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Prevalence of *Toxoplasma gondii* infection in diabetic patients in Makkah AL Mukarramah, Saudi Arabia

Khalil, M.[1*], Baothman, M.[1], Alserhan, F.[1], Almunyif, A.[1], Alsharbe, G.[1], Samaren, H.[1], Deqnah, N.[2], AL Malki, A.[2] and AL Harbi, W.[3]
[1]Epidemiology Department, Faculty of Public Health & Health Informatics, Umm AL Qura University
[2]Maternal and Children Hospital, Ministry of Health, Makkah, SA
[3]Physiology Department, Faculty of Medicine, Umm AL Qura University
[*]Corresponding author e-mail: kmismail@uqu.edu.sa; khali72@gmail.com
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Abstract. Toxoplasmosis is a parasitic and zoonotic disease caused by *Toxoplasma gondii*. The disease has worldwide distribution and all people maybe under the risk of getting infected by the parasite. The overall aim of this research was to detect the prevalence rate of anti-*Toxoplasma gondii* IgM and IgG among diabetic patients in Makkah, Saudi Arabia. Blood samples were collected from diabetic patients. Toxo IgM and IgG combo rapid test cassette were used to screen the samples, and the results were confirmed by using Enzyme-Linked Immunosorbent Assay (ELISA) to detect anti-IgM and anti- IgG antibodies on the plasma of 90 diabetic patients who attended the mobile clinic or AL Noor hospital in Makkah area. The subject was asked to complete a structured questionnaire. The questionnaire data and serological results were analyzed by using SPSS 20. *Chi*-square was used to compare different variables. Out of 90 samples, 39 (43.3%) were positive to anti-*Toxoplasma gondii* IgG wherein 33 (36.6%) of them were male and 6 (6.7%) were female. The age ranged between 13–85 years with the mean of 49.9 years. The study found that there were statistical differences between the age groups with higher prevalence in the 50-65 years age group. Detection of IgM against *T. gondii* gave negative results. The results of the study indicate that latent *T. gondii* in diabetic patient are relatively high especially among the 50-65 age group. There were significant associated between direct contact with a cat and infection by *T. gondii* ($p<0.05$).

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an intracellular obligate parasite that belongs to phylum Apicomplexa which causes Toxoplasmosis (Weiss & Dubey, 2009). Cats and other Felidae family members implicate as a definitive host of the parasite. Humans and other livestock are considered as an intermediate host of *T. gondii* (Tenter *et al.*, 2000; Pal *et al.*, 2014).

Humans usually become infected via consumption of raw or undercooked meat that contain cyst of *T. gondii* or through vertical transmission (Dubey, 2009; Hide, 2016). Infection also can take place by ingestion of contaminated food, water or through contaminated soil with oocyst of *T. gondii* that are excreted by cats (Tenter *et al.*, 2000; Tenter, 2009; Robert-Gangneux & Dardé, 2012).

Toxoplasmosis is usually asymptomatic in infected humans. However, some infected group such as immunocompromised patients, suffer from fatal and life-threatening outcome including encephalitis, pneumonia, retino-choroiditis, epilepsy and other neurological complication (Walker & Zunt, 2005; Weiss & Dubey, 2009; Butler *et al.*, 2013; Ngoungou *et al.*, 2015; Wang *et al.*, 2017).

In a global perspective, up to one-third of the human population has been estimated to be infected with toxoplasmosis (Dalimi & Abdoli, 2012; Flegr *et al.*, 2014). It is reported that toxoplasmosis is more common in some population where raw meat is regularly eaten and more prevalent in warm and humid climates (Bojar & Szymañska, 2010). The quality and hygiene of water also influenced by the prevalence of infection (Jones & Dubey, 2010; Saadatnia & Golkar, 2012).

Toxoplasmosis is implicated in numerous autoimmune diseases for example rheumatoid arthritis, polymyositis, thyroid autoimmunity and antiphospholipid syndrome (Shapira *et al.*, 2012).

