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Seroprevalence of *Toxoplasma gondii* Infection in Diabetic Patients at Kirkuk province

Sarah Farhad Nihad[a]*, Sadia Shahab Hamad[b]

[a,b]*Department of Biology / College of Science / Kirkuk University / Kirkuk / Iraq*

Abstract

Toxoplasma gondii is an important opportunistic parasite in immunocompromised people .On other hand , diabetes is the disease affecting in immune system and minimizes cellular and humoral immunity. Both toxoplasmosis and diabetes are very common in Iraq and other countries. The main aim of the current study was investigated the seroprevalence of anti-*Toxoplasma gondii* antibodies IgG and IgM in the blood of diabetic patients attending Azadi Teaching Hospital, Kirkuk General Hospital within Kirkuk province for the period of January 2016-February 2017 , with a group of non diabetic patients to serve as a control group . The Enzyme Linked Immuno Sorbent Assay (ELISA) technique was implemented to detect the antibodies for *Toxoplasma gondii* in the sera of 100 patient with diabetic and 45 non diabetic apparently healthy control. A total of 100 sample obtained from diabetic patients (44 male and 54 female with age range (20-60 years) were examined for the presence of *toxoplasma* antibodies the results showed , 31 cases (31%) were positive included 10% for IgM and 21% for IgG , None of the apparently healthy controls showed seopositivity for *T. gondii* IgM antibodies but seopositivity for *T. gondii* IgG antibodies were in 5 cases(11.11 %). The relationship between diabetes and *T. gondii* was evaluated using chi-square test ($P< 0.05$) and ($P< 0.01$). As long as the diabetes lowers the immune response, it may pave the way to toxoplasmosis so our hypothesis is that toxoplasmosis paves the way for diabetes and diabetes paves the way for diabetes but this depends on which one establishes first.

Keywords: Toxoplasma gondii ; Diabetes Mellitus; ELISA.

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* Corresponding author.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite, capable of infecting all warm-blooded organisms , including humans, birds, and rodents, as the intermediate host . Morever , it infects domesticate and wild cats as the definite host [1] . There are two main routes of *T. gondii* transmission to humans: ingestion of food or water contaminated with oocysts shed by *T. gondii* infected cats, and eating raw or undercooked meat containing tissue cysts [2]. Most *T.gondii* infections are asymptomatic but some infected individuals may develop clinical manifestations of toxoplasmosis including lymphadenopathy, chorioretinitis, and meningoencephalitis [3, 4]. A reactivation of a *T.gondii* infection in immunocompromised patients may lead to a life-threatening disease with involvement of the central nervous system [1,3] After infection *T. gondii* spreads to many organs of the host [5] The presence of *T.gondii* infection in pancreas has been reported in humans and animals. In humans, *T.gondii* infection may cause pancreatitis [6] Some studies have reported that tissue cysts of *T.gondii* as the cause of tissue necrosis and pancreatitis in cats and koalas [7] In pathological studies on acute toxoplasmosis , the death of infected mice owing to disseminated toxoplasmosis and involvement of liver , spleen , and pancreas have been reported [8] .

During the acute infection of toxoplasmosis , tachyzoites rapidly proliferate .In this stage , the parasite attacks the nucleated cell by active invasion . After proliferation the tachyzoites destroy and kill the host cells and disseminate to the central nervous system , eyes , cardiac and skeletal muscle , placenta by blood circulation. Continuation of the parasite proliferation leads to the host cell death. Finally the immune system response cause 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. In the chronic infection, necrosis always often found in brain and eye [9,10].

Some other studies have shown the relationship between diabetes insipid in children and congenital toxoplasmosis [11] based on the hypothesis that toxoplasmosis may act as a risk factor in development or activation of diabetes for the first time [12].

diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [13] Currently, the levels of IgG and IgM antibodies in serum can easily be measured using available techniques .Serologic methods are techniques used to measure the level of infection to *Toxoplasmasis* in humans and animals. Amongst these methods, the most common techniques are ELISA and IFA or indirect immuno-florescence [14]. The present study aims to clarify the relationship between *T. gondii* and IgM/IgG titers in diabetic patients .

2. Materials and methods

2.1 Selection of patients

This cross-sectional study was performed on men and women referred to Azadi teaching Hospital, Kirkuk general hospital Kirkuk City, Iraq during the period from January 2016-February 2017. More ever blood samples were obtained from 145 serum samples including 100 from diabetic cases and 45 from healthy non-diabetic controls were investigated. The samples (2-3 ml) were centrifuged and the sera were used in this study which have been kept at -20°C until have been used. Questioners have been given to all participants regarding the demographic characteristics and some risk factors of toxoplasmosis.

