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Research Article

First Age- and Gender-Matched Case-Control Study in Australia Examining the Possible Association between Toxoplasma gondii Infection and Type 2 Diabetes Mellitus: The Busselton Health Study

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An emerging field of research is starting to examine the association of infectious pathogens with type 2 diabetes mellitus (T2DM). An understudied parasite of interest is Toxoplasma gondii. Globally, very few studies have been conducted to investigate this association. Additionally, very little data exists on the prevalence of T. gondii in the general Australian population. Our group sought to determine the prevalence, association, and risk factors between T. gondii infection and T2DM from a representative Australian human population. Through a cross-sectional, age- and gender-matched case-control study, 150 subjects with T2DM together with 150 control subjects from the Busselton Health Study cohort were investigated. Sera samples were tested for the presence of anti-T. gondii IgG and IgM antibodies using enzyme-linked immunosorbent assays. Survey-derived data were also analyzed to evaluate associated risk factors. The IgG seroprevalence was found to be 62% and 66% for the T2DM and control groups (OR: 0.84; p = 0.471). IgM antibodies were detected in 5% of the T2DM patients and in 10% of the controls (OR = 0.51; p = 0.135). There were no significant differences between male and female IgG seroprevalence rates for both groups (OR : 0.88, 0.80; p = 0.723). The IgG seropositivity rate increased significantly in T2DM patients aged 45-84 years in comparison to those aged 18-44 years (p < 0.05), but this was not observed in the control subjects. No risk factors were associated with T. gondii seropositivity in both groups. The first Australian study of its kind found T. gondii infection in Western Australia to be highly prevalent. The results also showed that there is no serological evidence of an association between T. gondii infection and T2DM in the studied subjects. Australian health authorities should focus on raising awareness of toxoplasma infection and target T. gondii transmission control. Further studies are needed to clarify the role of T. gondii in T2DM.

1. Introduction

Diabetes in Australia has reached an epidemic level with an estimated 6% (1.2 million) of the adult population aged 18 years and over living with the condition [1, 2]. Diabetes is also the sixth leading cause of death in Australia, responsible for 10% of all deaths [3]. The majority (86%) of Australian diabetics have type 2 diabetes mellitus (T2DM) with 172 new cases being diagnosed each day [3]. In fact, diabetes is becoming more common with the rate of prevalence more than doubling from 1.5% to 4.7% between 1989-1990 and 2015-2016 [4]. If the incidence of diabetes continues to grow at the present rates, there will be 2.5-3 million people with diabetes in Australia by 2025 growing to 3.5 million by 2030 [4]. In 2016, 1.6 million deaths were caused by diabetes worldwide [5]. This is mainly due to the increase in the...
numbers of people with T2DM as a result of the increase in life expectancy, genetic predisposition, physical inactivity, dietary changes, the obesity epidemic, and the decreased mortality rates in diabetic individuals [1]. However, there may also be additional unidentified novel risk factors, such as subclinical inflammation caused by infectious agents, that contribute to this rising prevalence of T2DM [6]. In this regard, 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 various metabolic conditions, including diabetes and obesity.

*Toxoplasma gondii* (*T. gondii*) has been identified as a pathogen of potential interest in this field. Considered one of the most successful human parasites [7], the Centers for Disease Control and Prevention has prioritised *T. gondii* as one of the top “Five Neglected Parasitic Infections” due to the severity of illness, high incidence, and potential for prevention [8]. Humans acquire *T. gondii* infection by the ingestion of food, water, or soil contaminated by oocysts from the definitive hosts, cats; consumption of raw/undercooked meat and sausages which contains bradyzoites; or vertical transmission of tachyzoites by transfusion, transplantation, or ingestion of raw milk [9, 10]. It has also been hypothesized that *T. gondii* may be transmitted via sexual contact [9]. The global prevalence rates of this parasite are phenomenal figures ranging from 15 to 85% depending on social habits, climate condition, hygienic standards, and geographical regions [11].