A meta-analysis has been conducted to determine the possible association between DM and toxoplasmosis; they reviewed seven case-control studies among different diabetic groups which include diabetic pregnant women, and general diabetic patient. The overall odds ratio was 1.86 and for T1D 1.10 and T2D 2.39 which suggests that toxoplasmosis may increase susceptibility to acquiring diabetes (Majidiani *et al.*, 2016).

In Saudi Arabia, many studies were conducted on the prevalence of infection and the majority of studies were conducted among pregnant. In the south of Saudi Arabia, the prevalence ranged from 20.8% to 24.1% in Najran and Jazan Province, respectively and 38.8% in the southwestern (Almushait *et al.*, 2014; Aqeely *et al.*, 2014; El-Shahawy *et al.*, 2014). A recent study in Riyadh confirmed that up to 32.5% and 6.4% were positive to *T. gondii* IgG and IgM antibodies respectively (Alghamdi *et al.*, 2016). In the eastern region, in Dhahran the seropositive to *T. gondii* was 28.5% IgG and 3% IgM (Elsafi *et al.*, 2015). In AL Madinah Almunawwarah, a study using ELISA showed a prevalence of 21.3% (Imam *et al.*, 2016). In Makkah, a study carried out in 2002 found that 35.6% were positive (Ghazi *et al.*, 2002). A subsequent study in 2006 found that the seroprevalence was 29.4% (Al-Harthi *et al.*, 2006). Recent studies conducted among pregnant women in Makkah, the prevalence rate of *T. gondii* was 47.7% and 21.2% respectively (ElSawy *et al.*, 2016; Khalil *et al.*, 2016).

However, there is a lack of studies among diabetic patient in Saudi Arabia. This study aims to estimate the seroprevalence of *T. gondii* and the associated risk factors among diabetic patients in Makkah, Saudi Arabia.

MATERIALS AND METHODS

Study design and study area

The study was cross-sectional performed in both male and female who suffered from *Diabetes Mellitus* and attended either the outpatient in Al Noor hospital in Makkah or mobile clinic. The study was done between January and April in 2017.

Data and blood samples were collected from 90 diabetic patients who agreed to participate in the current study and signed the consent form. The target group was patients who had fasting blood glucose ≥7mmol/l or had a result of HbA1c 48 mmol/mol or above and on medication for raised blood glucose, with a history of diagnosis of diabetes and lives in Makkah.

Data collection

The purpose of this study was clarified to all participants, and signed consent obtained from them. Data collected using a standard questionnaire. The questionnaire included questions regarding to sociodemographic, food appetite (consumption of undercooked meat, raw fish, and drinking water, drinking unboiled milk), daily activity (interaction with cat, regular contact with soil, travel), and questions about previous blood transfusion or organ transplantation.

Ethical approval

A letter of approval was received from the Ethical Committee in the Faculty of Public Health and Health Informatics, Umm Al Qura University, Saudi Arabia.

Sample collection

Blood samples were collected from each participant by using 5 ml syringe into EDTA tube. The plasma was separated from the blood at room temperature by centrifugation and transferred to Eppendorf tubes to

measure IgM and IgG. Plasma was stored in a freezer at –80°C until used. Plasma samples were screened for *T. gondii* using Toxo IgG and IgM combo rapid test cassette and by ELISA IgG and IgM.

Combo Rapid Cassette Test

Combo Rapid Cassette Test is qualitative, lateral flow immunoassay for detection of IgG and IgM antibodies of *T. gondii* in serum or plasma. The procedures of the test done according to the manufacturing instructions.

ELISA IgG and IgM

Detection of IgG and IgM in plasma by using (Toxo IgG® and IgM®, Human Germany) was performed according to manufacturer instruction.

Data analysis

Data analysis achieved was using SPSS software (version 20). The chi-square test was used to compare the serology result value in relation to the characteristics of the subject. The *p-value* less than 0.05 were considered as the level of significance.

RESULTS

Socio-demographic profile

Of the study population, the study includes a total of 90 diabetic patients from a different clinic in Makkah area. Where 73(81.1%) of them were men and 17 (18.9%) were female. Their age ranged (13-85) years with the mean of 49.9 years. Furthermore, approximately the half of participants have high school 20 (22.2%) and 20 (22.2%) have bachelor's degree while the illiteracy rate was 9 (10%).

