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ORIGINAL ARTICLE

Diagnosis of acute toxoplasmosis by IgG avidity method in pregnant women referred to health centers in south-eastern Iran

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Abstract Toxoplasmosis, one of the most common parasitic infections, can cause abortions in human. The purpose of this study was to determine seroprevalence of toxoplasmosis and acute form of toxoplasmosis in pregnant women. 208 pregnant women who referred to health centers in south-eastern Iran were taken under study after signing the informed consent forms and filling out the checklists. For those with high levels of IgG or IgM anti-body titers, IgG Avidity test was performed to determine the acute infection. High level of IgG anti-body were found in sera of 81 pregnant women and 7 persons had borderline titer. IgM antibody results were positive in 33 and borderline in 4 cases. Then, the samples with positive and borderline results for IgM and IgG were evaluated by IgG avidity test. In this study, approximately 60% of pregnant women were not immune to risks posed by Toxoplasma gondii. Therefore, this group are at risk of acquiring primary infection of toxoplasmosis during gestation.

Keywords Acute toxoplasmosis · IgG avidity · Pregnant women · South-eastern Iran

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Introduction

Toxoplasmosis is a zoonotic parasitic disease caused by *Toxoplasma gondii* worldwide. Although toxoplasmosis is commonly asymptomatic among immunocompetent people, congenital transmission with different manifestation might occur during pregnancy (Skariah et al. 2010; Zarean et al. 2017). Despite the rise of transmission rate with increasing the gestational age, the severity of congenital effects is reduced. Then, the screening programs for toxoplasmosis are necessary for all the pregnant women, especially in the first trimester in where with high prevalence of *Toxoplasma* (Many and Koren 2006). Since, most of the pregnant cases could not be recognized clinically in the primary stage of infection, serological tests are recommended for prenatal diagnosis (Laboudi and Sadak 2017).

Although detection of IgM-specific antibody in human sera is the main indicator to diagnose the acute phase by indirect-fluorescent antibody test (IFA) or enzyme-linked immunosorbent assay (ELISA), it might persist for several months or even years (Petersen et al. 2005).Consequently, the only positive IgM results are not reliable to distinguish between the chronic and acute infection. Recently, another

confirmatory assay recommended performing is IgG avidity. Based on the rate of anti-body maturation and rise of strength between antibody-antigen complex by increasing the weeks after infection, the IgG avidity commercial kit can determine the time of acquisition of the toxoplasmosis. A high avidity more than 60% shows an infection acquired at least during last 3 to 5 months and a lower avidity than 40% indicates an initial step or acute infection (Pour Abolghasem et al. 2011; Shojaee et al. 2017).

Recent systematic review done on the seroprevalence of toxoplasmosis among pregnant women and girls in the childbearing age in Iran indicates high incidence of 33–43% and also high percentage of seronegative in these groups (Mizani et al. 2017).Hence, in order to reduce the serious clinical signs in fetal and neonatal, the serological screening tests would be performed on the majority of risk groups disposed to the infection.

The purpose of this study was to determine the seroepidemiology of Toxoplasmosis and determination of IgG avidity in pregnant women referred to health care centers in Saravan in 2017.

Materials and methods

Ethical consideration

All the procedures in this investigation have been reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences (Ethical code no.IR.MUMS.fm.REC.1396.459).

Study area

Saravan is a city in the capital of Saravan County located in Sistan and Baluchistan Province, south-eastern Iran. According to the most recent national census, its population was 58,652, in 10,078 families. Saravan with DMS 27°22′15″N, 62°20′3″E is close to the international border of Pakistan. The inhabitants of the city are Balochi and speak the Balochi language (Wikipedia 2018).

Collection of serum samples

The sample size was determined based on prevalence of toxoplasmosis in pregnant women (34.2%) obtained from previous study done in Bandar-Abbas, another city in Iran (Jahromi et al. 2003). This sample size could be investigated with 95% confidence level with a margin of error of less than 10%.

Between April 2015 and March 2016, 208 serum samples were collected from pregnant women referring to Saravan health centers in Sistan and Baluchistan province to perform this descriptive cross-sectional research. The basic demographic information was recorded for the people who signed the consent forms to participate. Then, the sera were transferred to the laboratory and stored at − 20 C.

ELISA

Based on manufacturer's instruction (EUROIMMUN, Germany), all the sera were examined for anti-Toxoplasma IgG and IgM antibodies. The level of antibody titer for IgG was measured semi-quantitatively while the positive results of IgM antibody were determined for the higher amount of optical density (OD) in comparison with the cutoff point. The OD of IgG and IgM antibody were measured at 450 and 620 nm, respectively.

Anti-*Toxoplasma* IgG titers were evaluated by calculating a ratio of extinction value of the control or patient samples over the extinction value of the calibrator (sample OD/calibrator OD). According to the kit interpretation, the ratio lower than 0.8 was considered as negative for anti-*Toxoplasma* IgG antibodies; while the results between 0.8 to 1.1 are interpreted as equivocal and the higher value than 1.1 was considered as positive.