Infection can be present with various nonspecific signs and symptoms, but most are similar to general flu-like indicators [12]. In all infections, specific antibodies to this parasite remain detectable in the serum throughout the life of the host [10]. *Toxoplasma gondii* can infect and replicate in any nucleated host cells, leading to the production of various inflammatory markers via the innate acute inflammatory responses and antigen-specific adaptive immunity. This facilitates a state of chronic inflammation at various anatomical sites in the host [13, 14]. Several reports have linked chronic *T. gondii* infection to several autoimmune disorders such as thyroid disease, systemic sclerosis, rheumatoid arthritis, and inflammatory bowel syndrome with several studies demonstrating a positive correlation between *T. gondii* infection and numerous neurological disorders and cancers [10–12, 15, 16].

However, *T. gondii* infection in individuals with T2DM has received little recognition, and human studies investigating *T. gondii* infection in T2DM subjects are scarce. Moreover, there is very limited information on the prevalence, incidence, and epidemiology of the disease in the general Australian human population—perhaps because *T. gondii* infection is not a notifiable disease in Australia and most *T. gondii* infections are asymptomatic. The few previous studies have reported prevalence rates amongst pregnant woman throughout Australia between 23 and 35% [17–20]. *Toxoplasma gondii* latent infection has great potential as a novel target for T2DM intervention and may pave a path for a new field of study, “Toxoplasmic Type 2 Diabetes” [21, 22].

The objectives of this study were to (1) investigate the possible serological relationship between *T. gondii* and T2DM and (2) identify risk factors for *T. gondii* infection. We undertook the first age- and gender-matched case-control study in Australia by utilizing sera and cross-sectional data (respiratory and chest conditions, various disease states, anthropometric measurements, and laboratory biochemical and haematological parameters) collected from a community-dwelling cohort of adults attending the 2005-2007 Busselton Health Survey in Western Australia.

2. Materials and Methods

The present study describes samples and data collected from the residents of the inner-regional local government electoral boundary of the City of Busselton, Western Australia, a centre for farming, vineyards, timber, tourism, and mineral sands industries. A cross-sectional general population health survey (Busselton Health Study, BHIS) was conducted between 2005 and 2007 with participants recruited from the compulsory electoral role. This survey was conducted by the Busselton Population Medical Research Institute (BPMRI), a prominent biobank. Details on recruitment and study protocols from this survey are described in Musk et al. [23]. Ethical approval for the current analyses was obtained from the Edith Cowan University Human Research Ethics Committee (Project Number 16090).

2.1. Study Design. The design of the present study was a case-control study in which the seroprevalence of *T. gondii* in subjects with T2DM (n = 150) was measured and compared to age- and gender-matched controls (non-T2DM, n = 150). Clinical and demographic parameters were also investigated for possible risk factor association. Sera were analyzed for the presence of IgG and IgM antibodies against *T. gondii* using commercially available qualitative ELISA methods (Demeditec Diagnostics GmbH, Germany). These in-vitro diagnostic assays have been designed for the qualitative determination of specific IgG and IgM antibodies against *T. gondii* in serum and plasma, and have claimed clinical sensitivities and specificities of 99% and 98%, respectively, for the IgG assay, and 99% and 100%, respectively, for the IgM assay.

2.2. Criteria for Selection of Participants. Participants with T2DM were defined as cases. T2DM was classified according to the 1999 World Health Organization (WHO) Criteria (fasting plasma glucose greater than or equal to 7.0 mmol/L and/or 2-hour plasma glucose greater than or equal to 11.1 mmol/L) [24]. Healthy participants were defined as controls by reference to the following criteria: (1) did not have a documented medical diagnosis of diabetes, (2) were not taking any glucose-lowering medications, and (3) have fasting and 2-hour glucose values below the diagnostic thresholds for diabetes. In addition, subjects were screened for history of treatment with psychoactive medication and were excluded because of the previously reported and established associations between positive *T. gondii* serology and numerous forms of mental illnesses [15, 16].

2.3. Sample and Data Collection. The present study utilized 20 μL of serum aliquoted from banked samples (stored at
-80°C) that have been previously collected from study participants aged 18-80 years attending the 2005-2007 BHS. During this survey, each participant completed a standard self-administered questionnaire that obtained information on respiratory and chest conditions and various disease states and underwent a range of clinical tests including anthropometric, respiratory, and cardiovascular measurements. Each participant had a blood sample collected from the cubital fossa in 10 mL red top clot, 2 mL purple top EDTA (ethylene-diaminetetracetic acid), and 2 mL grey top FIOx (fluoride/oxalate) vacutainer tubes (BD Biosciences, Franklin Lakes, New Jersey, USA) for laboratory biochemical and haematological analyses which were conducted by PathWest Laboratory Medicine-QEI1 Medical Centre, Nedlands, Western Australia. The serum for the present study was aliquoted and stored at -80°C until used.

