Anti-*Toxoplasma gondii* IgG, IgM, and IgA among Type-2 Diabetic Patients in Benghazi Libya: A Comparison Study

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Abstract

Toxoplasma gondii is an important opportunistic parasite in immune compromised hosts like Diabetes Mellitus (DM) patients. On other hand, diabetes is a systemic disease which affects the immune system, diabetes mellitus is an increasing problem in our country today and diabetics have been reported to be immunocompromised. Hence, detection and monitoring of anti-Toxoplasma antibodies are of a great interest in Diabetes Mellitus (DM) patients.

Aim: To identify the prevalence of *Toxoplasma gondii* in Type 2 diabetic Patients using ELISA methods in Benghazi, Libya.

Settings and Design: A case control study of Type 2 diabetic patients was conducted at Benghazi diabetic and endocrine centre between August to November 2016.

Materials and Methods: Two hundred and fifty serum samples including 200 from diabetic Type 2 patients (125 Female and 75 Male) and fifty samples from healthy non-diabetics (32 Female and 18 Male) were used as a control group. A total of 250 blood samples were taken from individuals aged from 18 to<51 years. The Enzyme-Linked Immunosorbent Assay (ELISA), were used to detect Levels of IgG, IgM, and IgA antibodies against *Toxoplasma gondii* in the patients' sera. Cases and controls were matched for detection of Toxoplasma (Ab) with demographic parameter such as gender, age, abortion and the presence of wild animals especially cats.

Statistical Analysis: Statistical analyses were performed using the Statistical Package for the Social Sciences version 17; SPSS Inc, Chicago, IL, USA).

Results: The overall Prevalence of infection with *Toxoplasma gondii* among Type 2 diabetic patients [IgM=21 (10.5%), IgG=83 (41.5%) and IgA=7 (3.5%)] was higher than non-diabetics individual group [IgM=(4%), IgG=(24%) and IgA=(0%)].

Conclusion: Anti-*Toxoplasma gondii* IgG, IgM, and IgA levels among Type 2 diabetic Patients was higher than non-diabetic in Benghazi, Libya.

Keywords: *Toxoplasma gondii*; ELISA; Diabetes; Benghazi; Libya

Introduction

Toxoplasmosis is a zoonotic infection caused by a ubiquitous intracellular protozoa parasite a *Toxoplasma gondii*, belonging to family Toxoplasmidae and Class Toxoplasmidae [1]. The protozoon infects different warm-blooded organisms, including human, birds, and rodents, as the intermediate hosts. Moreover, it infects domesticate and wild cats as the definite host [2,3].

Toxoplasma gondii generally, cause a self-limited, asymptomatic disease in immune competent patients, but is an important opportunistic parasite in immunocompromised people. Primary maternal infection during pregnancy can be transmitted to the foetus and result in serious sequelae (3) during the acute infection of toxoplasmosis, tachyzoites rapidly proliferate. In this stage, the parasite attacks the nucleated cell *via* active invasion. After proliferation, the tachyzoites destroy and kill the host cells and disseminate to the central nervous system, eyes, cardiac and skeletal muscles, and placenta *via* blood circulation. Finally, the immune system response causes transformation of the tachyzoites into bradyzoites, and the tissue cysts are developed. The tissue cysts remain alive and are able to sustain in the host body throughout the host lifespan. [4].

On the other hand, diabetes is the disease influences the cellular and humoral immunity predisposing the patient to many opportunistic parasites one of them is *Toxoplasma gondii*. It's well known that Both diabetes and toxoplasmosis can cause congenital malformation of the foetus.

Consequently, the aim of our study focused on the serologic detection of *Toxoplasma gondii* infection in diabetic patients and compared to non-diabetic control.

Materials and Methods

Study design and Patients

A case control study was conducted in Benghazi city. The study has been done for 250 serum samples including 200 of them Type 2 diabetic patients (125 Female and 75 Male) recruited from government diabetes and endocrine centre (Syde Hussein) and 50 cases of healthy non-diabetics (32 Female and 18 Male) control group has been recruited randomly from the Alhaya laboratory during the period from August to November 2016. Cases and controls were matched with demographic parameters such as age, gender, abortion and the presence of wild animals especially cat's and history of diabetic patients was recorded.

