Comparison of IIF, ELISA and IgG avidity tests for the detection of anti-Toxoplasma antibodies in single serum sample from pregnant women

Behrouz Naghili[1], Shamsi Abbasalizadeh[2], Shahin Tabrizi[1], Mehranghiz Rajaii[3], Masoumeh Akramiyan[1], Hossein Alikhah[4], Mohammad Naghavi-Behzad[5], Reza Piri[6], Farid Karkon-Shayan[7], Sepideh Tehrani-Ghadim[2]

[1]Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[2]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[3]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[4]Department of Emergency Medicine, Tabriz University of Medical Sciences, Tabriz, Iran;
[5]Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran;
[6]Medical Philosophy and History Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[7]Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Science, Tabriz, Iran

SUMMARY

A valid estimate of test efficiency is needed to choose adequate screening and detecting strategies in diagnosis of acute toxoplasmosis. Therefore, in the present study we evaluated the efficiency of diagnostic laboratory methods to detect anti-toxoplasma antibodies in single serum samples of pregnant women by indirect immunofluorescence test (IIF), enzyme-linked immunosorbent assay (ELISA) and IgG avidity tests in north-western Iran. In an analytical-descriptive study, during March 2010 to April 2013, 391 pregnant women aged 21 to 35-years who were referred by gynaecologists or infectious disease specialists for anti-toxoplasma antibody evaluation were studied. A peripheral blood sample was collected from individuals and serum was prepared immediately for anti-toxoplasma antibody evaluation by IIF, ELISA and IgG avidity tests. ELISA and IgG avidity tests were used as gold standard. Evaluation of anti-toxoplasma antibodies by IIF revealed that 280 cases (71.61%) were seropositive and 111 (28.38%) seronegative, while evaluation by ELISA revealed that 267 cases (68.28%) were seropositive and 124 (31.70%) seronegative; 65 (16.62%) were IgM positive by both IIF and ELISA tests. There were 45 (69.23%) and 7 (10.76%) IgM positive suspected cases respectively in IIF and ELISA confirmed by the IgG avidity test for recent toxoplasmosis. This study highlights how to manage and evaluate pregnant women who are suspected to be infected with toxoplasmosis by using diagnostic tests, especially in a single serum sample indication.

Keywords: toxoplasmosis, diagnostic tests, IIF, ELISA, IgG avidity.

INTRODUCTION

Toxoplasma gondii is a well-known zoonotic intracellular protozoan parasite which approximately one-third of the world's population is infected with [1-3]. It can infect a variety of animals including a wide range of mammals, birds, and humans. Domestic and free-ranging felines are known the main hosts in the transmission of *Toxoplasma gondii*, since they are the only hosts that can produce and shed resistant oocysts in environment through the feces [4-8].

Formation and titration of Anti-Toxoplasma IgG in adults as a diagnostic tool, depends on various factors such as age, geographical background,

Corresponding author
Mohammad Naghavi-Behzad
E-mail: dr.naghavii@gmail.com

and assay methods [9-11]. Toxoplasmosis is often asymptomatic in humans or is associated with mild, non-specific clinical symptoms in immunocompetent subjects, but in pregnant women seroconversion, reactivation of a chronic infection cause fetal damages which considered as congenital toxoplasmosis [12]. Significant fetal damage has been documented in offspring of women who were entirely asymptomatic or who had no apparent exposure to the parasite during gestation [13]. Classically serodiagnosis includes titration of specific immunoglobulin G (IgG), showing previous exposure and screening for specific IgM, which may be evidence of recent exposure or ongoing active infection [14]. Pregnant women need to be systematically screened for *Toxoplasma gondii* IgG and IgM antibodies during their first medical visit (ideally in their first trimester) in obstetrical care [15, 16]. Serial specimens facilitate the unequivocal diagnosis of a recently acquired infection by demonstration of seroconversion or a significant rise in IgG Toxoplasma antibodies accompanied by the presence of detectable IgM titers [17]. The problem with serologic diagnosis is further complicated by the fact that antibodies to *Toxoplasma gondii* may persist for years in healthy people [18]. Therefore, positive IgG and IgM test results do not demonstrate recently acquired infection. Accurate diagnosis of recently acquired infection with *Toxoplasma gondii* is important for the proper clinical management of pregnant women. It is very important to determine whether a pregnant woman was recently infected or not. In Iran, diagnosis is often made by serological tests using a single sample of serum [19]. Also, it is critical to determine that the infection was acquired prior to gestation or during gestation. For these reasons, several assays must be performed for determination of toxoplasma serological profiles (TSPs) by ELISA, ISAGA, IgG avidity, IIF and Dye test. The aim of present study was to compare ELISA and IIF as an acceptable or gold standard method and IgG avidity test as a confirmatory assay for diagnosing acute toxoplasmosis in pregnant women.