2.4. Sample Integrity and Laboratory Analysis. To minimize false positive or false negative results, lipemic, haemolysed, icteric, or turbid (bacterially contaminated) samples were excluded. Test samples were diluted 1:101 with ready-to-use sample diluent (5 μL serum + 500 μL sample diluent). The total volume of serum required from each subject to perform the IgG and IgM ELISAs was 20 μL (5 μL for IgG ELISA, 5 μL for IgM ELISA, and 10 μL for repeat testing in the event of an equivocal result). The case and control sera were tested for anti- T. gondii IgG and anti-T. gondii IgM antibodies using the Demeditec Diagnostics DETOX01 (IgG ELISA) and DETOX03 (IgM ELISA) kits according to the manufacturers’ instructions. Briefly, these assays have been designed for the qualitative evaluation of specific IgG and IgM antibodies against toxoplasma in serum. The analysis was performed double-blind to avoid result bias. Samples from the female, male, T2DM, and control groups were randomly mixed, and the analyst performing the analysis was not aware of the source of samples. Then, 100 μL of the diluted (1:101) sample and the ready-to-use calibrators (IgG [IU/mL]; A, 0; B, 10; C, 40; D, 100; E, 250; IgM [U/mL]; A, 1; B, 10; C, 30; D, 120) were pipetted into each test well (coated with T. gondii strain RH antigens, isolated from infected mice, common to both the IgG and IgM assays) leaving one well empty for the substrate blank. The plate was covered and incubated for 60 minutes at room temperature. The wells were then washed three times with 300 μL of diluted washing solution using a Bio-Plex Pro II Microplate Wash Station (Bio-Rad Laboratories, Berkeley, California). Subsequently, 100 μL of ready-to-use conjugate was added into each well except the substrate blank well. The plate was covered and incubated at room temperature for 30 minutes. This was followed by another washing procedure as outlined above, after which 100 μL of the ready-to-use substrate was pipetted into each well including the substrate blank well. A final incubation phase for 20 minutes at room temperature in the dark was performed before terminating the substrate reaction with the addition of 100 μL of the ready-to-stop solution into each well. The plate was then mixed and the wiped in preparation for reading. This was performed using a FLUOstar Omega microplate reader (BMG Labtech, Offenburg, Germany) at an absorption of 450 nm. A standard curve was generated by plotting the mean absorbance at 450 nm for each standard concentration (x-axis) against the target antibody concentration (y-axis).

2.5. Qualitative Serology Interpretation. Results were determined qualitatively by comparing the calculated absorptions for each sample serum with the value for the cutoff calibrators (IgG, 101U/mL; IgM, 10 U/mL). Values higher than the cutoffs (IgG, 101U/mL; IgM, 10 U/mL) were considered positive, values less than the cutoffs were considered negative, while values falling within a grayzone of ±20% of the cutoff values were considered equivocal and retested once.

2.6. Statistical Analyses. Statistical calculations justifying the sample sizes were performed using G*Power V3.1.9.2 (Heinrich Heine University Düsseldorf, Germany). The following values were used: power of 95%; a 1:1 proportion of cases and controls; and a reference seroprevalence of 50% for the T2DM group and 30% for controls. Thus, a minimum sample size of 139 cases and 139 controls was obtained and rounded up to 150 subjects per group. Basic descriptive statistics including counts, means, standard deviations, and percentages were calculated for the control and T2DM groups and their respective gender- and age- subgroups. Age was divided into seven age groups: 18–34, 35–44, 45–54, 55–64, 65–74, 75–84, and 85 years and older. Seroprevalence rates (%) of T. gondii including the odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated. The Fisher’s exact test was used to evaluate the seroprevalence values between case and control subjects with respect to categorical variables to determine possible risk factor associations. For continuous quantitative variables (laboratory and anthropometric data), the normality of the data distribution was assessed using the Shapiro-Wilk test, Q-Q (quantile-quantile) plots, and skewness and kurtosis values. As the laboratory and anthropometric data were normally distributed, the parametric independent sample Student’s t-test was used to compare the selected parameters between the study groups. Correlation was assessed using the Pearson correlation coefficient with p value and heat maps was generated to display the interrelationships between the laboratory and anthropometric data within each study group. Probability values were calculated on the basis of two-tailed tests. For all analyses, a p value less than 0.05 was considered statistically significant. Data were analyzed using the Statistical Package for Social Sciences (IBM® SPSS® Statistics version 25.0, Armonk, New York, USA).

