the obese and diabetic individuals.

Infection with *T. gondii* may also act as a stimulus for motivational- and reward- driven behaviours (over-eating for example) via altered dopamine pathways. Indeed, animal models have shown that both dopamine release and availability of the rate-limiting enzyme in dopamine synthesis are influenced by *T. gondii* [50]. It has also been shown that dopamine antagonists can block behaviour changes in rats infected with *T. gondii* [50]. Thus, the behavioural changes in the host which may be induced by *T. gondii*, may be driven by survival needs which ultimately could have inadvertent effects on eating patterns that promote obesity [64]. Accordingly, the association between *T. gondii* infection, T2DM, and obesity is very significant and could alter the current views on T2DM management.

Lastly, it has been argued that the simple explanation for the relationship between *T. gondii* and T2DM may be that diabetic patients have an increased susceptibility to parasitic infections due the possibility of a suppressed immune system, decreased arterial perfusion, and neuropathy [65-69]. However, there is evidence to suggest otherwise. A study looking at parasitic infections in 200 diabetic (type 1, n = 16; type 2, n = 184) and 1,024 nondiabetic individuals did not find a significant difference in the incidence of intestinal parasites between the T2DM and control groups (47% and 56%, respectively) [66].

In light of the above reports, there is a strong clinical and laboratory evidence to support the notion of a genuine association between chronic latent *T. gondii* infection and T2DM development. Further studies incorporating inflammatory biomarker analysis are required to elucidate possible pathogenic mechanisms of *T. gondii* infection and T2DM. *T. gondii* latent infection may have potential as a novel target for T2DM intervention and may pave a path for the newly coined term: “Toxoplasmic Type 2 Diabetes” [31].

**CONCLUSION**

In conclusion, T2DM is characterised by impaired pancreatic β cell function resulting in insulin resistance with an underlying subclinical inflammation. Many reports suggest that insulin resistance is associated with elevated circulating levels of inflammatory cytokines suggesting that metabolic abnormalities in T2DM may be instigated by, or are exacerbated by, an overproduction of cytokines. A possible contributor to this prolonged low-grade inflammation that subsequently leads to the clinical expression of T2DM is the protozoan parasite *T. gondii*. Cellular immunity has been established as the key component of the host's immune response to infection by *T. gondii*. To date, only a handful of studies have examined the risk of T2DM following *T. gondii* infection, and none cover Europe, African, Australian, and the American regions. In addition, apart from Turkey and Iran, the majority of Asian and middle-eastern countries also remain overlooked. However, regardless of the
limited number of studies and a lack of data, *T. gondii* infection should continue to be regarded as a possible novel T2DM risk factor. In the present study, subjects with T2DM had significantly higher seroprevalence rates of *T. gondii* compared with those without T2DM. Human studies investigating inflammatory biomarkers in T2DM subjects with *T. gondii* infection are urgently needed in order to provide a more robust link between *T. gondii* infection and T2DM.

If the serological associations between *T. gondii* and T2DM are replicated, it will warrant the need for further research to clarify whether *T. gondii* increases the risk of developing T2DM, and mechanisms behind this relationship, if any. Current reports are limited to association studies meaning that authors are not able to find out if there is a causal relationship between *T. gondii* and T2DM, including the possibility of reverse causality (i.e. T2DM prompting the risk of *T. gondii* infection) or shared causality (i.e. common factor causing both T2DM and *T. gondii* infection). In addition, studies measuring only *T. gondii* IgG and IgM seropositivity cannot shed any light on the possible mechanisms of action between *T. gondii* infection and T2DM. Therefore, future studies specifically designed to investigate the role of *T. gondii* in T2DM using, for example, inflammation as the main outcome may provide a more robust link between *T. gondii* infection and T2DM. To this extent, studies examining selected inflammatory biomarkers that are altered in the serum during, and in the absence of chronic *T. gondii* infection in subjects with T2DM vs. non T2DM healthy controls are required. The study design should be population-based case-control with samples representative of all age groups matched for all possible confounding parameters. Standard criteria to define various parameters, especially the diagnosis of T2DM, in addition to defining inclusion and exclusion criteria in concordance with the latest definitions of diabetes by the WHO should be employed. Lastly, subjects presenting with psychiatric conditions and personality disorders should be excluded from these studies because of the previously reported link between positive *T. gondii* serology and the various forms of serious mental illness.

**DECLARATIONS**

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