PATIENTS AND METHODS

In an analytical-descriptive study from March 2010 to April 2013, 391 pregnant women who were referred to Imam-Reza Educational-Clinical center of Tabriz University of Medical Sciences (which is a referral center in the north-west of Iran) by gynecologists and infectious disease specialists for anti-toxoplasma antibody evaluation by IIF, ELISA, and IgG avidity tests were studied. These individuals were in the different stages of pregnancy. A questionnaire was prepared for evaluating the demographic, epidemiologic and pregnancy information. Serum samples were prepared in the same condition and subjected to anti-toxoplasma antibodies evaluation by IIF, ELISA, and IgG avidity tests.

IIF test

The IIF was performed to demonstrate IgG or IgM antibodies to *Toxoplasma gondii*. Antigen-coated slides, conjugated polyvalent anti-human immunoglobulin and serum controls were purchased from Baharafshan Company Tehran, Iran. Antihuman serum conjugated with fluorescin isothiocyanate (FITC) was used at a dilution of 1:20 in Phosphate Buffered saline (PBS) in 0.1% Evans blue. Diluted sera were placed on antigen spots then incubated at 37 °C for 30 minutes. The slides were rinsed in PBS (pH: 7.6) two times for 5 minutes and dried. A drop of diluted FITC-AHG was added to each spot. Slides were incubated at 37ºC for 30 minutes, and after washing mounted with buffered glycerol. The slides were evaluated using a fluorescent microscope. Positive samples were determined by complete peripheral or whole parasite. A negative result showed only apical staining. For screening of toxoplasma-specific IgG by IIF, serum dilution of 1/100 was used as starting dilution, if necessary higher dilutions, including 1/200, 1/400, 1/800 were used. For screening of toxoplasma-specific IgM only 1/100 serum dilution was prepared, but in suspected cases it was prepared 1/50 and 1/200 dilutions, respectively.

ELISA test

The sensitivity and specificity of Toxoplasma specific-IgG test (RADIM, Italy) have been estimated to be 97.1% and 99.1%, respectively. All the procedures including Toxoplasma IgG and IgM, IgG avidity tests were done as instructed by the manufacturer. The optical density (OD) of 15 IU/ml were considered for both negative control and cut-off calibrator in IgG test. The presence or absence of anti-Toxoplasma IgG antibodies is defined by comparison of the sample absorbance

with the cut-off calibrator absorbance. Samples with lower OD from the 15 IU/ml (cut-off) were considered as negative results for anti-Toxoplasma IgG antibodies, while samples with OD higher than the cut-off calibrator were considered positive for anti-Toxoplasma IgG antibodies. Samples with absorbance values varying within ±10% of the cut-off calibrator considered as suspected results (gray zone). Measurement of anti-Toxoplasma IgG antibodies was done quantitatively based on 30, 60, 120 and 240 IU/ml concentrations of calibrators.

The Toxoplasma IgM antibodies were detected by toxoplasma specific IgM antibodies kit (RADIM, Italy). The sensitivity and specificity of Toxoplasma IgM test have been estimated to be 98.1% and 98.2%, respectively. For IgM antibodies, OD calculation of samples were performed. The presence or absence of anti-Toxoplasma IgM antibodies is defined by comparison of the sample absorbance to the absorbance of the cut-off control. Samples with lower OD than the cut-off control OD were considered as negative results for anti-Toxoplasma IgM antibodies, while samples with higher OD than the cut-off control OD were considered as positive results for anti-Toxoplasma IgM antibodies. Samples with absorbance values ranging within ±10% of the cut-off control were considered as suspected results and re-tested for confirmation (gray zone).

IgG Avidity test

The sensitivity and specificity of the Toxoplasma specific IgG avidity EIA-test (RADIM - Italy) have been estimated to be 89% and 90%, respectively. The avidity percentage less than 20% (<20%) is considered as low avidity and greater than 30% (>30%) was considered as high avidity, and 20-30% was considered as suspected results (gray zone).

Ethical issues

All patient had filled a written consent form before entering the study. All procedures performed during this study was a necessary part of their routine therapeutic process and no additional fees were added regarding the study. During the study, all the patient's information was anonymous and the information was labeled with codes. The study protocols were confirmed by Ethics Committee of Tabriz University of Medical Sciences which follows Helsinki Declaration.