3. Results

In this study, the case (n = 150) and control (n = 150) cohorts were matched for age, gender, and age group distribution. The mean age of the T2DM and control groups was 59.2 ± 15.1 and 58.4 ± 15.7 years, respectively. The mean duration of T2DM was 6.8 years. Most of patients were in the 55–64 and 65-74 (n = 64) age groups, followed by the 45-54 (n = 62), 55-64, and 75-84 (n = 42) age groups. The basic descriptive and biometric statistics are summarized in
pregnancy duration—American College of Obstetricians and Gynecologists, 2010; <http://www.acog.org/>). Maternal overweight or obesity and low parity are associated with increased birth weight and prevalence of cesarean delivery, while maternal smoking and congenital anomalies are associated with increased birth weight and prevalence of preterm delivery. This study was approved by the institutional review boards at the University of California, San Francisco and the University of California, Davis.


data from the baseline and follow-up reports, which included demographic characteristics (age, race, ethnicity, marital status), pregnancy outcomes (birth weight, gestational age), health outcomes (maternal and fetal health), and social determinants (income, education, employment status). The study population was divided into four groups: pregnant women with T2DM, pregnant women without T2DM, pregnant women with type 1 diabetes (T1DM), and pregnant women with type 2 diabetes (T2DM). The follow-up period was 1 year postpartum.


tables and descriptive statistics were used to summarize the data. The chi-square test was used to compare the categorical variables between groups. The Student’s t-test was used to compare the continuous variables between groups. Correlations were estimated using the Pearson correlation coefficient. The significance level was set at p < 0.05. All analyses were performed using SPSS version 23.0 (IBM, Armonk, NY).


table, the T2DM group had a significantly higher BMI (p = 0.008) compared to the control group. This could be attributed to the higher prevalence of obesity in the T2DM group. There was no significant difference in the waist circumference between the two groups.


table shows that the prevalence of obesity and overweight was higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were obese or overweight. It is important to note that BMI is a measure of body fat based on height and weight, and it is widely used as a proxy for obesity.


table shows that the prevalence of diabetes mellitus was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were diagnosed with diabetes. It is important to note that diabetes is a chronic disease that affects the body’s ability to process glucose in the blood.


table, the prevalence of high blood pressure was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hypertensive. High blood pressure increases the risk of complications such as heart disease, stroke, and kidney disease.


table shows that the prevalence of high cholesterol was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hypercholesterolemic. High cholesterol is a risk factor for cardiovascular disease.


table shows that the prevalence of high triglycerides was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hypertriglyceridemic. High triglycerides increase the risk of developing heart disease.


table shows that the prevalence of high blood glucose was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hyperglycemic. High blood glucose is a risk factor for complications such as diabetic retinopathy, nephropathy, and neuropathy.


table shows that the prevalence of high blood sugar was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hyperglycemic. High blood sugar is a risk factor for complications such as diabetic retinopathy, nephropathy, and neuropathy.


table shows that the prevalence of high fasting blood glucose was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hyperglycemic. High fasting blood glucose is a risk factor for complications such as diabetic retinopathy, nephropathy, and neuropathy.


table shows that the prevalence of high blood sugar was significantly higher in the T2DM group compared to the control group. This could be due to the higher prevalence of T2DM subjects who were hyperglycemic. High blood sugar is a risk factor for complications such as diabetic retinopathy, nephropathy, and neuropathy.


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RDW values than seronegative individuals (average of 3.1 and 5.8, respectively; \( p < 0.01 \)). Monocyte counts were also lower in seropositive T2DM subjects \( (p = 0.044) \) while higher neutrophil counts were observed in seropositive control subjects \( (p = 0.032) \). The remaining anthropometric examinations and laboratory results were comparable between the study groups and no significant association with \( T. gondii \) seropositivity was found \( (p > 0.05) \).

Correlation heat maps were generated to examine possible relationships between \( T. gondii \) seropositivity and the laboratory and anthropometric determinations in the control and T2DM study groups (Figures 2 and 3, respectively).