IgG avidity assay

The IgG avidity test was done on all positive IgG patients using Anti-*Toxoplasma gondii* ELISA for avidity determination (IgG kit, EUROIMMUN, Germany). The presence of low avidity antibodies in a patient's serum has been proved if the ELISA extinction value is considerably reduced by urea treatment. The relative avidity index (RAI) was calculated in percent using the extinction values with and without urea treatment. The upper limit of the range of low avidity antibodies (cut-off value) is 40% RAI. The values below the cut-off point were considered as an indication of low avidity antibodies, values between 40 and 60% RIA as equivocal and values above 60% was considered as an indication of high avidity.

Statistical analysis

The correlations between different obtained data were determined using Chi square test and analyzed by SPSS software (IBM SPSS Statistics 18) with 95% confidence interval.

Result

A total of 208 samples were taken from pregnant women with an average age of 27.5 who 63 women (30.3%) in the first trimester of pregnancy, 46 women (22.1%) in the

second trimester of pregnancy and 99 women (47.6%) were in the third trimester of pregnancy.

The positive results of evaluation of the sera by IgG were 88(42.3%) of which 7 persons had borderline titer and 81 patients had high titer antibody. High level of IgM antibodies was seen in 3cases (15.8%) and the Sera with yielding borderline and low results for IgM were 4 and 171, respectively (Table 1). Then, the samples with positive and borderline antibody titers for IgM and IgG were evaluated by IgG avidity test. The results of IgG avidity indicate that 5 pregnant women were in the acute phase (low avidity less than 40%), 25 cases had high avidity (more than 60%) and one sample shows the equivocal result (between 40 to 60%).

No significant relation was found between consumption of fresh meat and positive *Toxoplasma* IgM and IgG test results. The use of contaminated vegetables as a risk factor was positive for high titer of IgM antibodies but there was no significant correlation between positive and negative results of IgG antibodies between consumption of infected vegetable and seroprevalence of toxoplasmosis. The most important factor in toxoplasmosis was contact with cats, which had the greatest chance among other factors. The clinical, laboratory test and epidemiological feature information employed for pregnant women are present in Table 2.

Discussion

Toxoplasma gondii is known as one of the opportunistic infection in human (Zarean et al. 2017).The interpretation of IgG and IgM antibodies levels against *Toxoplasma* have been challenging to diagnose acute infection during pregnancy. Furthermore, prenatal toxoplasmosis leads to sever tragic effects on the fetus and newborn (Emelia et al. 2014).

According to the last systematic review done in Iran, the seroprevalence of toxoplasmosis in pregnant women has been reported about 43%(Mizani et al. 2017). In present study, the same prevalence (43.2%) was found in pregnant women regarding 42.3% for anti-*Toxoplasma* IgG, 15.8% for anti-*Toxoplasma* IgM, and 14.9% for both antibodies. The high seroprevalence of toxoplasmosis seen in this investigation demonstrated that toxoplasmosis is still considered a silent threatening disease in this group in Saravan. However, a study performed by Ebrahimzadeh et al. in Zahedan,the capital of Sistan and Baluchistan Province, showed a lower prevalence of toxoplasmosis about 30.8% for IgG and 1.4% for both IgG and IgM (Ebrahimzadeh et al. 2013). Therefore, application of IgG-avidity test as a reliable diagnosis method for the acute phase and making decision for treating toxoplasmosis seems to be essential in cases who have high titer level anti-*Toxoplasma* IgM antibody (Petersen et al. 2005). IgG avidity above 60% (IgG+ , IgM+) could be ascribed to the high half-life of IgM and chronic infection or the existence of rheumatoid factor.

In this study, only 2.4% of pregnant women had a low avidity (less than 40%) for IgG by IgG-avidity test and showed the acuteness of infection. A number of researchers stated a positive percentage of 55% for IgG and IgM among this group with a greater number of low avidity about 7.1% (Pour Abolghasem et al. 2011; Tlamcani et al. 2013). It could be related to susceptibility of environment for survival time and infectivity of oocysts and behavior of population in the regions under study.

However, avidity test was described to be specific and sensitive for identification of recent infection, the follow-up one sample with borderline-avidity antibody is necessary to confirm acute phase. Similarly, the serological follow-up has to be done for sera which only have high or borderline titer levels of IgM (Iqbal and Khalid 2007).

In the current study, the rate of seropositivity was higher in the first and second semester compared to the third trimester while the result of Mizani et al. (2017) study showed a high seropositivity rate only in the first semester. Since anti-*Toxoplasma* seroconversion detection during pregnancy is important for the clinician to make a decision for early treatment, the screening serological methods are ideally recommended in the first trimester (Montoya and Remington 2008).