Overall prevalence

The seroprevalence of *T. gondii* of diabetic patients who tested using combo test cassette and then confirmed the result by using ELISA displayed in Table 1. The result showed that seropositive rate of Toxo test of anti IgG was 0% while ELISA revealed that 39 (43.3%) of diabetic patients were positive to anti-IgG. The result did not show seropositivity to IgM whether by Toxo test or by ELISA. Among diabetic patient, 33 (36%) of men were positive to anti *T. gondii* IgG and 6 (6.7%) of female were positive to anti *T. gondii* IgG.

The impact of socio-demographic factors on the seropositivity of *T. gondii* presented in Table 2. The result highlighted that there is no significant difference between male and female ($p>0.3$). At the same time, age showed statistically significant ($p<0.05$) which infection was observed higher in diabetic patients aged between 50 to 65 years old (20%). No significant difference identified between educational level and infection with *T. gondii*.

Risk factors

The participants asked about factors that have known as risk factors of acquiring *T. gondii* Table 3. With respect to cats as there are no significant difference between positive and negative diabetic patient with regard to own or breeding a cat ($p>0.2$). Nonetheless, in term of direct contact with a cat about 35(38.9%) of positive diabetic patients have direct contact with cat and this showed significant ($p< 0.05$).

Regarding to food appetite as another important source of infection, the study was investigated and showed that there is no significant difference between seropositivity

Table 1. Prevalence of toxoplasmosis diabetic people using screening and confirmatory tests

Gender	Screening Test		Confirmatory Test (ELISA)		%
	IgM	IgG	IgM	IgG	
Male	0 (0.0%)	0 (0.0%)	0 (0.0%)	33/73	36.6%
Female	0 (0.0%)	0 (0.0%)	0 (0.0%)	06/17	06.7%
Total	0 (0.0%)	0 (0.0%)	0 (0.0%)	39/90	43.3%

Table 2. Sociodemographic information association with infection by *Toxoplasma gondii*

Variable	Target Group		X^2	*p*-value
	IgG Positive	IgG Negative		
Gender				
Male	33 (36.7%)	40 (44.4%)	0.552	0.322
Female	6 (6.7%)	11 (12.2%)		
Age				
<20	3 (2.2%)	2 (1.8%)		
20–35	5 (5.6%)	8 (8.9%)		
35–50	8 (8.9%)	22 (24.4%)	10.048	0.040
50–65	20 (22.2%)	17 (18.9%)		
65–80	5 (6.7%)	3 (2.2%)		
Education				
Uneducated	7 (7.9%)	2 (2.2%)		
Primary	8 (9.0%)	9 (10.1%)		
Secondary	3 (3.4%)	10 (11.2%)		
High School	10 (11.2%)	10 (11.2%)	8.761	0.188
University	6 (6.7%)	14 (15.7%)		
Higher	2 (2.2%)	2 (2.2%)		
Other	2 (2.2%)	4 (4.5%)		

Table 3. Association between infection with *Toxoplasma gondii* and related risk factors

Variable	Target group		X^2	*p-value*
	IgG Positive	IgG Negative		
Own cat				
Yes	5 (5.6%)	3 (3.3%)	1.314	0.219
No	34 (37.8%)	48 (53.3%)		
Contact cat				
Yes	35 (38.9%)	35 (38.9%)	5.701	0.015
No	4 (4.4%)	16 (17.8%)		
Blood transfusion				
Yes	4 (4.4%)	4 (4.4%)	0.159	0.723
No	35 (38.9%)	47 (52.2%)		
Undercooked meat				
Yes	6 (6.7%)	7 (7.8%)	0.049	0.528
No	33 (36.7%)	44 (48.9%)		
Drinking water				
Yes	13 (14.4%)	9 (10.0%)	2.944	0.071
No	26 (28.9%)	42 (46.7%)		
Gardening				
Yes	12 (13.3%)	21 (23.3%)	1.031	0.380
No	27 (30.0%)	30 (33.3%)		
Unboiled milk				
Yes	19 (21.1%)	22 (24.4%)	0.278	0.671
No	20 (22.2%)	29 (32.2%)		
Travel abroad				
Yes	6 (6.7%)	6 (6.7%)	0.251	0.757
No	33 (36.7%)	45 (50.0%)		

and eating undercooked meat ($p>0.5$), drinking rainwater and well water ($p>0.07$), drinking unboiled milk ($p>0.6$). Also, other ways of acquiring infection such as blood transfusion ($p>0.7$), gardening ($p>0.3$), and travel abroad ($p>0.7$) showed no statistically significant impact on the seropositivity of *toxoplasmosis*.