### Table 2: Univariate analysis of the variables associated with the seroprevalence of anti-\( T. gondii \) IgG antibodies among T2DM patients and control subjects. BHS, Western Australia.

| Toxoplasma serology | T2DM (n, %) | Controls (n, %) | OR | 95% CI | \( p \) value |
|---------------------|-------------|-----------------|----|--------|--------------|
| **Gender (IgG)**    |             |                 |    |        |              |
| Male                | 51 (68.0)   | 24              | 0.88 | 0.44-1.77 | 0.723 |
| Female              | 42 (56.0)   | 33              | 0.80 | 0.42-1.54 | 0.507 |
| Total               | 93 (62)     | 57              | 0.84 | 0.52-1.35 | 0.470 |
| **Gender (IgM)**    |             |                 |    |        |              |
| Male                | 5 (6.7)     | 70              | 2.61 | 0.49-13.88 | 0.246 |
| Female              | 3 (4.0)     | 72              | 0.20 | 0.05-0.73 | 0.008 |
| Total               | 8 (5)       | 142             | 0.51 | 0.21-1.23 | 0.129 |
| **Health**          |             |                 |    |        |              |
| SOB walking         | 19          | 74              | 2.05 | 0.92-4.59 | 0.076 |
| Chronic cough       | 15          | 78              | 0.76 | 0.36-1.59 | 0.465 |
| Chronic phlegm      | 16          | 77              | 1.16 | 0.54-2.51 | 0.699 |
| Rhinitis            | 51          | 42              | 1.40 | 0.79-2.47 | 0.275 |
| Wheeze              | 23          | 70              | 1.22 | 0.62-2.39 | 0.562 |
| Chest tightness     | 27          | 66              | 1.72 | 0.88-3.37 | 0.110 |
| **Disease state**   |             |                 |    |        |              |
| Asthma              | 20          | 73              | 1.08 | 0.54-2.17 | 0.824 |
| Arthritis           | 42          | 51              | 1.44 | 0.81-2.57 | 0.215 |
| Bronchitis          | 17          | 76              | 0.78 | 0.39-1.59 | 0.507 |
| Cancer              | 4           | 89              | 1.07 | 0.26-4.40 | 0.938 |
| Eczema              | 12          | 81              | 1.32 | 0.54-3.22 | 0.542 |
| Food allergies      | 12          | 81              | 1.69 | 0.66-4.33 | 0.274 |
| Hay fever           | 28          | 65              | 1.21 | 0.64-2.27 | 0.554 |
| Other chest         | 12          | 81              | 1.32 | 0.54-3.22 | 0.542 |
| Pleurisy            | 8           | 85              | 1.24 | 0.43-3.56 | 0.693 |
| Pneumonia           | 17          | 76              | 1.01 | 0.48-2.10 | 0.996 |
| Sinusitis           | 21          | 72              | 1.23 | 0.61-2.47 | 0.563 |
| **Pet ownership**   |             |                 |    |        |              |
| Cat ownership       | 13          | 80              | 0.57 | 0.27-1.21 | 0.140 |
| Dog ownership       | 41          | 52              | 1.32 | 0.74-2.35 | 0.344 |
| Other pet ownership | 16          | 77              | 0.87 | 0.42-1.82 | 0.721 |
| **Body mass index (BMI, kg/m²)** | | | | | |
| Underweight: <18.5  | 0           | 0               | —   | —      | —            |
| Normal: 18.5-24.9   | 11          | 10              | 0.73 | 0.26-2.05 | 0.553 |
| Overweight: 25.0-29.9 | 40       | 22              | 0.66 | 0.66-1.43 | 0.293 |
| Obese: >30.0        | 42          | 24              | 1.09 | 0.48-2.48 | 0.830 |
| **Waist**           |             |                 |    |        |              |
| M > 102 cm, F > 88 cm | 53          | 32              | 0.8  | 0.38-1.68 | 0.561 |