Statistical analysis

SPSS software version 16.0 was used to perform Chi-square test for data analysis. Frequency (percentage) was used to express descriptive data. Sensitivity, specificity, positive and negative predictive values, and accuracy of tests were calculated. P value less than 0.05 was considered statistically significant.

RESULTS

In the present study, 391 patients were screened for Toxoplasma specific IgG and IgM antibodies by IIF and ELISA tests, then these results were used to discriminate their infection status. Finally, suspected patients were subjected to IgG avidity test. Of all 391 patients, 215 (54.98%) were in first trimester, 102 (26.08%) were in second trimester and 74 (18.92%) were in third trimester of gestation.

Frequencies of positive and negative results of toxoplasma antibodies by IIF and ELISA tests are shown in Table 1; as it is seen, both IIF and ELISA tests have presented the same positive IgM and positive IgG samples. Although IIF test has less IgG negative samples than ELISA test, this difference was not statistically significant (p=0.21). Of all 391 women in IIF test, 111 (28.39%) were IgG negative, which 42 samples (10.74%) were weakly positive and 69 remaining samples (17.64%) were negative.

Table 2 presents frequency of positivity for each dilution in IIF and ELISA tests; of all 65 IgM and IgG positive samples in ELISA test, 58 samples (14.83%) were definite positive for IgM and 7 samples (1.79%) were doubtfully positive or in

Table 1 - Frequency of positive & negative results of toxoplasma antibodies by IIF and ELISA tests.

		IIF	*ELISA*
Seronegative		111 (28.39%)	124 (31.71%)
Seropositive	IgG (only) Positive	215 (54.99%)	202 (51.67%)
	IgG & IgM Positive	65 (16.62%)	65 (16.62%)
TOTAL		391 (100%)	391 (100%)

IIF: Indirect Immunofluorescent test; ELISA: Enzyme Linked Immunosorbent Assay.

Table 2 - Frequency of positivity for each dilution in IIF and ELISA tests.

Test	*IgG*		*IgM*
IIF	Dilution*	Frequency (N=391)	Frequency (N=65)
	<1/100	111 (28.39%)	
	1/100	77 (19.69%)	
	1/200	94 (24.04%)13	13 (3.32%)
	1/400	90 (23.01%)37	37 (9.46%)
	1/800	19 (4.86%)15	15 (3.83%)
ELISA	Concentration**	Frequency (N=391)	Frequency (N=65)
	<30 IU/ml	124 (31.71%)	
	30-60 IU/ml	71 (18.15%)	
	60-120 IU/ml	51 (13.04%)	
	120-240 IU/ml	38 (9.71%)	15 (3.83%)
	240-480 IU/ml	107 (27.36%)	50 (14.83%)

IIF: Indirect Immunofluorescent test; ELISA: Enzyme Linked Immunosorbent Assay.
*<1/100 was considered negative.
**<30 IU/ml was considered negative.

Table 3 - Confirmatory role of IgG avidity test in discrimination of recent and past infection of toxoplasmosis.

Test			*Frequency*	*IgG avidity index**
IIF	Weakly IgG positive		42 (10.74%)	+
	IgM positive	Definite	45 (11.50%)	-
		Gray zone	20 (5.11%)	-
ELISA	IgM positive	Definite	58 (14.83%)	-
		Gray zone	5 (1.27%)	-
			2 (0.51%)	+

IIF: Indirect Immunofluorescent test; ELISA: Enzyme Linked Immunosorbent Assay.
*IgG avidity index more than 30% was considered positive and less than 20% was considered negative.

gray zone. In IIF test, of all 65 IgM and IgG positive samples, 20 samples (5.11%) were definite positive for IgM and 45 samples (11.5%) were doubtfully positive or in gray zone. As it is presented, definite positive IgM samples were more in ELISA test than IIF test (p=0.01).

Results of IgG avidity test confirmatory role in discrimination of recent and past infection of toxoplasmosis is shown in Table 3; all IgM positive samples (definite and gray zone) in IIF had avidity index less than 20%, indicating a recent infection, while all weakly positive IgG samples in IIF test proved to have IgG avidity more than 30% which indicates a past infection. In ELISA test of all 65 positive IgM samples, all samples proved to have IgG avidity index less than 20%, except 2 samples which had higher than 30% IgG avidity index (false positive cases of ELISA test). Sensitivity and specificity of each test is presented in Table 4; ELISA test if associated with IgG avidity test is the most reliable test (sensitivity =100% and specificity =100%).

