Determine the IgG and IgM antibodies created against *T. gondii* infections using the ELISA method in diabetic pregnant women in compared with non - diabetic pregnant women in Sanandaj, Kurdistan, west of Iran

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Abstract

Background: *Toxoplasma gondii* is an opportunistic parasitic protozoan, which is a causative agent of serious complications such as abortion or fetal still birth in pregnant women. The purpose of this study was evaluating the seroprevalence of toxoplasmosis and risk factors in diabetic pregnant women (case) in comparison with non-diabetic pregnant women (control) from Sanandaj, Kurdistan, west of Iran.

Methods: In this case-control study *Toxoplasma gondii* antibodies were tested

in 91 cases as well as 90 controls from referred the Toohid hospital diabetes center using ELISA (enzyme-linked immunosorbent assay) method during June 2018 to October 2019 in Sanandaj, Kurdistan, west of Iran. The collected data were analyzed by SPSS 19 and using Chi-Square and Fisher tests.

Results: The seropositivity for anti-Toxoplasma IgG in case group was 33% and 15.6% in the control group ($p = 0.006$) showing a statistically significant difference between groups, while for anti-Toxoplasma IgM ($p = 0.31$) showing no statistically significant differences between two groups. A significant relationship was observed between age ($p = 0.024$) and abortion history ($p = 0.05$) with anti-Toxoplasma IgG antibodies in cases and controls.

Conclusion: The results of current study indicated statistically significant relationship between diabetes and toxoplasmosis. The abortion history and increase of age was found to be a major *T. gondii* infection risk factor.

Background

Toxoplasmosis is a zoonotic disease caused by an obligate intracellular protozoan parasite called *Toxoplasma gondii*, with a worldwide distribution. Felids are the definitive hosts of the parasite, while many other mammals and birds serve as intermediate hosts (1). *Toxoplasma gondii* has different forms of tachyzoite, oocyst and tissue cyst. In cat epithelial cells, the parasite sexual produces millions of oocysts which are released in the feces (2). *T. gondii* is transmitted through contaminated food, meat, water and the placenta during pregnancy. Blood transfusion and organ transplantation have also been mentioned as possible infection risk factors (3). This parasite can lead to chronic infection in individuals, serious problems and death in immune compromised patients, hydrocephalus in congenitally infected newborns, intrauterine fetal death, and spontaneous abortion during pregnancy (4). Several studies found a positive correlation between toxoplasmosis with schizophrenia (5), and depression(6).

About 20% to 90% of the world's adult population in different regions is reported to have had contact with the parasite but only about 10% of infected individuals develop clinical signs and symptoms (7). High prevalence of the infection has been reported among pregnant women from different foci: in Africa, the prevalence varies between 50%-70% (8), in Europe, between 9%-67% (9), whereas in Asian countries indicated between 0.8% to 28.3% (10). Seropositivity of *T. gondii* among Iranian women was 48% -74.6%

in the northern area (11), 33% - 44% in the northwest (12), 22% -37% in the southwest (13), and 41.4% in central parts of Iran (14). Overall, there are different reports and several hypotheses demonstrating that toxoplasmosis increases susceptibility to acquiring diabetes and, on the other

hand, diabetic patients are more susceptible to a variety of infections such as toxoplasmosis (15-16). WHO (World Health Organization) defines diabetes as a chronic disease when the pancreas does not produce enough insulin, or when the body cannot effectively use insulin. GDM (Gestational diabetes mellitus) is a subtype of DM and is defined as glucose intolerance with onset or first recognition during pregnancy (17). There are serologic techniques to measure the level of *T.gondii* antibodies in humans and animals. ELISA is a most common standard method among serologic methods which is for examining anti-*T. gondii* antibodies. This method is easy to perform, widely available, and has high sensitivity and specificity (18). The aim of this study was to determine the IgG and IgM antibodies created against *T. gondii* infections using the ELISA method in diabetic pregnant women in compared with non-diabetic pregnant women in, Kurdistan, Sanandaj, west of Iran.