2.2 Serological Technique

The sera 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) method. The ELISA kit was provided by a commercial manufacturer (Biochek , USA). The procedure was done according to the instructions of the manufacturer of the kit.

2.3 Statistical analysis

Data were analyzed using SPSS software (version 19). The chi-square test was used to compare the seroprevalence values, related to the characteristics of the subjects. $P < 0.05 - 0.01$ was considered as the level of significance.

3. Results

The result of table 1 showed that 31% and 5% of the diabetic patients and control group respectively the seropositive for *toxoplasmosis* IgG was 21% of the diabetic patient and 5% of healthy control group while seropositive *toxoplasmosis* for IgM antibody was 10% of the diabetic patient and non of the healthy control individuals showed seropositivity for *T.gondii* IgM antibodies and the difference between the two groups was not significant.

Table 1: Percentage seropositivity of the anti- *Toxoplasma gondii* IgG and IgMantibodies in diabetic patients and healthy controls

Group	Number tested	Number of positive	Anti –toxoplasma IgG	Anti-toxoplasma IgM
Diabetic patient	100	31	21	10
Control group	45	5	5	0
Total	145		26	10
P- value = 0.360				

Table 2 shows the effect of the gender on the seropositivity rate with the anti-*T. gondii* IgG and IgM among the diabetic patient .The results showed that the seropositivity rate was significantly higher (P< 0.05) in females

42.9% than in males 11.36%.

Table 2: Relationship between toxoplasmosis and gender in the diabetic patient

Gender	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
male	44	5	11.36	4	9.09	1	2.27
female	56	24	42.9	15	26.78	9	16.07
P-value = 0.0007							

Regarding the impact of the age on the seropositivity rate, table 3 shows the age group (30-39) showed the highest rate 66.66% followed by the age groups (40-49) 52.38%, (50-59) 30%, (20-29) 25% and above 60 years 5.55% and significant differences ($p < 0.05$) were recorded in the prevalence of the parasite according to age .

Table 3: Relationship between positive anti –*toxoplsma* and IgG and IgM according age in diabetic patients.

Age range	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
20-29	8	2	25	2	25	0	0
30-39	15	10	66.66	6	40	4	26.66
40-49	21	11	52.38	9	42.85	2	9.52
50-59	20	6	30	2	10	4	20
Above 60 years	36	2	5.55	2	5.55	0	0
P value = 0.021							

The results showed that no significant difference in the seropositivity rate was found according to the type of residence , Individuals living in rural areas showed higher seropositivity rate 51.61% than their counterparts living in the urban areas 22.38% .

Table 5 shows the effect of the blood group on the seropositivity rate with the anti-*T. gondii* among the diabetic patient and the results showed that the highest rate 41.66% in the blood group A followed by 28.57% in blood group B, 26.47% in O and 11.11% in the AB and significant differences ($P<0.01$)were recorded in the prevalence of the *T.gondii* according to blood group .

Table 4: prevalence of *T.gondii* in diabetic patient according to type of residence

Type of residence	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
rural	31	16	51.61	10	32.25	6	19.35
urban	67	15	22.38	11	15.94	4	5.79
P value = 0.880							

Table 5: prevalence of *T.gondii* in diabetic patient according to blood group

Blood group	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
A	36	15	41.66	14	38.88	1	2.77
B	21	6	28.57	3	14.28	3	14.28
AB	9	1	11.11	0	0	1	11.11
O	34	9	26.47	4	11.76	5	14.70
P- value = 0.002							

Table 6 showed that no significant difference in the seropositivity rate was found according to occupationUnemployed individuals showed higher seropositivity rate 31.25% than Employed individuals 30% .

Table 6: prevalence of *T.gondii* in diabetic patient according to occupation

occupation	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
Employed	20	6	30	5	25	1	5
Unemployed	80	25	31.25	16	20	9	11.25
P value = 0.477							

Table 7 showed that the seropositivity rate of *T.gondii* according to education level , uneducated individuals showed higher seropositivity rate 45.94 % than Educated individuals 22.22% .

Table 7: prevalence of *T.gondii* in diabetic patient according to education level

Education level	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
Educated	63	14	22.22	7	11.11	7	11.11
Uneducated	37	17	45.94	14	37.83	3	8.10
P value = 0.054							

Table 8 showed the effect of the drinking milk in the seropositivity rate of *T.gondii* antibodies IgG and IgM among the diabetic patient group and the result showed that the seropositivity rate was higher in the individuals who drinking non pasteurised milk 33.33% than in the individuals who drinking pasteurised milk 28.76%.