Table 4 - Sensitivity and specificity and confidence intervals of IIF & ELISA & and IgG avidity tests.

Test	*Sensitivity*	*Specificity*
IIF only	100 (83.0-100%)	87.9 (84.1-91.0%)
ELISA only	100 (94.0-100%)	98.5 (96.5-99.5%)
ELISA+ avidity	100 (94.4-100%)	100 (98.9-100%)

IIF: Indirect Immunofluorescent test, ELISA: Enzyme Linked Immunosorbent Assay.

DISCUSSION

Prevalence of toxoplasmosis differs greatly from one country to another or even from one region to another. Contamination by *Toxoplasma gondii* can vary depending on cultural customs and eating habits, resulting in a prevalence ranging from less than 10% in certain regions of northern Europe to more than 90% in Africa [20-22]. Several studies demonstrated distribution pattern of toxoplasmosis in different parts of Iran, which ranges from 25-70% [16, 23-27].

Histological examinations of biological samples, show the lack of reliability when the animals are infected with few parasites [28]. Serological tests are the most common diagnostic methods used nowadays; especially Dye test and IIF requiring intact tachyzoites, because during the infection, the first significant increase in IgM and IgG antibodies is observed against cuticular antigens [29]. Diagnosis of recent toxoplasmosis has been traditionally done by detection of specific IgM antibodies and/or by demonstrating a significant increase in specific IgG antibodies by different methods. High IgG antibodies titers against *Toxoplasma gondii* among normal individuals in most populations and persistence of specific IgM antibodies in some cases have complicated the interpretation of serological tests when toxoplasmosis is suspected [30]. Two situations occur frequently: cases with a positive IgM with negative IgG and cases with positivity for both IgG & IgM. In the first situation (M+, G-), it is recommended to evaluate a second serum sample after two weeks [31]. We conducted the present study to compare IIF, ELISA and IgG avidity tests for the detection of anti-Toxoplasma antibodies in single serum sample in pregnant women. During this study, 391 pregnant women were evaluated. Based on findings of present study, according to IIF test results; 280 (71.61%) women were seropositive and IgM were positive in 65 (16.62%) individuals. On the other hand, according to findings of ELISA test, 267 (68.29%) were seropositive and IgM were positive in 65 (16.62%) individuals too.

In a study by Mohammad et al. about seroprevalence of *Toxoplasma gondii* between couples in Ramadi city (Iraq) using ELISA, the seroprevalence in wives was only 30.7%, while that in husbands was 13.1% only; this frequency is far less than what was reported in our study, this difference might be due to the difference in populations, because in present study women were just pregnant women [32].

In another study by Kalantari et al., a serological study of toxoplasmosis in pregnant women in Babol (Iran), it was shown that about 60% of pregnant women were seropositive; this frequency was less than what we found in present study [33]. In the present study, IgG Avidity test was promising in clarifying the final diagnosis in 42 IgG weak positive suspected cases and 45 IgM weak positive cases. Thus, we have repeated the tests with 1/50 and 1/200 of serum dilutions (starting dilution was 1/100) and we found completely different results. Forty-two cases of IgG weak positive in repeated tests with1/50 and 1/200 were shown positive and negative (real weak positive) respectively, but in IgM suspected cases which have very high titer antibody or prozone phenomena, the results of 1/50 and 1/200 titrations were negative and positive respectively. In 42 IgG weak positives cases, IgG avidity index were >30%, confirming chronic or past infection but in 45 IgM weak positive, IgG avidity index were ≤20% which confirmed a recent infection. Five IgM weak positive cases with ELISA which were in gray zone with suspected results, (IgM positive in IIF) were re-confirmed with IgG avidity test as a recent infection (≤20%). The two cases of IgM positive (ELISA) persistently positive for more than one year, IIF revealed them as IgM negative cases and IgG avidity index >30% so they were confirmed as a chronic or past infection or false positive IgM cases by ELISA test. In our study sensitivities and specificities of three tests (Table 4) were compared with each other, suggesting that, ELISA and IgG avidity would be the reliable tests for evaluation and checking of pregnant women especially when a single serum sample is available such as our condition [16, 26]. By contrast, low avidity results may persist for more than 1 year: for these reasons a panel of serologic tests must be performed (by ELISA, ISAGA, IgG avidity, IIF, Day test) for IgM, IgA, IgE.