Methods

In this case-control study totally 181 blood samples were collected who 91 cases were registered as diabetic pregnant women(case) and 90 individuals were collected as non-diabetic pregnant women(control) referred Toohid hospital diabetes center during the period of June 2018 to October 2019 in Sanandaj, Kurdistan, west of Iran. Five mL of blood was taken from each person. Then, samples were centrifuged at 3000 rpm for 10 minutes. The sera were separated and kept at -20 °C until the assay. All subjects completed a questionnaire including demographic information and risk factors.

Questionnaire

The applied questionnaire anchored in demographic data and risk factors including age, education, contact to animals, contact to cats and soil, consumption of half-cooked, raw and frosty meat, blood transfusion, gestational age, history of abortion, use of well and pipe water, residence area and the number of parity was prepared before the collection of blood samples and evaluated in case and control groups.

ELISA Test

Serum samples were tested for level of anti-*toxoplasma* IgM and IgG antibodies according to the manufacturer's instructions ELISA kit (Pishtaz Teb Co, Iran). Based on the ELISA kit, If IgG and IgM antibodies concentration was > 1.1 were defined as positive results, and < 0.9 were defined as negative results.

Statistical analysis

The results of this study were analyzed using SPSS software (version 19). Determination of statistical significance was performed by Chi-square and Fisher tests to compare the seroprevalence values. $P \leq$

0.05 was considered in a significant level.

Results

In this study a total of 181 diabetic and non-diabetic pregnant women age ranging from 17 to 40 years had participated. The mean age was 32.89 ± 4 . Sociodemographic data of patients and controls are shown in Table 1, which showed patients consuming frosty meat were significantly higher ($p = 0.024$) than those of the control group (16.1%, vs. 6.7% respectively). Although no significant relationship was found between level of education in two groups ($p = 0.59$) but there was an increase in elementary phase in case group 24(26.4%) versus control group 11(12.2%). Distribution of the patients and control studied and their correlation with *T. gondii* seroprevalence was shown in Table 2; anti-*T. gondii* IgG antibodies were found in 30 (33%) of the cases and in 14 (15.6%) of the control group. The seroprevalence of *T. gondii* infection was significantly higher in cases than in controls ($p = 0.006$). Anti- *T. gondii* IgM antibodies were found in 6(6.6%) and 3(3.3%) in case and control groups respectively. There was no statistically significant difference in the frequency of IgM seropositivity among cases and controls ($p = 0.31$). Table 3 revealed significant correlation was found between age ($p = 0.024$) and abortion history ($p = 0.05$) with IgG seropositivity anti-*T. gondii* in two groups. We found no statistically significant relationship between all characteristics and risk factors and the IgM *T. gondii* antibodies.

Table 1
Socio-demographic data of patients and controls

		Case N %	Control N%	P value
Age groups (yr)	<=20	2(2.2)	0	0.324
	21–30 years	30(33.0)	35(38.9)	
	> 30 years	59(64.8)	55(61.1)	
Education	Illiterate	8(8.8)	5(5.6)	0.059
	Elementary	24(26.4)	11(12.2)	
	High school	12(35.3)	22(24.4)	
	Diploma	29(31.9)	29(32.2)	
	University	18(19.8)	23(25.6)	
Meat	Frosty	15(16.1)	6(6.7)	0.024*
	Half cooked	1(1.1)	3(3.3)	
	Raw	3(3.3)	0	
	Cooked	72(79.1)	81(90.0)	
Gestational age	1st trimester4119	21(23.1)	23(25.6)	0.419
	2nd trimester	27(29.7)	33(36.7)	
	Third trimester	43(47.3)	34(37.8)	
Residence area	Urban	69(75.8)	10(11.1)	0.66
	Rural	12(13.2)	73(81.1)	
	Margin	10(11.0)	7(7.8)	
History of abortion	Yes	24(26.4)	29(32.2)	0.38
	No	67(73.6)	61(67.8)	
Contact Cat	Yes	2(2.2)	4(4.4)	0.44
	No	89(97.8)	86(95.6)	
Contact soil	Yes	25(27.5)	24(26.7)	0.99
	No	66(71.6)	66(73.3)	
Contact animal	Yes	14(15.4)	9(10.0)	0.28
* Statistically significant at P < 0.05				

