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ORIGINAL ARTICLE

Seroprevalence of toxoplasmosis in diabetic pregnant women in southwestern of Iran

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Abstract To investigate anti-*Toxoplasma gondii* IgG and IgM antibodies in diabetic pregnant women in Ahvaz, southwest of Iran this experiment was performed. In current study the sera of 110 diabetic pregnant women as well as 110 non diabetic pregnant women referred to the hospitals affiliated with the Ahvaz Jundishapur University of Medical Sciences were assessed for anti-*T. gondii* IgG and IgM antibodies by ELISA and IFA methods. The ELISA assessments showed that 47 (42.7 %) and 3 (2.7 %) of diabetic women were positive for IgG and IgM antibodies, respectively. However, in the control group, 24 individuals (21.81 %) were positive for IgG antibody but no detection for IgM antibody. According to IFA method, 46 (41.8 %) and 3 (2.7 %) of diabetic women were positive for IgG and IgM antibodies, respectively, while in control group, 21 individuals (19.09 %) were positive for IgG antibody. In this method, IgM antibody was negative for all samples of control group (0 %). In both methods, the values obtained in the case group were significantly higher than those in the control group ($p < 0.05$). Prevalence of anti-*Toxoplasma* IgG and IgM antibodies in diabetic pregnant women was higher than that in non-diabetic pregnant women. It seems that screening tests for seeking patients and teaching the transmission routes should be considered as prenatal cares for diabetic women.

Keywords Seroprevalence · *Toxoplasma gondii* · Diabetes · Pregnancy

Introduction

Toxoplasmosis is a parasitic infection caused by a protozoan called *Toxoplasma gondii* is an intracellular parasite that has a worldwide distribution. Cats and felids are its definitive hosts and humans, many warm-blooded vertebrates and birds are its intermediate hosts. *T. gondii* has different forms of trophozoite, tissue cyst and oocyst. Oocysts are formed in the intestine of the definitive host and are excreted in the feces. Humans are infected through the consumption of undercooked meat containing tissue cysts or the ingestion of food or water contaminated with oocyst defecated by cats. Toxoplasmosis in immunocompetent persons is often clinically asymptomatic. Ingesting contaminated foods, bradyzoites and sporozoites are released in the intestine, enter small intestine through epithelial and transform into tachyzoites which undergo replication and spread in the body. This parasite can lead to chronic infection in adults, death in immunocompromised people and miscarriage in pregnant women. The prevalence of *T. gondii* varies in different societies depending on the geographical region (Robert-Gangneux and Darde 2012). For example, the prevalence rate of *T. gondii* in pregnant women in Portugal is 59.8 % and in pregnant women in Turkey (52.1 %) (Ocak et al. 2007; Varella et al. 2003). In addition, this coccidian protozoan is widely distributed in Iran and the prevalence rate in Khuzestan ranged from 21.5 to 47.25 % (Daryani et al. 2014; Khademvatan et al. 2013;

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Saki et al. 2013, 2015; Soltani et al. 2013; Yad et al. 2014). It seems that the oocytes excreted by cats find the opportunity to become infectious due to its warm-humid seasons in khuzestan (Khademvatan et al. 2014).

Appropriate diagnosis of toxoplasmosis is crucial among the persons: Pregnant women who have acquired this infection during pregnancy, infants who have congenitally acquired toxoplasmosis during the prenatal period, patients with weakened immune system, and chorioretinitis patients (Ghasemian et al. 2007; Saki et al. 2015). The probability of prenatal transmission increases as the fetal age increases; so that it is 10–15, 30–54 and 60–65 % during the first, second and third trimesters of pregnancy, respectively. The infected fetus may exhibit a wide range of clinical symptoms of varying severity, from miscarriage and severe infection of neonates to asymptomatic infections (Neva and Brown 1994). This parasite acts as an opportunistic pathogen in immunocompromised people such as those with HIV, encephalitis and systemic infection (Zangerle et al. 1991).

