International Journal of Epidemiologic Research
doi:10.15171/ijer.2018.06 2018 Winter;5(1):24-29 http://ijer.skums.ac.ir

Original Article

Electrochemiluminesence Epidemiologic Detection of *Toxoplasma gondii* Infection in Pregnant Women With Direct and Indirect Diagnostic Techniques (ELISA Avidity Plus Biochemical Assay)

Ehsan Shariat Bahadory[1*], Ali dalir Ghaffari[2], Somayyeh Namrood[3], Seyyedeh Somayyeh Mosavipour[4], Javid Sadraei[5]

[1]Department of Medical Parasitology, Faculty of Medical Sciences, Tarbiat Modaress University, Tehran, Iran
[2]Department of Medical Parasitology, Faculty of Medical Sciences, Tarbiat Modaress University, Tehran, Iran
[3]Assistant professor, Department of Environmental sciences, Faculty of Fisheries and Environmental Sciences, University of Agricultural Sciences & Natural Resources, Gorgan, Iran
[4]Department of Nursing, Faculty of Medical Sciences, Tehran Medical Sciences University, Tehran, Iran
[5]Department of Medical Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Abstract

Background and aims: Toxoplasmosis is a very common disease in the world. Two types of acquired toxoplasmosis have been detected. In the chronic toxoplasmosis, the abnormality of tissue function is little but, in acute toxoplasmosis, function of RES system becomes interrupted. The assessment of toxoplasma antibody with tissue enzymes in this stage is very important. Furthermore, serum ferritin in some conditions became high in acute phase of infectious disease.

Methods: This study was based on comparative abundance study. Materials consisted of 980 serum and amniotic fluid samples collected from human blood with high level of IgG antibody against *Toxoplasma gondii* in Rajaie center, Tehran, Iran. The standard test was ELISA assay to detect these antibodies and the main test was measurement of liver transaminases (SGOT, SGPT) bilirubin and ferritin to evaluate acute toxoplasmosis. Data analysis was done by SPSS version 18.0. This study was done during March to June, 2017.

Results: Results showed that in some patients with high level of IgG AVIDITY antibody against *T. gondii* the level of liver transaminases, serum bilirubin and ferritin became increased. For example in 120 patients with acute toxoplasmosis the mean serum levels of SGOT were 108 IU/L and in 80 patients the mean serum bilirubin were about 5 mg/dL.

Conclusion: In acute congenital toxoplasmosis, the evaluation of IgG AVIDITY was first step and then the measurement of biochemical factors such as serum transaminases, serum Bilirubin and serum Ferritin were important.

Keywords: Toxoplasmosis, Bilirubin, Electrochemiluminesence, Ferritin, IgG Avidity, Liver function

*Corresponding Author:
Ehsan Shariat Bahadory,
Tel/Fax: 09124189060,
Email:
e_shari2000@yahoo.com

Received: 17 June 2017
Accepted: 12 December 2017
ePublished: 4 January 2018

Introduction

Toxoplasma gondii is an intracellular parasite of many kinds of tissue, including muscle, liver, and intestinal epithelium. In heavy acute infections, the organism can be found free in the blood and peritoneal exudates. It may inhabit in nucleus of host cell but usually lives in the cytoplasm. The life cycle includes intestinal-epithelial and extra intestinal stages in domestic cats and other felines but only extra intestinal stages in other hosts such as human. Seropositivity to toxoplasma was comparable among liver disease patients and those in control group. Raw shellfish consumption and domestic cat exposure are risk factors for acquiring acute *T. gondii* diseases. In the United States, exposure to certain raw or undercooked foods and kittens are risk factors for *T. gondii* infection.[1-3]

In spite of the fact that antibody to *toxoplasma* is widely prevalent in hosts throughout the world, the clinical toxoplasmosis is less common. A general use of the IgG avidity toxoplasma test can diagnose IgG positive in congenital toxoplasmosis. It is clear that most infections are asymptomatic or mild. In 1976, the global prevalence of toxoplasmosis was estimated over 500 million. In acute phase, tachyzoites proliferate in many tissues and this rapid reproduction tends to kill host cells at a faster rate than what the normal turnover of such cells does. As infections become chronic, the zoites infecting brain,

heart, liver, and skeletal muscles are multiplied much more slowly than in the acute phase. *T. gondii* causes many necrosis of liver cells and chorioretinitis.[4-7] In many immune suppressed hosts, *T. gondii* became acute. Acute toxoplasmosis may lead to congenital toxoplasmosis with fetal complications results during pregnancy. The Toxoplasma infection may lead to adverse pregnancy results; therefore, developing good habits of life and health is an effective way to avoid adverse pregnancy results.[8-10]