		Case N %	Control N%	P value
Infusion Blood	No	77(84.6)	81(90.0)	0.72
	Yes	3(3.3)	4(4.4)	
	No	88(96.7)	86(95.6)	
Use of water	Pipeline	88(96.7)	83(92.2)	0.21
	well	3(3.3)	7(7.8)	
N. Parity	0–2	83(91.2)	86(95.6)	0.24
	≥ 3	8(8.8)	4(4.4)	
* Statistically significant at P < 0.05				

Table 2
Distribution of patients and control groups
according to anti-*Toxoplasma* IgG, IgM

	Case NO (%)	Control NO (%)	P value
Anti-Toxo IgG			0.006*
Positive	30(33.0)	14(15.6)	
Negative	61(67.0)	76(84.4)	
Anti-Toxo IgM			0.31
Positive	6(6.6)	3(3.3)	
Negative	85(93.4)	87(96.7)	
* Statistically significant at P < 0.05			

Table 3
Prevalence rate of IgG *T. Gondii* antibody in diabetic pregnant women

		Case N %	Control N%	P value
Age groups (yr)	21–30 years	7(23.3)	8(57.1)	0.027*
	> 30 years	23(76.7)	6(42.9)	
Education	Illiterate	4(13.3)	1(7.1)	0.44
	elementary	13(43.3)	3(21.4)	
	High school	2(6.7)	2(14.3)	
	Diploma	6(20.0)	3(21.4)	
	University	5(16.7)	5(36.7)	
Meat	Frost	4(13.3)	0	0.37
	Half cooked	0	0	
	cooked	25(83.3)	14(100)	
	raw	1(3.3)	0	
Gestational age	1st trimester4119	3(10.0)	1(7.1)	0.64
	2nd trimester	14(46.7)	9(64.3)	
	Third trimester	13(43.3)	4(28.6)	
Residence area	Urban	19(63.3)	11(78.6)	0.69
	Rural	6(20.0)	2(14.3)	
	Margin	5(16.7)	1(7.1)	
History of abortion	Yes	8(26.7)	8(57.1)	0.05*
	No	22(73.3)	6(42.9)	
Contact Cat	Yes	1(3.3)	0	0.99
	No	29(96.7)	14(100)	
Contact soil	Yes	11(36.7)	3(21.4)	0.49
	No	19(63.3)	11(78.6)	
Contact animal	Yes	6(20.0)	2(14.3)	0.99
	No	24(80.0)	12(85.7)	

* Statistically significant at P < 0.05

		Case N %	Control N%	P value
Infusion Blood	Yes	1(3.3)	1(7.1)	0.54
	No	29(96.7)	13(92.9)	
Use of water	Pipeline	28(93.3)	14(100)	0.99
	well	2(6.7)	0	
N. Parity	0–2	25(83.3)	12(85.7)	0.99
	≥ 3	5(16.7)	2(14.3)	
* Statistically significant at P < 0.05				

Discussion

This is the first study to investigate at the level of *T. gondii* antibodies (IgM and Ig

G) in diabetic pregnant women in this region. In our study, the seroprevalence of IgG *T. gondii* antibody in diabetic pregnant women was 33% which was higher than reported by previous studies in Iran (27%) (19) China (16.5%) (20) and Yemen (21.1%) (21) but lower than the results of studies from Egypt (45%) (22) and turkey (53%) (23). In addition, our study showed that the prevalence rate of IgM *T. gondii* antibody in diabetic pregnant women was 6.6% which was in harmony with those the results reported from Brazil (5.3%) (24), higher than as reported by Iran (2.7%) (25), and lower than as reported from Saudi Arabia (12%) (26). These differences in studies may be due the existence of variations in cultures and traditions between different nations as well as different food habits (27). Toxoplasmosis may occur in diabetic patients due acquired acute infection, or they may suffer from the spread of an active latent infection. (28).

seroprevalence of *T. gondii* infection was significantly higher in cases than control group ($p = 0.06$). It has been found that diabetic patients were more susceptible to have toxoplasmosis than control group which was in line with the results reported from china (20), and Egypt (22), However it was in contrast to the results performed on Iran (29). In this study there was a significant higher prevalence of seropositivity (IgG) between age group above 30 years old for the case and control groups (76.7% and 42.9%) respectively ($p = 0.027$). Similarly was reported from previous studies in geographical settings. (19, 30). It could be explained that these women are exposed to the risk factors for a longer period of time than other women (31).