Several studies have shown that in diabetic patients, leukocyte cytotoxicity to eliminate pathogenic factors is reduced and the opsonic activity of immune system is significantly diminished; therefore, diabetic pregnant women are one of the groups susceptive to such opportunistic infections (Rayfield et al. 1982). Confirmation of diagnosis of *T. gondii* infection is necessary by laboratory tests. Although, the number of cases with toxoplasmosis is high, a sensitive and suitable method is not applied for detection of this infection in most diagnostic laboratories, which will lead to mismanagement of the involved cases. Among serologic methods, Enzyme-linked Immunoadsorbent Assay (ELISA), immunofluorescence assay (IFA), and indirect hemagglutination Test (IHT) are standard methods for examining anti-*T. gondii* antibodies (Robert-Gangneux and Darde 2012); therefore, using ELISA and IFA methods in this study, we intend to compare two mentioned methods as well as investigate IgG and IgM antibodies created against *T. gondii* in diabetic pregnant women of Ahvaz city, southwest of Iran country.

Materials and methods

In this case–control study blood samples were collected from women, who were registered as diabetic patients and underwent monthly follow ups by genecology specialist in the state and private hospitals in Ahvaz city southwest of Iran. A total of 110 serum samples were collected from diabetic pregnant women referred to the clinics of Golestan, Imam Khomeini, Razi and Aria hospitals (Ahvaz, Iran). In addition, 110 serum samples were collected as control group from non-diabetic pregnant women referred to the above-mentioned hospitals. 5 mL of blood was taken from each person. Then, sera were separated and kept at −20 °C till tested. All subjects completed a questionnaire including demographic information such as age, habitat, month of pregnancy and duration of diabetes. Anti-*Toxoplasma* IgM and IgG antibodies were measured using ELISA kit (Biotech Co. England) and IFA kit (Euroimmun Co, England) as formerly described (Saki et al. 2015). Sensitivity (%) and specifity (%) of IFA and ELISA are as following (Gharavi et al. 2008):IFA: 97.3 and 96 %; ELISA IgG: 97.3 and 92 %; ELISA IgM: 92 and 100 %. Results were analyzed using *t* test with the statistical package of SPSS version 19.

Results

The results of IFA and ELISA methods were close to each other and the agreement between the two methods was 97 %. The ELISA assessments showed that 47 (42.7 %) and 3 (2.7 %) diabetic women were positive for IgG and IgM antibodies, respectively. However, in the control group, 24 individuals (21.81 %) were positive for IgG antibody. IgM antibody was negative for all samples of control group (0 %). According to IFA method, 46 (41.8 %) and 3 (2.7 %) diabetic women were positive for IgG and IgM antibodies, respectively, while in control group, 21 individuals (19.09 %) were positive for IgG antibody. In this method, IgM antibody was negative for all samples of control group (0 %). In both methods, the values obtained in the case group were significantly higher than those in the control group ($p < 0.05$) (Table 1).

Comparing the prevalence rate in terms of the month of pregnancy in diabetic group showed that IgG in the subgroups of the first and third trimesters of pregnancy is more prevalent than that in its similar subgroups in the control group. In diabetic group, IgM was 1.5 and 7.1 % positive in the subgroups of the first and third trimesters of pregnancy, respectively, while no positive case was identified in its similar groups in the control group (0 %) (Table 2). In this study, comparing the prevalence rate in terms of region showed that IgG in diabetic group and in urban and rural subgroups was more prevalent than that in its similar subgroups in the control group. In addition, 3.42 % of individuals in diabetic group in the urban subgroup were positive for IgM, while it was negative in similar subgroup in the control group (0 %).

In this study, diabetic patients were divided into three groups: The first group with duration of diabetes 0–2 years, the second group with duration of diabetes 2–4 years, and the third group with duration of diabetes more than 4 years. Comparing the prevalence rate in terms of the duration of

Table 1 Prevalence of anti-*T. gondii* IgG and IgM antibodies in diabetic and non-diabetic pregnant women using ELISA and IFA methods

Groups	Samples	ELISA		IFA	
		IgG	IgM	IgG	IgM
Diabetic pregnant women	110	47 (42.7 %)*	3 (2.7 %)*	46 (41.8 %)*	3 (2.7 %)*
Non diabetic pregnant women	110	24 (21.81 %)*	0 (0 %)*	21 (19.09 %)*	0 (0 %)*

* $p < 0.05$

Table 2 Comparison of the prevalence rate of IgG and IgM antibodies in diabetic pregnant women in terms of the trimesters of pregnancy using ELISA method

Trimesters	Diabetic pregnant women			Non diabetic pregnant women		
	NO	IgG	IgM	NO	IgG	IgM
First trimester	63	30 (47.6 %)*	1 (1.5 %)*	22	5 (22.7 %)*	0 (0 %)*
Second trimester	19	1 (5.2 %)*	0 (0 %)*	35	4 (11.4 %)*	0 (0 %)*
Third trimester	28	16 (57.1 %)*	2 (7.1 %)*	53	15 (28.3 %)*	0 (0 %)*
Total	110	47 (42.7 %)	3 (2.7 %)*	110	24 (21.81 %)	0 (0 %)*

NO number of the serum

* $p < 0.05$

diabetes showed that the prevalence rate of IgG is directly associated with the history of affliction (the more the duration of affliction, the higher the prevalence of IgG) (Table 3).

