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Short note

Is there a positive association between *Toxoplasma gondii* seropositivity and obesity in diabetic patients?

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ABSTRACT. The objective of this study was to determine the possible association between *Toxoplasma gondii* infection and obesity in Iraq. A cross-sectional study was performed on subjects with type 1 and type 2 diabetes referred to diabetes centers in Iraq. The results showed that diabetic individuals (T1DM and T2DM) with positive anti-*T. gondii* IgG antibodies had significantly higher probability of being obese compared to seronegative diabetic individuals ($P<0.001$). In addition, diabetic individuals (T1DM and T2DM) with positive *T. gondii* serology had significantly higher probability of being overweight compared to seronegative diabetic individuals ($P<0.001$). Moreover, in both obese and overweight groups, females were significantly more ($P<0.05$) prone to be obese and overweight than males. In conclusion, the results of the present study showed that the relationship between *T. gondii* infection, diabetes, and obesity is significant and may change the present views on diabetes, toxoplasmosis and obesity management.

Keywords: toxoplasmosis, obesity, seropositivity, anti-*Toxoplasma gondii* antibodies

Introduction

Toxoplasmosis, diabetes and obesity are very common worldwide and it has been reported that about 57% of the world's adult population is projected to be obese or overweight by 2030, emphasizing the need for more future studies to be conducted on obesity pathogenesis in order to identify novel targets for intervention [1].

Although the role of *T. gondii* in the pathogenesis of type 2 diabetes (T2DM) is still mysterious, the inflammatory-mediated destruction of pancreatic β cells during the infection with *T. gondii* which contributes to the failure of the β cell to produce enough insulin, would increase the risk of developing acute and chronic pancreatitis as well as type 2 diabetes [2]. In addition, Waree [3] reported that *T. gondii* infection can cause pancreatic tissue necrosis. Accordingly, it has been suggested that individuals infected with *T. gondii* may be more at risk to develop diabetes than uninfected individuals. On the other hand, Gonzalez-Perez et al. [4] reported that T2DM is associated with an increased risk of developing acute pancreatitis and consequently, it has been suggested that diabetic patients are more susceptible to parasitic infections, including toxoplasmosis, due to the possibility of a suppressed immune system, decreased arterial perfusion, and neuropathy [5]. These reports clearly revealed the presence of a strong clinical and laboratory evidence to support the notion of a genuine association between chronic latent *T. gondii* infection and development of T2DM.

Moreover, Majidiani et al. [6] conducted a systematic review and meta-analysis to identify the possible association between chronic toxoplasmosis and diabetes mellitus and found only seven publications (3 on T1DM and 4 on T2DM) meeting the inclusion criteria. The authors concluded that (despite the few numbers of studies) this meta-analysis suggests chronic toxoplasmosis as a possible risk factor for T2DM but no statistically significant association was observed between *T. gondii* and T1DM. More recently, Molan and Ismail [7] in a case-control study investigated the seroprevalence of the anti-*Toxoplasma gondii* IgG and IgM antibodies in the blood of diabetic and non-diabetic individuals and their results showed

that 66.6% of the diabetic patients were found seropositive for IgG while 33.4% of the apparently healthy individuals (control group) were found seropositive for the same antibody and the difference between the two groups was highly significant. As long as the diabetes and toxoplasmosis lower the immune response, the authors concluded that toxoplasmosis paves the way for diabetes and diabetes paves the way for diabetes but this depends on which one establishes first.

Although many previous studies have confirmed the link between toxoplasmosis and diabetes (T1DM and T2DM) [7–9], the link between toxoplasmosis and obesity [10], and the relationship between obesity and diabetes [11], this study seems to be the first to evaluate the possible link between obesity, diabetes (both T1DM and T2DM) and toxoplasmosis together.