Methods

Antibody detection: Antibody detection using Elisa test to detect specific Toxoplasma antibodies and IgA**.**

Blood samples and preparation of sera: Blood samples were collected from the venepuncture of the patient into plain sterile tubes, left to clot at room temperature an hour, and centrifuged subsequently at 3000 RPM for at least 20 min at room temperature. the separated sera were stored at -20ºC or below until analysis of *T. gondii* by ELISA [5-7].

Determination of antibodies to *T. gondii*: All 250 serum samples were tested using Euroimmun enzyme-linked immunosorbent assay (ELISA) kits (PerkinElmer Company Euroimmun, Lübeck, Germany) for the presence of Toxoplasma IgG, IgM and IgA antibodies. Positive and negative controls were used with each series of anti *T. gondii* IgG/IgM/IgA test IgG and IgM and IgA indexes of<0.8 were regarded as the negative results, between 0.8 to 1.1 as the borderline, and ≥ 1.1 as the positive results, Positive IgG titers and negative IgM titers were considered a latent infection. Sera with concurrent positive IgG and IgM titers were further analysed by Elias test, results were obtained by reading the optical density at 450 nm using spectrophotometer and values were interpreted comparing with the cut-off value.

Data processing and analysis: Data was colleted and analyzed with Statistical Package for Social Sciences (SPSS) version 17 on Windows XP. Chi-square test was used to compare the differences in prevalence of *Toxoplasma gondii* Ab between the age groups, gender, abortion and the presence of wild animals. Data were considered statistically significant when the P-value was<0.05.

Results

In the present study out of 200 (38 positive and 162 negative) among Patients with Type 2 diabetic subjects who were tested

(41.5%) were seropositive for Toxoplasma - IgG antibodies, 21/200 (10.5%) and 7/200 (3.5%) were seropositive for IgM and IgA antibodies, respectively, while in control group, 50 individuals 12 (24%) were positive for IgG and 2 (4%) IgM antibody. IgA antibody was negative for all samples of control group (0 %) (**Table 1 & Figure 1**).

Table 1: Prevalence of Antibodies IgG, IgM and IgA Anti-*Toxoplasma gondii* among studied group's diabetic and non-diabetic patients.

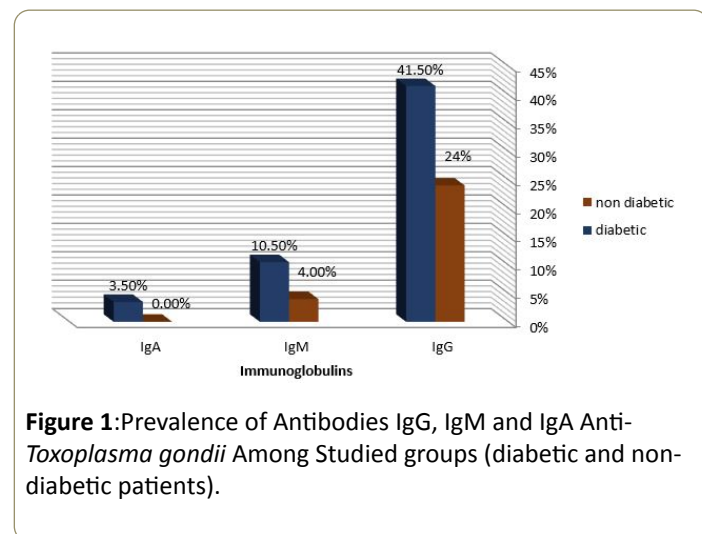| Diabetic			
Immunoglobulins	**Negative**	**Positive**	**Total**
IgG	117 (58.5%)	83 (41.5%)	200
Ig M	179 (89.5%)	21 (10.5%)	200
IgA	193 (96.55%)	7 (3.5%)	200
Non diabetic			
Immunoglobulins	**Negative**	**Positive**	**Total**
IgG	38 (76.0%)	12 (24%)	50
IgM	48 (96.0%)	2 (4.0%)	50
IgA	50 (100%)	0 (0.0%)	50

Figure 1:Prevalence of Antibodies IgG, IgM and IgA Anti-*Toxoplasma gondii* Among Studied groups (diabetic and non-diabetic patients).

The result shows no statistical difference in antibodies prevalence according to age either in diabetic or healthy non-diabetic control except for IgG level in diabetics where significantly higher in younger diabetic's (IgG: X2=10.779a, P=0.013). Similarly, no statistical difference in antibodies prevalence according to sex in either group is shown in (**Tables 2-4**).