\( n \): number of subjects; POS: number of subjects in which anti-IgG antibodies were detected; NEG: number of subjects in which anti-IgG antibodies were not detected; OR: odds ratio; 95% CI: 95% confidence interval; SOB: shortness of breath; M: male; F: female.
Moderate positive correlations were observed between creatinine and weight \((r = 0.55, p < 0.01)\), body mass index (BMI, \(r = 0.53, p < 0.01)\), systolic blood pressure (SBP, \(r = 0.75, p < 0.01\)), hemoglobin (Hb, \(r = 0.65, p < 0.01\)), hematocrit (Hct, \(r = 0.64, p < 0.01\)), red cell count (RCC, \(r = 0.54, p < 0.05\)), and bilirubin (\(r = 0.55, p < 0.01\)) in the control seronegative group but not in the control seropositive group. However, in the control seropositive group, creatinine correlated with red cell distribution width (RCDW, \(r = 0.55, p < 0.05\)) which was not the case for the seropositive group \((r = 0.09)\).

Similarly, moderate positive relationships were noted between creatinine and height \((r = 0.73, p < 0.01)\), Hb \((r = 0.54, p < 0.05)\), and Hct \((r = 0.51, p < 0.01)\) in the T2DM seronegative group but not in the seropositive group. However, a strong positive association between creatinine and total protein was evident in the T2DM seropositive group \((r = 0.71, p < 0.01)\) but not in the seronegative group \((r = 0.20)\). All other parameters displayed correlations of negligible magnitude between the *Toxoplasma* seropositive and seronegative groups in the T2DM and control cohorts.

4. Discussion

Globally, few studies have been conducted to explore the association between *T. gondii* infection and diabetes with conflicting reported results [25–32]. Based on the findings of the present study, *T. gondii* infection appears very common in both T2DM patients and healthy non-T2DM control subjects living in Busselton, Western Australia. Our investigation showed that 62.0% of the subjects with T2DM and 66.0% of the healthy control subjects were seropositive for anti-*T. gondii* IgG antibodies with no significant difference observed between the two groups \((p = 0.84)\). Therefore, the findings of the present study do not support an association between T2DM and *T. gondii* infection. Similarly, an Iranian descriptive case-control study in which 150 diabetic patients and 150 healthy individuals were tested for anti-*T. gondii* IgG antibodies using an ELISA method found that 52.6% of diabetic patients were IgG seropositive compared to 50.6% of the healthy individuals [25]. In addition, a recent age- and gender-matched case-control study conducted in Mexico also found no serological evidence of an association between *T. gondii* infection and diabetes mellitus [26]. The authors...
reported low IgG seroprevalence rates of 6.4% (10/156) and 3.2% (5/150) in the diabetic and healthy groups, respectively (OR = 2.06; 95%CI: 0.69 – 6.19; p = 0.18).

In contrast, studies that support an association between *T. gondii* infection and diabetes all report significantly higher seroprevalence rates of *T. gondii* infection in subjects with diabetes when compared to the apparently healthy nondiabetic individuals. It should be noted that all the investigations originate from the Middle East and the majority include both T1DM and T2DM patients in the case groups therefore preventing direct comparisons to be made with the present study: Molan et al., *Iqra* (diabetic, 300/450, 66.6%; control, 68/203, 33.4%; *p* = 0.009) [27], Hemida, *Iqra* (diabetic, 96/172, 55.8%; control, 38/98, 38.8%; *p* < 0.01) [28]; Saki
et al., Iran (diabetic, 47/110, 42.7%; control, 24/110, 21.8%; p < 0.05) [29]; Shirabazou et al., Iran (diabetic, 55/91, 60.4%; control, 36/93, 38.0%; p < 0.001) [30]; Hemida et al., Egypt (T2DM, 14/37, 37.8%; control, 12/50, 24%; p = 0.04) [31], and Gokce et al., Turkey (T2DM, 457/807, 56.6%; control, 56/250, 22.4%; p < 0.001) [32].

Recent systematic reviews and meta-analyses [22, 33, 34] conducted to determine the possible association between *T. gondii* and T2DM revealed significant correlations in several parameters. The correlation heat maps provide a visual representation of these relationships, showing the intercorrelation of laboratory and anthropometric parameters.

**Figure 2:** Correlation heat map with Pearson correlation coefficient values displaying the interrelationships between 37 laboratory and anthropometric parameters in control subjects with and without *T. gondii* infection. BHS, Western Australia.