Materials and Methods

Selection of patients

The present study was performed on individuals (males and females aged 10–79 years) referred to diabetes center of Baquba City and the National Centre of Diabetes in Baghdad City, Iraq during the period from July to November 2016. Medical examination was done by a general practitioner who evaluated diabetic patients in these centers. After 12 hours fasting by each participant, blood samples were collected by the professional staff at the diabetes centers into sterile Vacutainer glass tubes and the sera were separated by centrifugation and used on the same day to confirm the diagnosis of diabetes. The leftover sera samples (2–3 ml) from confirmed types 1 and 2 diabetic subjects were stored at –80°C for two weeks and then used in this study.

Each individual was anonymised using a specific ID number which enabled tracking of individuals throughout the study period. Questionnaires were given to all participants regarding the demographic characteristics and some risk factors of toxoplasmosis. This study has been reviewed and approved by the College of Sciences (Diyala University) Human Ethics Committee, application 2/2016. All the participants gave their written informed consents.

In order to measure the body mass index (BMI) for patient, the weight and height of each patient were measured. The weight was measured using digital weight scale while the patient minimally clothed without shoes and the height was measured using tape measure in standing position without shoes. The BMI was calculated by dividing the weight (kg) by the height (meters) squared and categorized on the basis of the World Health Organization (WHO) classification: underweight was defined as (BMI < 18.5 kg/ m^2), normal weight (BMI 18.5–24.9 kg/m^2), Overweight (BMI ≥ 25–29.9 kg/m^2) and obesity as (BMI ≥ 30 kg/m^2).

The inclusion criteria were male and female subjects between the ages of 10 and 80 years, with type 1 or type 2 diabetes mellitus and must be or his/her parents willing and able to provide written informed consent and to provide the required information in the questionnaires. The exclusion criteria were: a history or presence of clinically significant cardiovascular, respiratory, gastro-intestinal, endocrine diseases or diagnosis of any form of cancer. Other exclusion criteria were: a positive HIV, hepatitis B of hepatitis C serology. On the other hand, control subjects were defined by reference to the following criteria: 1) did not have a documented medical diagnosis of any type of diabetes, and 2) were not taking any glucose-lowering medications.

Enzyme-Linked Immunosorbent Assay (ELISA)

Sera collected from diabetic men and women were tested for the presence or absence of the specific anti-*Toxoplasma gondii* IgG and IgM antibodies by the Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA kit was provided by a commercial manufacturer (Acon, USA) and the procedure was performed according to the instructions of the manufacturer and as described previously. The cut-off value of ELISA test was 0.286 (0.034–0.456).

Statistical analysis

The data were analysis using Microsoft office Excel 2007 and SPSS (statistical package of social science) program version 16. $P<0.05$ was considered as the level of significance. The association between different variables was calculated by logistic regression. The dependent variable was the stable infection (seropositive/seronegative); all other variables were considered independent.

Results

The results showed that 75.3% type 1 and 65.1% of type 2 diabetic patients were found seropositive for anti-*Toxoplasma gondii* IgG while 33.4% of the

Table 1. Seropositivity of the anti-*Toxoplasma gondii* IgG and IgM antibodies in diabetic type 1 (T1DM) and type 2 (T2DM) diabetes patients and non-diabetic subjects (control group)[a]

	Number tested	Number positive		Seropositivity %	
		IgG	IgM	IgG	IgM
Diabetic patients					
T1DM	69	52	0.0	75.3	0.0
T2DM	381	248	0.0	65.1	0.0
Control group	203	68	0	33.4	0.0
P-value (diabetic vs. non-diabetic)				0.009	

[a]Enzyme-Linked Immunosorbent Assay (ELISA) was used for determination of anti-*T. gondii* antibodies. Cut-off value: 0.286.

apparently healthy individuals (control group) were found seropositive for the same antibody and the differences between the diabetic patients and non-diabetic healthy subjects were highly significant (P<0.009) and neither the diabetic patients nor the apparently healthy controls showed seropositivity for *T. gondii* IgM antibodies (Tab. 1).