In current study we found significant relationship between the seroprevalence of history of abortion and *T. gondii* infection ($p = 0.05$). The obtained results of some of studies were opposite with our results (26, 32) but a study in north of Iran (33) reported the frequency of anti-*Toxoplasma* IgG (34.21%) in pregnant women with a history of abortion. Although the previous studies have shown that living in rural areas had a higher risk of Toxoplasmosis than in urban areas (34), we found no statistical difference in

seroprevalence between rural and urban areas ($p = 0.66$) which agreed with what has been found with the other reports (12, 35).

High seroprevalence among individual from rural areas may be due poor socio-economic conditions and poor health.

In our study there was an increasing pattern of infection rates with level of education in elementary phase in case versus control group (43.3% v 21.4%) but the trend was not statistically significant ($p = 0.44$). Other studies confirmed our findings (36–37). Contact with cats is often accepted as risk factors for infection with toxoplasmosis (25). In present study no significant relationship was found between the seroprevalence of *T. gondii* infection and contact to cats ($p = 0.99$), animals ($p = 0.99$), and soil ($p = 0.49$) which was similar to other studies (22, 31). This could be explained due the climate of Kurdistan province is cold and this temperature is not suitable for the sporulation and survival of *Toxoplasma* oocysts.

Several outbreaks and epidemiological studies have indicated the consumption of undercooked or raw meat as a source of toxoplasmosis (38). Although no

correlation was found in this study ($p = 0.37$), agreement with previous reports (39), reported an association of raw meat consumption with *Toxoplasma* in Cameroon and Japan (40–41).

In this study no significant relationship between toxoplasmosis and gestational age ($p = 0.64$) was found but IgG in third trimesters of pregnancy in case group was more prevalent than in its similar subgroup control group (43.3% v 28.6%)

, which was compatible to other studies (36, 42). If the mother is infected to *T. gondii* during the second and third trimester of pregnancy, the probability of prenatal transmission will be increased to 80%. Early detection and specific treatment of mothers can reduce the risk of fetal infection by up to 50%.

(42). Contaminated drinking water is also a potential source of *T. gondii* infection (25). In our study, no association between the sources of drinking water and *Toxoplasma* infection was observed ($p = 0.99$). The results a study in Nigeria was inconsistent with the present investigation (43) but other studies confirmed our finding (20, 32). Blood transfusion as well as number of parity can also be a source of infection. The present study showed no apparent relationship between IgG-*Toxoplasma* infection and blood transfusion ($p = 0.54$) and number of parity ($p = 0.99$). Other studies agreement with our results (25, 44).

Conclusion

The study results showed a statistically significantly higher proportion (33%) of seropositivity anti-*Toxoplasma* IgG antibodies among diabetes pregnant women compared to (15.6%) the control group. Also results of this study showed that 67% of pregnant women in sanandaj city did not have immunity against toxoplasmosis, therefore they are at risk of congenital toxoplasmosis for their fetuses and it is

require awareness on the disease and its transmission, designing the preventive measures and care to avoid parasite exposure during their pregnancies.

Declarations

- **Ethics approval and consent to participate**

- The study protocol was approved by Kurdistan University of Medical Sciences Ethical

- **Consent for publication:**

- are applicable

- **Competing interests:**

- The authors declare that they have no competing interests.