In a study by Jian-fang et al. trying to differentiate pregnant women with Toxoplasma serologic profiles indicative of recently acquired infections from those with TSPs indicative of infections acquired in the distant past, it was concluded that ELISA test for serodiagnosis of toxoplasmosis, the total concordance (including positive and

negative sera) was 93.2% and 95.7% for the detection of IgG and IgM antibodies, respectively [34]. In another study, Bel-Ochi et al. conducted a study about the use ELISA to detect *Toxoplasma gondii*-specific IgG antibodies in human sera and saliva, and it was concluded that serum-based ELISA detected specific IgG with 100% sensitivity and specificity; this sensitivity and specificity is almost the same we presented in current study [35]. In conclusion, IIF needs an experienced technician for working with fluorescent microscopy and interpretation of suspected results. On the other hand, because of higher sensitivity and specificity of ELISA, easier technique and lower expense, it is the preferred test to screen toxoplasmosis infection. However, in some literature IIF is mentioned as a standard technique. Finally, we propose to perform similar studies in this field in a large sample and in other medical centers and with consideration of relevant factors. Educational plans regarding risk of toxoplasmosis at fertility ages are recommended to be implemented in the national maternity health care programs. Results of the present study may be used in modifying guidelines to improve screening and therapeutic processes.

Conflict of interest. The authors declare that there is no conflict of interests regarding the publication of this manuscript, and this manuscript is an original contribution and is not being reviewed or published elsewhere.

ACKNOWLEDGMENTS

This study was financially supported by Tropical and Infectious Diseases Research Center (TIDRC), Tabriz, Iran.

REFERENCES

[1] Dubey J.P., Jones J.L. *Toxoplasma gondii* infection in humans and animals in the United States. *Int. J. Parasitol.* 38, 1257-1278, 2008.

[2] Elmore S.A., Jones J.L., Conrad P.A, Patton S., Lindsay D.S., Dubey J.P. T*oxoplasma gondii:* epidemiology, feline clinical aspects, and prevention. *Trends Parasitol.* 26, 190-196, 2010.

[3] Lehmann T., Marcet P.L., Graham D.H., Dahl E.R., Dubey J.P. Globalization and the population structure of *Toxoplasma gondii. Proc. Nat. Acad. Sci.*103, 11423-11428, 2006.

[4] Afonso E., Thulliez P., Gilot-Fromont E. Transmission of *Toxoplasma gondii* in an urban population of domestic cats (Felis catus). *Int. J. Parasitol.* 36, 1373-1382, 2006.

[5] Dubey J.P. The history of *Toxoplasma gondii*-the first 100 years. *J. Eukaryot. Microbiol.* 55, 467-475, 2008.

[6] Jones J.L., Kruszon-Moran D., Sanders-Lewis K., Wilson M. *Toxoplasma gondii* infection in the United States, 1999 - 2004, decline from the prior decade. *Am J. Trop. Med. Hyg*. 77, 405-4010, 2007.

[7] Saeij J.P.J., Boyle J.P., Boothroyd J.C. Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. *Trends Parasitol*. 21, 476-481, 2005.

[8] Dubey J.P., Su C., Cortes J.A., et al. Prevalence of *Toxoplasma gondii* in cats from Colombia, South America and genetic characterization of *T. gondii* isolates. *Veter. Parasitol.* 141, 42-47, 2006.

[9] Sensini A. *Toxoplasma gondii* infection in pregnancy: opportunities and pitfalls of serological diagnosis. *Clin. Microbiol. Infect.* 12, 504-512, 2006.

[10] Thaller R., Tammaro F., Pentimalli H. Risk factors for toxoplasmosis in pregnant women in central Italy. *Infez. Med.* 19, 241-247, 2011.

[11] Russo M., Pergola G., Pedicini G. Ocular toxoplasmosis: our experience. *Infez. Med*. 13, 160-167, 2005.

[12] Varella I.S., Canti I.C., Santos B.R., et al. Prevalence of acute toxoplasmosis infection among 41,112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. *Memorias do Instituto Oswaldo Cruz*. 104, 383-388, 2009.

[13] Di Carlo P., Mazzola A., Romano A., et al. Postnatal follow-up of infants born to mothers with certain *Toxoplasma gondii* infection: evaluation of prenatal management. *Infez. Med.* 13, 72-78, 2005.

[14] Benard A., Petersen E., Salamon R., et al. Survey of European programmes for the epidemiological surveillance of congenital toxoplasmosis. *Euro Surveill*. 13, 15, 2008.