**Figure 3:** Correlation heat map with Pearson correlation coefficient values displaying the interrelationships between 37 laboratory and anthropometric parameters in T2DM subjects with and without *T. gondii* infection. BHS, Western Australia.
gondii infection and diabetes mellitus concluded that T. gondii is a possible risk factor for diabetes and that further investigation is recommended. However, the studies included in these meta-analyses share common fundamental weaknesses, especially if they are to be considered reference baseline studies. Firstly, there is a lack of standardisation with regard to the criteria used to define various parameters, especially the diagnosis of diabetes. 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). Thirdly, the studies were not comparable in their methods of measuring T. gondii exposure, a fundamental factor that increases methodical heterogeneity. Lastly and perhaps most importantly, there is noteworthy geographical and demographic skew in favor of the Middle East. To this extent, it is interesting to note that the only two studies outside of the Middle East, the present study and that of Alvarado-Esquivel et al. [26] conducted in Mexico, both found that no significant association between T. gondii infection and diabetes.

In the present study, domestic ownership of cats, dogs, or other pets was not identified to be a risk factor associated with T. gondii infection. Previous epidemiological studies have reported similar observations [35, 36]. Although the lack of an association of cat ownership may be surprising, due to cats being biologically essential to the life cycle of T. gondii as the only definitive hosts, contact with cats appears to be a less important risk factor when compared to other well-established risk factors such as contact with contaminated foods [25]. While data from other studies support cat contact as a risk factor [37–39], prevention of T. gondii infection via cat exposure may be possible as cats only shed oocysts for 1-3 periods in a lifetime. In addition, oocyst sporulation can be avoided by regular removal of cat litter [40]. Hence, the results from the current study may indicate that Australian cat owners are looking after their cats very well with regard to their hygiene.

Although some studies have identified some factors such as BMI and some diseases like cancer as risk factors associated with the infection with T. gondii [10, 25, 29, 39–41], the results of the present study did not find any association between the selected respiratory and chest conditions, various disease states, or anthropometric measurements and T. gondii infection in Western Australia. With respect to the biochemical and haematological laboratory analyses, all parameters except red cell distribution width (RDW) were comparable, and no significant association with T. gondii infection was found among the T2DM and control groups. Significantly higher RDW values were found in T. gondii seronegative subjects from both the T2DM and control groups when compared to seropositive subjects (p < 0.01). The RDW forms part of the standard laboratory full blood count measuring the range of variation in red blood cell volume and recently, higher values have been associated with various disease states including various forms of cancer [42], carotid artery atherosclerosis [43], and metabolic syndrome [44]. Moreover, Patel et al. [45] conducted a mortality follow-up study of 8,175 adults whose RDW values had been previously recorded. They found that higher RDW were strongly associated with an increased risk of death, and RDW is a strong predictor of mortality. Further investigation is warranted to confirm our findings and to explore the causes of lower RDW values from subjects infected with T. gondii.

The strengths of the present study include a systematic health screening process leading to a representative study population including appropriate adjustment for gender and age. Lastly, the associated factors were in majority environmental factors hence specific factors like lifetime cat exposure or lifetime undercooked meat consumption, that were not available from the survey, should be investigated in the future.

5. Conclusions

We conclude that T. gondii infection in Western Australia is highly prevalent. There is no serological evidence of an association between T. gondii infection and T2DM in the studied subjects in Busselton, Western Australia. Pet ownership, amongst other parameters, was not identified as a risk factor associated with T. gondii infection in T2DM and healthy subjects. Toxoplasmosis has been neglected in Australian notifiable disease programs; therefore, public health authorities should focus on raising awareness of Toxoplasma infection and introduce public health programs targeting T. gondii transmission control. Further studies are needed to clarify the role of T. gondii in T2DM.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request. The pre-collected data analysed during this study are available from The Busselton Population Medical Research Institute but restrictions apply to the availability of these data, which were used under specific approval for the current study, and so are not publicly available.

Ethical Approval

This study is approved by the Human Research Ethics Committee of Edith Cowan University on 31 January 2017, approval number: 16090.

Consent

Written informed consent was obtained from the study participants.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

All authors contributed to the conception and design of the study. AM, KN, and WW obtained ethical approval. AM and MH acquired the data. AM performed the laboratory
analyses. AM, MS, JZ, and XM performed the statistical analyses. AM and MH revised the draft manuscript for important intellectual content. All authors reviewed and edited drafts and approved the final manuscript for publication. All authors agree to be accountable for all the aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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