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- **Authors' contributions:**

NB designed the study and wrote the protocol. NSH, FF managed the study and NB and MZ were the contributors to the analysis and interpretation of the data. All authors read and approved the final manuscript

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- **Abbreviations:** ELISA (enzyme-linked immunosorbent assay), WHO (World Health Organization), GDM (Gestational diabetes mellitus), *T. gondii* (*Toxoplasma gondii*), IgG

(Immunoglobulin G), IgM (Immunoglobulin M)

Abbreviations

ELISA (enzyme-linked immunosorbent assay), WHO (World Health Organization), GDM (Gestational diabetes mellitus), *gondii* (*Toxoplasma gondii*), IgG (Immunoglobulin G), IgM (Immunoglobulin M)

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	Cooked	72(79.1)	81(90.0)	
Gestational age	1st trimester	41 19 21(23.1)	23(25.6)	0.419
	2nd trimester	27(29.7)	33(36.7)	
	Third trimester	43(47.3)	34(37.8)	
Residence area	Urban	69(75.8)	10(11.1)	0.66
	Rural	12(13.2)	73(81.1)	
	Margin	10(11.0)	7(7.8)	
History of abortion	Yes	24(26.4)	29(32.2)	0.38
	No	67(73.6)	61(67.8)	
Contact Cat	Yes	2(2.2)	4(4.4)	0.44
	No	89(97.8)	86(95.6)	
Contact soil	Yes	25(27.5)	24(26.7)	0.99
	No	66(71.6)	66(73.3)	
Contact animal	Yes	14(15.4)	9(10.0)	0.28
	No	77(84.6)	81(90.0)	
Infusion Blood	Yes	3(3.3)	4(4.4)	0.72
	No	88(96.7)	86(95.6)	
Use of water	Pipeline	88(96.7)	83(92.2)	0.21
	well	3(3.3)	7(7.8)	

N. Parity	0-2	83(91.2)	86(95.6)	0.24
	≥3	8(8.8)	4(4.4)	

* Statistically significant at P < 0.05

Table 2: Distribution of patients and control groups according to anti-*Toxoplasma* IgG, IgM

	Case NO (%)	Control NO (%)	P value
Anti-Toxo IgG			0.006*
Positive	30(33.0)	14(15.6)	
Negative	61(67.0)	76(84.4)	
Anti-Toxo IgM			0.31
Positive	6(6.6)	3(3.3)	
Negative	85(93.4)	87(96.7)	

* Statistically significant at P < 0.05

Table 3: Prevalence rate of IgG *T. Gondii* antibody in diabetic pregnant women

		Case N	%	Control N%	P value
Age groups (yr)	21-30 years	7(23.3)		8(57.1)	0.027*
	> 30 years	23(76.7)		6(42.9)	
Education	Illiterate	4(13.3)		1(7.1)	0.44
	elementary	13(43.3)		3(21.4)	
	High school	2(6.7)		2(14.3)	
	Diploma	6(20.0)		3(21.4)	
	University	5(16.7)		5(36.7)	
Meat	Frost	4(13.3)		0	0.37
	Half cooked	0		0	
	cooked	25(83.3)		14(100)	
	raw	1(3.3)		0	
Gestational age	1st trimester	41	19	3(10.0)	0.64
	2nd trimester			14(46.7)	
	Third trimester			13(43.3)	
Residence area	Urban	19(63.3)		11(78.6)	0.69
	Rural	6(20.0)		2(14.3)	
	Margin	5(16.7)		1(7.1)	
History of abortion	Yes	8(26.7)		8(57.1)	0.05*
	No	22(73.3)		6(42.9)	
Contact Cat	Yes	1(3.3)		0	0.99
	No	29(96.7)		14(100)	
Contact soil	Yes	11(36.7)		3(21.4)	0.49
	No	19(63.3)		11(78.6)	
Contact animal	Yes	6(20.0)		2(14.3)	0.99
	No	24(80.0)		12(85.7)	
Infusion Blood	Yes	1(3.3)		1(7.1)	0.54
	No	29(96.7)		13(92.9)	
Use of water	Pipeline	28(93.3)		14(100)	0.99
	well	2(6.7)		0	
N. Parity	0-2	25(83.3)		12(85.7)	0.99

≥ 3	5(16.7)	2(14.3)
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* Statistically significant at P < 0.05