Discussion

In this investigation being the first study to evaluate the status of *T. gondii* antibodies in diabetic pregnant women in Iran, the prevalence rate of both IgG and IgM antibodies in diabetic pregnant women was significantly higher than that in non-diabetic pregnant women. Our results is accord with the study of Gokce et al. in India that showed the prevalence rates of IgG in diabetic patients and control group were 56.6 and 22.4 %, respectively. In addition, the study showed that the prevalence rate of IgM in diabetic patients and control group were 2.4 and 1.6 %, respectively (Gokce et al. 2008).

In this study, the prevalence rate of IgG antibody was directly associated with the duration of diabetes, probably due to the weakened immune system of diabetic patients. Cellular immunity in particular with macrophages, T lymphocytes and "natural killer" (NK) cells involvement is the key component of the host's immune reaction in the event of attack by *Toxoplasma* (Lindberg and Frenkel 1977). The leukocyte cytotoxicity as well as the complement opsonic activity to eliminate *Staphylococcus* and *E. coli* in diabetic patients is significantly reduced compared to the control group (Rayfield et al. 1982). The intestinal parasitic infections in diabetic patients are more prevalent than that in the control group or non-diabetic patients (Akhlaghi et al. 2005).

Current study showed that IgG in the subgroups of the first and third trimesters of pregnancy in diabetic group is more prevalent than that in its similar subgroups in the control group. The prevalence of toxoplasmosis is 29.35 % in pregnant women in Khuzestan (Yad et al. 2014). Toxoplasmosis in pregnant women causes miscarriage, microcephaly, hydrocephalus and severe neurological disorders in fetus. If the mother is infected to *T. gondii* during the first trimester of pregnancy, complications will be severe and included miscarriage or stillbirth, although the probability of transmission is 10–15 %. If the mother is

Table 3 Comparison of the prevalence rate of IgG and IgM antibodies in diabetic pregnant women in terms of the duration of affliction by ELISA

Duration of affliction (year)	Total number	IgM+	IgG+	Mean OD IgG
0–2	66	2 (3.03 %)	27 (40.9 %)	1.09
2–4	30	0 (0 %)	13 (43.3 %)	1.14
>4	14	1 (7.1 %)	7 (50.0 %)	1.29
Total	110	3	47	1.73

infected to *T. gondii* during the second and third trimester of pregnancy, the probability of prenatal transmission will be increased to 80 %; however, this leads to symptoms including chorioretinitis, strabismus, blindness, epilepsy, mental retardation etc. (Neva and Brown 1994). In individual with immune deficiency, chronic form of the infection can convert to acute, following rupture of a cyst contain the latent bradyzoites and change to tachyzoites, the proliferation of which is not effectively controlled by the immune response of the host, leading to severe brain damage (Sullivan and Jeffers 2012). Therefore, considering the weakened immune system in diabetic people, diabetic pregnant women are placed in a high risk group of infection. It is important to detect both above-mentioned antibodies in people with weakened immune system. Diagnosis of acute toxoplasmosis in pregnant women can prevent damages of fetus, such as microcephaly, hydrocephalus, mental retardation or miscarriage (Neva and Brown 1994).

In conclusion, the current study indicated significantly higher prevalence of anti-*T. gondii* IgM and IgG antibodies in diabetic pregnant women in comparison to control non diabetic pregnant women. In addition, there is a direct relationship between the prevalence of these antibodies and the duration of diabetes; therefore, considering the susceptibility of diabetic pregnant women to opportunistic infections such as toxoplasmosis, it is necessary to perform screening to quickly diagnose and treat infected diabetic pregnant women. Instructions about the disease and its transmission routes should be given to diabetic women and be considered as prenatal cares.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Statement Before collecting blood samples a written informed consent was obtained from all individual participants included in the study. It is worth mentioning that present study was approved by the ethic committee of Ahvaz Jundishapur University of Medical Sciences (No. ETH-1392.121).

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