In our study, however, the prevalence of infection was significantly increased in women who had an abortion

Table 1 Analysis of antibody titers of IgG, IgM, and measurement of IgG avidity in pregnant women referred to health centers in south-eastern Iran

Antibody	IgG	IgM	IgG avidity
Negative	120 (57.7%)	171(82.2%)	25 (high avidity)
Equivocal	7(3.4%)	4(1.9%)	1
Positive	81(38.9%)	33(15.8%)	5 (low avidity)
Total	208	208	31(14.9%)

Table 2 The clinical, laboratory test and epidemiological features of pregnant women referred to health centers in south-eastern Iran

	Pregnant women (total)	Whole no.	IgG +	*p* value	IgM +	*p* value	IgM + IgG +	*p* value
Gestational trimesters	First trimester	63 (30.3%)	22 (25%)	<0.001	15 (84%)*	0.01	7(33.4%)*	<0.001
	Second trimester	46 (22.1%)	17 (19.3%)*				5(23.8%)*	
	Third trimester (reference)	99 (47.6%)	49(55.7%)		9 (11%)* 13 (5%)		9(42.8%)	
Educational stage	Elementary school	158 (76%)	64(40.5%)*	<0.001	31(19.6%)*	<0.001	18(85.8%)*	<0.001
	Secondary school	42 (20.2%)	20(47.6%)		4(9.5%)*		2(9.5%)*	
	Post-secondary education (reference)	8 (3.8%)	4(5%)		2(25%)		1(4.7%)	
Abortion history	Yes	54 (26%)	21(23.9%)*	<0.001	9(24.3%)*	<0.001	5(23.8%)*	<0.001
	No	154 (74%)	67(76.1)		28(75.7%)		16(76.2%)	
A history of contact with cat	Yes	38 (18.3%)	23(26.1%)*	0.012	8(21.6%)*	<0.001	7(33.3%)	0.06
	No	170 (81.7%)	65(73.8%)		29(78.3%)		14(66.7%)	
Consumption of meat (semi-cooked or raw)	Yes	153 (73.6%)	30 (34%)*	0.032	13(35.1%)*	<0.001	9(42.9%)	0.072
	No	55 (26.4%)	58(65.9%)		24(64.8%)		12(57.1%)	
Consumption of vegetable (semi-cooked or raw)	Yes	68 (32.7%)	26(29.5%)*	<0.001	11(29.7%)*	<0.001	7(33.3%)*	<0.001
	No	140 (67.3%)	62(70.4%)		26(71.3%)		14(66.7%)	

*The group has a single asterisk if the Chi-square showed a statistically significant difference compared to other groups in each row

history (55%), 30% of them had only IgM positive and need to be repeated after 3 weeks by serological tests. Similar to Abedi et al. (2015) study, an association between toxoplasmosis and miscarriage history has been observed among the majority of seropositive women taken understudy.

Despite other published papers, an increasing rate of *T. gondii* seropositivity by declining the level of education has been observed. It could be due to the women participated in the present investigation, were much less likely to have a postsecondary degree (Hung et al. 2015; Mizani et al. 2017).

The domestic cats are one of the main risk factors for toxoplasmosis. The infected cats could excrete millions of oocysts in the soil during a day and speculated forms of oocysts might stay for several months or even years in ideal status in the environment. The results obtained from this study indicated that there is a meaningful correlation between close contact with cats and high amount of anti-Toxoplasma antibody in pregnant women. This finding has an agreement with other previous studies (Lopes et al. 2009; Sroka et al. 2010).

Contaminated meat containing bradyzoites in tissue cysts is one of the other sources of infection. Consumption of raw or semi-cooked meat causes to raise the risk of parasite transmission. The data showed the rate of seropositivity has a significant association in individual who consumed infected raw and semi-cooked meet or vegetables contaminated with oocysts. These results also have been reported by Lopes et al. (2009), Mizani et al. 2017 and Gelaye et al. 2015.

Conclusion

The percentage of seronegative women during gestation has been calculated about 58%. Concerning more than half of the pregnant women were at risk of infection and congenital toxoplasmosis, this group should be monitored during gestation. Therefore, it seems that by using health

education methods and increasing the level of health awareness through public media, it will be possible to decrease the disease burden of toxoplasmosis on Iranian population while the newborn complications of congenital toxoplasmosis impose heavy costs for treatment.

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Author contributions MR-B and MZ constructing an idea or hypothesis for research, MR-B and MZ planning methodology to reach the conclusion, SAS organising supervising the course of the project or the article and taking the responsibility, HM and SMHS providing personnel, environmental and financial support and tools and instruments that are vital for the project, SH-P, EM, OA and YS taking responsibility in execution of the experiments, patient follow-up, data management and reporting, LJ taking responsibility in logical interpretation and presentation of the results, BRHF and MZ reviewing the article before submission not only for spelling and grammar but also for its intellectual content and taking responsibility in the construction of the whole or body of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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