Table 8: prevalence of *T.gondii* in diabetic patient according to drinking milk

Drinking milk	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
Pasteurised milk	73	21	28.76	15	20.54	7	9.58
Non Pasteurised milk	27	9	33.33	6	22.22	3	11.11
P value = 0.935							

The result showed that the seroprevalence of *T.gondii* was higher within the individuals who was with contact with soil 40% than the individuals who wasnt with contact with soil 29.41% .

Table 9: prevalence of *T.gondii* in diabetic patient according to contact with soil

Contact with soil	Number tested	Number of positive	%	Anti –toxoplasma IgG		Anti-toxoplasma IgM	
				No	%	No	%
Yes	15	6	40	4	26.66	2	13.33
No	85	25	29.41	17	20	8	9.41
P value = 0.686							

4. Discussion

The main objective of the present study was to investigate the seroprevalence of the anti-*Toxoplasma gondii* IgG and IgM antibodies in the blood of diabetic patient. This study is the first to be conducted in these Provinces. The results of the current study revealed that 31% of the diabetic subjects were found seropositive for the *T. gondii*. The risk factor for *T. gondii* infection in diabetic patients was about two folds higher than in healthy controls. Therefore, patients infected with *T. gondii* may be more at risk to develop diabetic than uninfected individuals [15]. Therefore, these findings suggest that *Toxoplasmosis* patients are more susceptible to be diabetics than those without. Destruction of the pancreas occurs in three phases of *Toxoplasma gondii*:

1. Hyperactive phase (hyper-period) in which β-cell destruction of nerve cells and less interference in the insect in a hyperactive state of the pancreas, insulin secretion is sometimes excessive, often resulting in low blood sugar, or a too low blood sugar, this stage is often during adolescence.

2. Disordered phase (compensatory phase), in which neurons and pancreatic β-cells have a considerable amount of damage, under normal circumstances, secretion of insulin will be inadequate, the body will start the compensatory function. Thus, this phase of insulin secretion over time, when few in the disordered state.

3. Decline phase (recession), in which nerve cells and β-cells destruction of more compensatory function reached its limits.

The risk factor for *T. gondii* infection in diabetic patients was higher than in healthy controls. Therefore, patients infected with *T. gondii* may be more at risk to develop diabetic than uninfected individuals [16].Regarding the effect of gender on the seropositivity rate of toxoplasmosis in diabetic patient , the results of the current study revealed that the seropositivity rate among the female was significantly higher (P<0.05) than that among males. Similarly, Siyadatpanah and his colleagues [12] and Modrek and his colleagues [16] found that the seropositivity rates with anti-*T. gondii* antibodies were significantly higher in females than in male diabetic subjects in Iran. In contrast, Sharad and Al-Hamairy[17] reported that the seropositivity rates with anti-*T. gondii* antibodies were significantly higher in males than in female diabetic subjects in Iraq. The controversy between different studies may be due to the size of the samples and the residency of the tested participants, whether from rural or urban areas and the contact with the risk factors. The results of the present study clearly showed that the highest seropositivity rate was found among the women of old age . This may be related to the fact that old women involved heavily in the house work, especially dealing with meats, preparing salads, cooking, cleaning and consequently be more exposed to the risk factors of toxoplasmosis and other diseases. Regarding the residency of the patients and its relation with seropositive of *T.gondii* the result showed no significant differences between *Toxoplasma* Abs distribution on both rural and urban areas through which the rate were 51.61% and 22.38% respectively . this may be due to the hygiene and the differences in samples and races and the exposure to the infectious in the rural higher than urban. The present study showed that the number of unemployed individuals infected with *T.gondii* was higher than employed individuals , but with no significant differences this may be due to that the specimens in this study most of them were from unemployed individuals compared to employed individuals. Result showed that the number of infected uneducated individuals with toxoplasmosis was higher than educated individuals this may be to the lower levels of education are associated with socioeconomic status or may be due to sample size. The result showed the individuals who drinking non pasteurised milk infected with *T.gondii* higher than the individuals who drinking pasteurised milk , this may be due to the contaminated tools used for drinking milk or milk itself by *T.gondii.* milk may serve as a potential source for human toxoplsmosis . Regarding to contact with soil the high seropsitivity rate recorded within the individuals who contact with soil , Contact with soil was identified as a risk factor for *toxoplasma* infection Cats excrete oocysts up to 10 million oocysts per day**s** for only two weeks of their life, when they first acquire infection. Oocysts become infective one to five days after excretion, are spread by surface water, and can survive for more than a year [18].

5. Conclusion

A strong association has been found between toxoplasmosis and both types of diabetes. As long as the diabetes lowers the immune response, it may pave the way to toxoplasmosis so our hypothesis is that toxoplasmosis paves the way for diabetes and diabetes paves the way for diabetes but this depends on which one establishes first.

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