DISCUSSION

The seropositivity of the present study is in good agreement with a survey done by Saki *et al.* (2016); they were investigating the seropositivity of anti *T. gondii* IgG and IgM antibodies among pregnant diabetic and non-diabetic patients. Their results confirmed that 42.7% of pregnant diabetic patients were positive to IgG and 2.7% to IgM. In addition, results obtained by Abdelsalam, (2013) are highly comparable with the current study which they found that 45% of patients with *Diabetes mellitus* type 1 infected with *T. gondii*.

Also, seropositivity was noted nearly to the present study in some studies by Goekce *et al.* (2008), Sharad & AL-Hamairy (2015), Gokce *et al.* (2008) who examined the seroprevalence of IgG *T. gondii* and found that 56.26%, 51%, and 56.6% respectively. Conversely, seropositivity of IgG recorded in the current study among diabetic patients was lower than that stated by Molan & Ismail (2016), Shirbazou *et al.* (2013) and Jafari Modrek *et al.* (2015) which were 66%, 60.43%, and 70.7% respectively. The difference between the studies may be due to the geographic variation of the prevalence of *T. gondii*.

In the present study, no one of the diabetic subjects presented positive of IgM against *T. gondii*; the same results obtained by Goekce *et al.* (2008), Molan & Ismail (2016), and Alvarado-Esquivel *et al.* (2017).

Crucially, current study apparently showed that age plays a significant a role ($p<0.04$) in infection with *T. gondii* which high seropositivity rate recorded among old age between 50–65 and this is corroborated with previous results by Jafari Modrek *et al.* (2015) and Molan & Ismail (2016). The possible reason for impact of age on the prevalence of *T. gondii* is that older adults are accumulation exposed to different risk factors of *T. gondii*.

Concerning to the effect of contact with cats on the prevalence of *T. gondii*, the result of our study suggest the seropositivity with *T. gondii* on diabetic patients who contact with a cat has statistically significant risk ($p<0.01$) than who not. This indicates that the contact with a cat was the main source of acquiring the infection in diabetic patient in the current study area. Other risk factors which investigated in this study did not show any statistically significant of increased risk of acquiring infection.

The possible role of *T. gondii* in diabetes that inflammation destruction of pancreatic β cells induces decline in β cells mass and that will lead to failure of β cells to produce sufficient insulin (Molan *et al.*, 2016). Also, a study based on diabetic rat show that after infected with *T. gondii* they observed a significant increase in the number of *T. gondii* cysts in the brain compared to uninfected diabetic rat (Hassanain *et al.*, 2014).

The current study indicates that diabetic patients have latent toxoplasmosis. So, bradyzoites will remain detectable in the host for the entire life of the host in several body sites, such as central nervous system (Kamerkar & Davis, 2012). And it has been reported that some microorganisms become more virulence in diabetic cell (Geerlings & Hoepelman, 1999; Casqueiro *et al.*, 2012).

Consequently, diabetic patients considering as weakened immune system, may face significant neurological problem due to reactivation of latent infection such as schizophrenia, road traffic accident and epilepsy. Also, they are more vulnerable due to their immune state to ocular complication such as chorioretinitis and cataract.

It plausible that, some of limitations could have influenced the results obtained such as the ability of diabetic patients to participate in the study.

CONCLUSION

In this study, we found that latent *T. gondii* in diabetic patients in Makkah, Saudi Arabia is relatively high especially among age group 50–65 and when diabetes decreased their immune system, it may cause several complications. The evidence from this study suggests that diabetic patient should avoid contact with cats and screen for anti *T. gondii* IgG to prevent the risk of ocular complication associated with toxoplasmosis.

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