Table 2 : Prevalence of Antibodies IgG, Ig M and IgA Anti-*Toxoplasma gondii* among diabetic patients according to age groups.

Age group	No. of exam	IgM infected	IgG infected	IgA infected	Statistical difference of toxoplasma antibodies between age groups
(18-28)	79	9 (11.4%)	23 (29.1%)	3 (3.8%)	IgG: X2 10.779a, P=0.013
(29-39)	84	10 (11.9%)	39 (46.4%)	4 (4.8%)	IgM: X2 1.812a, P=0.612
(40-51)	29	1 (3.4%)	15 (51.7%)	0 (0%)	
(>51)	8	1 (12.5 %)	6 (75.0%)	0 (0%)	IgA: X2 1.759a, P=0.624
Total	**200**	**21 (10.5%)**	**83 (41.5%)**	**7 (3.5%)**	

Table 3: Prevalence of Antibodies IgG and IgM Anti-*Toxoplasma gondii* among non-diabetic patients according to age groups.

Age group	No. of exam	IgM infected	IgG infected	Statistical difference of toxoplasma antibodies between age groups
(18-28)	6	0 (0.0%)	0 (0.0%)	IgG: X2 7.502a, P=0.058
(29-39)	11	0 (0.0%)	3 (27%)	
(40-51)	10	0 (0.0%)	1 (10%)	IgM: X2 1.437a, P=0.697
(52-62)	23	2 (100%)	8 (34.8%)	
Total	**50**	**2**	**12**	

Diabetic with domestic cat contact they have significantly higher antibodies (IgG, IgM, IgA) seropositivity compared to diabetic with no history of domestic cat contact. (IgM: X2=20.231a, df=1, P=0.00; IgG: X2=48.445a, df=1, P=0.000; IgA: X2=11.991a, df=1, P=0.001).While in non-diabetic only IgG was statistically different. (IgM: X2=.136a, df=1 P=.712; IgG: X=212.348a, df =1, p= 0.000) in (**Tables 5 & 6).**

Table 4: Prevalence of Antibodies IgG, Ig M and IgA Anti-*Toxoplasma gondii* according gender among diabetic and non-diabetic participants.

		Diabetic			
Sex	No. exam	IgM infected	IgG infected	IgA infected	Statistical difference of toxoplasma antibodies between age groups
M	75	9 (12.0%)	31 (41.3%)	1 (1.3%)	Ig M: X2 0.287a, P=0.592
F	125	12 (9.6%)	52 (41.6%)	6 (4.8%)	IgG: X2 0.001a, P=0.970
Total	**200**	**21 (10.5%)**	**83 (41.5%)**	**7 (3.5%)**	IgA: X2 1.668a, P=0.197

		Non-Diabetic			
M	18	1 (5.6%)	6 (33.3%)	0	IgM: X2 3.391a, P=0.066
F	32	1 (3.1%)	6 (18.8%)	0	IgG: X2 0.233a P=0.630
Total	**50**	**2**	**12**	**0**	

Table 5: Prevalence of Antibodies IgG, IgM and IgA Anti-*Toxoplasma gondii* and contact with domestic cat among diabetic patients.

	No. exam	IgG infected	IgM infected	IgA infected
Contact with cat	40	36 (90.0%)	12 (30.0%)	5 (12.5%)
Non-contact	160	47 (29.4%)	9 (5.6%)	2 (1.3%)
Total	**200**	**83**	**21**	**7**

Table 6: Prevalence of Antibodies IgG and IgM Anti-*Toxoplasma gondii* and contact with domestic cat among non-diabetic participants.

	No. exam	IgG infected	IgM infected
Contact with cat	15	6 (40.0%)	1 (6.7%)
Non-contact	35	6 (17.1%)	1 (2.9%)
Total	**50**	**12**	**2**

The clinical inference of antibody status of all participant show (41.5%) have probably previous exposure to toxoplasmosis and (56%) seronegative and (10.5%) of recent infection, (3.5%) only IgA positivity of acute infection and others of probably of reactivated infection (**Table 7**).

Table 7: The antibody status of the participant and their clinical inference.