The present study also investigated the possible association between obesity and toxoplasmosis in diabetic subjects and the results are shown in tables 2 and 3. The body mass index (BMI) data obtained in this study were used to generate three groups of body weights: obese (BMI ≥ 30 kg/m^2), overweight (BMI= ≥ 25–29 kg/m^2), and non-obese/overweight

Table 2. Distribution of anti-*Toxoplasma gondii* IgG seropositivity status according to the gender and body mass index (BMI) of the participant type 2 diabetic individuals (T2DM)

	Anti-*Toxoplasma gondii* IgG seropositivity			
Character	Positive	Negative	Total	P-value
	N (%)	N (%)	N (%)	(positive vs. negative)
Gender				
Males	101 (55.5)	81 (44.5)	182 (47.8)	P<0.046
Females	147 (73.9)	52 (26.1)	199 (52.2)	P<0.0001
Total	248 (65.1)	133 (34.9)	381	P<0.0001
BMI				
Normal weight				
Males	14 (19.7)	57 (80.3)	71	P<0.0001
Females	12 (46.2)	14 (53.8)	26	P<0.041
Total	26 (26.8)	71 (73.2)	97	P<0.0001
Overweight				
Males	60 (75.0)	20 (25.0)	80	P<0.0001
Females	73 (70.9)	30 29.1()	103	P<0.0001
Total	133 (72.7)	50 (27.3)	183	P<0.0001
Obese				
Males	27 (87.1)	8 (12.9)	31	P<0.0001
Females	62 (88.6)	4 (11.4)	70	P<0.0001
Total	89 (88.1)	12 (11.9)	101	P<0.0001

(BMI 18.5–24.9 kg/m^2). The results showed that the gender was associated with obesity in that in both the anti-*T. gondii* IgG seropositive and seronegative type 2 diabetic individuals (T2DM), the females had significantly higher (P<0.01) probability of being overweight and obese than males (Tab. 2).

Of 381 T2DM individuals, 101 (26.5%; 80 females and 31 males) were obese and of those 89 (88.1%) were seropositive for anti-*T. gondii* IgG antibodies and only 12 (11.9%) were seronegative for the same antibody and the difference between the two groups was highly significant (P<0.001). Among the T2DM obese females, 62 (88.6%) were seropositive for anti-*T. gondii* IgG antibodies and only 8 (11.4%) were seronegative with a highly significant difference between the two groups (P<0.001). Among the T2DM obese males, 27 (87.1%) were seropositive for anti-*T. gondii* IgG antibodies and only 4 (12.9%) were seronegative and the difference between the two groups was highly significant (P<0.0001). A similar trend was found among the overweight T2DM patients in that the females had significantly higher (P<0.05) probabilities of being overweight than males (Tab. 2). In addition, the females and males who were seropositive for the anti-*T. gondii* IgG antibodies had significantly higher (P<0.0001) probabilities of being overweight than their counterparts who were seronegative for these antibodies.

It can be seen from table 3 that of 69 type 1 diabetic individuals (T1DM), 26 (37.7%) were obese, 24 (34.8%) were overweight and 19 (27.5%) were with normal weight. Among the 26 obese T1DM individuals, 24 (92.3%) were seropositive for anti-*T. gondii* IgG antibodies and only 2 (7.7%) were seronegative for the same antibody and the difference between the two groups was highly significant (P<0.0001). The anti-*T. gondii* IgG seropositive male and female diabetic patients were significantly more (P<0.001) prone to be obese and overweight than their counterparts who were seronegative for *T. gondii* IgG antibodies (Tab. 3).

Table 3. Distribution of anti-*Toxoplasma gondii* IgG seropositivity status according to the gender and body mass index (BMI) of the participant type 1 diabetic individuals (T1DM)

Character	Anti-*Toxoplasma gondii* IgG seropositivity			
	Positive N (%)	Negative N (%)	Total N (%)	P-value (positive vs. negative)
Gender				
Males	21 (73.5)	9 (26.5)	30 (47.8)	P<0.0001
Females	31 (79.5)	8 (20.5)	39 (52.2)	P<0.0001
Total	52 (75.4)	17 (24.6)	69	P<0.0001
BMI				
Normal weight				
Males	3 (33.3)	6 (66.7)	9	NS
Females	5 (50.0)	5 (50.0)	10	NS
Total	9 (47.4)	10 (52.6)	19	P<0.0001
Overweight				
Males	8 (80.0)	2 (20.0)	10	P<0.0001
Females	12 (85.7)	2 (14.3)	14	P<0.0001
Total	20 (83.3)	4 (16.7)	24	P<0.0001
Obese				
Males	10 (90.9)	1 (9.1)	11	P<0.0001
Females	14 (93.3)	1 (6.7)	15	P<0.0001
Total	24 (92.3)	2 (7.7)	26	P<0.0001