[15] Pappas G., Roussos N., Falagas M.E. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int. J. Parasitol.* 39, 1385-1394, 2009.

[16] Rajaii M., Pourhassan A., Asle-Rahnamaie-Akbari N., et al. Seroepidemiology of toxoplasmosis in childbearing women of Northwest Iran. *Infez. Med.* 21, 194-200, 2013.

[17] Montoya J.G., Remington J.S. Management of *Toxoplasma gondii* infection during pregnancy. *Clin. Infect. Dis.* 47, 554-566, 2008.

[18] Di Carlo P., Romano A., Schimmenti M.G., Mazzola A., Titone L. Materno-fetal *Toxoplasma gondii* infection: critical review of available diagnostic methods. *Infez. Med*. 16, 28-32, 2008.

[19] Press C., Montoya J.G., Remington J.S. Use of a single serum sample for diagnosis of acute toxoplasmosis in pregnant women and other adults. *J. Clin. Microbiol.* 43, 3481-3483, 2005.

[20] Hamilton C.M., Gray R., Wright S.E., Gangadharan B., Laurenson K., Innes E.A. Prevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in red foxes (Vulpesvulpes) from around the UK. *Vet. Parasitol.* 130, 169-173, 2005.
[21] Jones J.L., Muccioli C., Belfort R., Jr., Holland G.N., Roberts J.M., Silveira C. Recently acquired *Toxoplasma gondii* infection, Brazil. *Emerg. Infect. Dis.* 12, 582-587, 2006.
[22] Pappas G., Roussos N., Falagas M.E. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int. J. Parasitol*. 39, 1385-1394, 2009.
[23] Daryani A., Sharif M., Hosseini S.H., Karimi S.A., Gholami S. Serological survey of *Toxoplasma gondii* in schizophrenia patients referred to Psychiatric Hospital, Sari City, Iran. *Trop. Biomed*. 27, 476-482, 2010.
[24] Fallah M., Rabiee S., Matini M., Taherkhani H. Seroepidemiology of toxoplasmosis in primigravida women in Hamadan, Islamic Republic of Iran, 2004. *East. Med. Health J.* 14, 163-171, 2008.
[25] Mostafavi S.N., Ataei B., Nokhodian Z., Yaran M., Babak A. Seroepidemiology of *Toxoplasma gondii* infection in Isfahan province, central Iran: A population based study. *J. Res. Med. Sci*. 16, 496-501, 2011.
[26] Salahi-Moghaddam A., Hafizi A. A serological study on *Toxoplasma gondii* infection among people in South of Tehran, Iran. *Korean J. Parasitol*. 47, 61-63, 2009.
[27] Sharif M. Gholami S., Ziaei H., et al. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. *Vet. J*. 174, 422-424, 2007.
[28] Hill D., Dubey J.P. *Toxoplasma gondii:* transmission, diagnosis and prevention. *Clin. Microbiol. Infect.* 8, 634-460, 2002.
[29] Iqbal J., Khalid N. Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. *J. Med. Microbiol.* 56, 1495-1499, 2007.
[30] Holec-Gasior L. *Toxoplasma gondii* recombinant antigens as tools for serodiagnosis of human toxoplasmosis - the current status of studies. *Clin. Vacc. Immunol.* 20, 9, 1343-1351, 2013.
[31] Paquet C., Yudin M.H., Allen V.M., et al. Toxoplasmosis in pregnancy: prevention, screening, and treatment. *J. Obstet. Gynecol. Canada* 35, 78-79, 2013.
[32] Husain J.H., Abdulatef Y. Seroepidemological study on infected women by *Toxoplasma gondii* in Al-Ramadi City. *International Journal*. 2, 343-347, 2014.
[33] Kalantari N., Ghaffari S., Bayani M., et al. Serological study of toxoplasmosis in pregnant women in Babol, northern Iran 2012-2013. *SJIMU* 22, 102-108, 2014.
[34] Dai J.F., Jiang M., Qu L., et al. *Toxoplasma gondii*: Enzyme-linked immunosorbent assay based on a recombinant multi-epitope peptide for distinguishing recent from past infection in human sera. *Exp.Parasitol.* 133, 95-100, 2013.
[35] Chahed Bel-Ochi N., Bouratbine A., Mousli M. Enzyme-linked immunosorbent assay using recombinant SAG1 antigen to detect *Toxoplasma gondii*-specific immunoglobulin G antibodies in human sera and saliva. *Clin. Vacc. Immunol.* 20, 468-473, 2013.