Inference	No.	%	Antibodies Status
Previous exposure	83	41.5	IgG (+) IgM (-)
Recently infection	21	10.5	IgM (+) IgG (-)

The IgA test is used by some laboratories as an additional marker of acute infection	7	3.5	IgA (+)
Reactivated?	6	3	IgA (+) IgG (+) IgM (+)
Reactivated or chronic *T. gondii* infection	16	8	IgG (+) IgM (+)
Seronegative	112	56	IgG (-) IgM (-)

Discussion

This is the first study aiming to determine anti toxoplasma antibodies in diabetic patients attending at government diabetes and endocrine centre in the city of Benghazi. In the present study out of 200 (38 positive and 162 negative) among Patients with type 2 diabetics who were tested, 41.5% were seropositive for Toxo- IgG antibodies,10.5% (21/200) and 3.5% (7/200) were seropositive for IgM and IgA antibodies, respectively, while in control group, 50 individuals 12 (24%) were positive for IgG and 2 (4%) IgM antibody. IgA antibody was negative for all samples of control group (0%) therefore; these findings suggest that diabetics are more susceptible to Toxoplasmosis than those without.

This result in agreement of which they found with Shirbazou et al. the prevalence of IgG antibodies against *Toxoplasma gondii* in diabetic patients and healthy controls were 60.43% and 38% respectively. Also in accordance with of Which demonstrate with Entsar J. Saheb that the seroprevalence of toxoplasmosis in diabetic patients was found to be comparatively higher than in non-diabetic patients [8,9].

The relation between diabetes and *T. gondii* infection is still poorly understood, in a study conducted by Arshad et al. the analysis revealed that Toxoplasma infection was more prevalent in diabetic patients than non-diabetic individuals. Results also showed that insulin level was higher in persons with chronic infection [10].

While in anon comparison study in Iran has shown that 70.3% among diabetic patients were seropositive for Toxoplasma antibodies [11]. Similarly, Gocke et al. study confirmed that 56% of diabetic patients were positive for anti-*T. gondii* IgG. [12].

The value of 24% seroprevalence of anti-*T. gondii* IgG in control group is lower to a study by Akinbami et al. in Nigeria 40.8 %.in non-diabetic pregnant women [13].

While the result of the current study is lower than 81.1% reported by Zemene et al. In the South western and Central Ethiopia respectively [14,15].

Similarly the results found in our study of anti-*T.gondii* IgM antibodies was 10.5% (21/200) was in higher compared to 5.3 % reported by Silva et al. [12,16].

IgA and IgM class antibodies are associated with recent infections (acute infection) while IgG class antibodies are associated with chronic infection. However, the IgA antibody test is not commonly used, and mostly used to detect the disease in cases of congenital infection. Thus, the IgA antibody test is an important marker for *T. gondii* infection in new born babies. In our study the anti-*T. gondii* IgA was low 3.5% compared to 69.4% reported by Fernando et al. in high risk pregnant female. However, in the current study the anti-*T. gondii* IgA was 0% in healthy control group which is similar to finding of Carmen et al among healthy individuals in Slovakia [17,18].

In contrast in a case control study in Durango City, Mexico they conclude that there is no serological evidence of an association between *T. gondii* infection and diabetes mellitus in the studied subjects [19].

A systematic review and Meta-analysis of case-control studies was published the researcher conclude that chronic toxoplasmosis as a possible risk factor for type 2 DM. However, based on random effects model no statistically significant association was observed between *T. gondii* and type 1 (DM).

The result shows no statistical difference in antibodies prevalence according to sex either in diabetic or non-diabetics, this in contrast to finding of Entsar J. Saheb [9]. The same finding with age except for IgG level in diabetics where significantly higher in younger diabetic's (X2=10.779a, P=.013) which in agreement with Entsar J. Saheb.

Diabetic with history of cat contact they have significantly higher Toxoplasma antibodies (IgG, IgM, IgA) seropositivity compared to diabetic with no history of domestic cat contact, while in non-diabetic only IgG was statistically different.

A Meta-Analysis published at 2016, show that cat contact can increase the risk of infection by *T. gondii* in various populations, including the general population, immune-suppressed/pregnant individuals, those with special occupations, and cat(s) owners, but while significant, cat contact may not be the most important risk factor for infection by *T. gondii* in cat owners Wei HX et al. [20,21].

Conclusion

Anti-*Toxoplasma gondii* IgG, Ig M, and IgA levels among Type 2 diabetic Patients was higher than non-diabetic in Benghazi, Libya. Gender and age were not determining variable except for younger diabetic were the IgG was higher. History of domestic cat contact was risk variable for diabetics.

Acknowledgement

Disclosure

Conflict of interest

No conflict of interest.

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