NS: Not significant

For example, of 15 obese females with T1DM, 14 (93.3%) were found seropositive for anti-*T. gondii* IgG antibodies while only one female (6.7%) was seronegative for the same antibody. Among obese males with T1DM, 10 out of 11 (90.9%) showed positive serology for anti-*T. gondii* IgG antibodies and only one male (9.1 %) was found seronegative (Tab. 3).

Discussion

As far as we know, this study is the first study to identify an association between *T. gondii* seropositivity and obesity among individuals with type 1 (T1DM) and type 2 (T2DM) diabetes. The results showed that the gender was significantly associated with BMI (P<0.001) in both types of diabetes and the females were significantly more (P<0.001) prone to be obese than males. Ali et al. [11] conducted a cross-sectional study based on questionnaire survey and medical examination of 311 patients who attended the Sulaimani Diabetic Centre, Sulaimani, Iraq to assess the relationship between obesity and type 2 diabetes mellitus and their results showed that the gender was significantly associated with BMI (P<0.05) and the females were significantly more (P<0.05) prone to be obese than the males.

The results of the current study showed that diabetic individuals (T1DM and T2DM) with positive *T. gondii* serology had significantly higher the odds of being overweight and obese compared to seronegative diabetic individuals (P<0.001). In addition, in both obese and overweight groups, females were significantly more prone to be obese and overweight than males (P<0.001). Reeves et al. [10] estimated the possible association between *T. gondii* infection and obesity in a sample of 999 psychiatrically healthy adults and their results showed that individuals with positive anti-*T. gondii* IgG antibodies had twice the odds of being obese when compared to seronegative individuals and that individuals who were obese had significantly higher *T. gondii* IgG titers compared to individuals who were non-obese.

It is well known that obesity is one of the risk factors for the development of T2DM and it is believed that the significant increase in the prevalence of diabetes over the last two decades was largely due to the prevalence of obesity [10,12,13]. In addition, chronic inflammation is associated with obesity, insulin resistance, and T2DM [14]. On the other hand, Carter [15] reported that infection with *T. gondii* may be associated with obesity due to the ability of the parasite to live in fatty tissues and to alter inflammatory fat distribution. In addition, excessive gestational weight gain was reported during pregnancy in women infected with toxoplasmosis compared with uninfected pregnant women [16. Furthermore, excess weight gain was detected in a murine pregnancy model that mimics toxoplasmosis complications in humans [17]. On the other hand, Picard et al. [18] reported that *T. gondii* may modulate weight gain by decreasing muscle lipoprotein lipase and altering tissue lipoprotein lipase activity during chronic toxoplasmosis to elevate triglyceride distribution in the adipose tissues.

Animal studies have provided evidence which indicates the presence of a possible connection between parasites survival and obesity as some parasites may alter host appetite, food intake and levels of the appetite hormone, leptin [19]. It is well known that the bradyzoites of *T. gondii* are present within tissue cysts in mostly every organ including the brain, and the brain cysts have greater distribution in the amygdala and nucleus accumbens, brain regions that may be primarily targeted to influence fear signaling to promote survival and these regions also influence reward driven behavior such as eating related behaviors [20].

As long as obesity, diabetes, and toxoplasmosis are associated with a chronic systemic inflammatory response, each of them may pave the way for the other but this depends on which one establishes first to pave the way for the others. In addition, the results of the present study showed for the first time that the relationship between *T. gondii* infection, diabetes, and obesity is significant and may change the present views on obesity management, diabetes, and toxoplasmosis.

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