Toxoplasma gondii is one of the infectious agents of congenital TORCH infections and causes severe clinical outcomes in fetuses and newborns. Infection with *T. gondii* during pregnancy can lead to severe fetal squeal. Moreover, ocular toxoplasmosis is a disease caused by the infection by *T. gondii* through congenital or acquired routes. The different laboratory methods used for diagnosing the congenital toxoplasmosis have variable sensitivity and specificity levels such as IgM Western blotting (WB), IgA enzyme immunoassay (EIA), and DNA amplification by real-time polymerase chain reaction (PCR). In acute stage of toxoplasmosis, the level of IgG avidity antibody is high and in these conditions the level of tissue enzymes such as transaminases (SGOT, SGPT), serum bilirubin, and ferritin became high. In addition, total malfunction of RES system was observed. An increase in serum transaminases, bilirubin, and ferritin can be important in acute phase of intracellular infection.[11-15]

Objective

The purpose of this survey was to evaluate the biochemical factors using electrochemiluminescence and ELISA AVIDITY test in patients with acute congenital toxoplasmosis (IgG avidity positive) in an Iranian population.

Methods

The study was based on comparative study. Samples were serum and amniotic fluid specimens collected from 980 patients with high level of IgG avidity antibody against *T. gondii* in Rajaie center, Tehran, Iran. The gold standard test was ELISA assay to detect IgG avidity toxoplasma antibody. These patients have positive ELISA titer for *toxoplasma* antibody measured by laboratory technicians in Rajaie clinical laboratory. The main test to detect serum liver transaminase and bilirubin was based on electrochemiluminescence assay and measurement of serum ferritin was based on ELISA assay.

ELISA Method to Detect Toxoplasma Antibody or Serum Ferritin

1. 100 λ of blood serum, amniotic fluid sample, or calibrator 2 was added to ELISA avidity wells.
2. Samples incubated for 30 minutes at 37°C.
3. Pellet washed for 3 times with PBS (ELISA WASHER).
4. 100 λ enzyme conjugate added to these plates.
5. The mixture incubated for 30 minutes at 37°C.
6. Then 100 λ substrate was added (TMB).

After 15 minutes, the added solution stopped, then read at 450 nm ELISA reader.

The formula of ELISA avidity (semi-quantitative):

$$\frac{\text{OD patients serum or amniotic samples}}{\text{OD calibrator 2}}$$

The normal range is <0.8 but the result >1.1 is positive for congenital toxoplasmosis.

Formula of SGPT measurement:

$$\text{L-alanine} + \text{2-oxoglutarate} \xrightarrow{\text{SGPT}} \text{l-glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{l-lactate} + \text{NAD}^+$$

The reagent 1 to measure SGPT consists of TRIS buffer, l alanine, and LDH.

The reagent 2 to measure SGPT consists of 2-oxoglutarate andNADH.

Formula of SGOT measurement:

$$\text{L-aspartate} + \text{2-oxoglutrate} \xrightarrow{\text{SGOT}} \text{l-glutamate} + \text{oxoglutarate}$$

$$\text{Oxoglutarate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{l-malate} + \text{NAD}^+$$

The reagent 1 to measure SGOT consists of TRIS buffer, l-aspartate, malate dehydrogenase (MDH,) and LDH.

The reagent 2 to measure SGOT consists of 2-oxoglutrate and NADH.

In both measurements, we mixed 100 λ human serum + 1000 λ reagent 1 and after 5 minutes, we added 250 λ reagent 2, then read OD of reaction tubes against blank tube at 340 λ wave-length. The normal range (activity) of SGOT and SGPT in this study was: <31 U/L. The measurement of serum bilirubin based on photometric study by auto analyzer Cobas e411 electrochemiluminescence instrument and measurement of serum ferritin were done by ELISA method.

This study was a comparative study based on studies during March to June 2017. The calculation of results performed by SPSS software version 9.0.

Results

Results showed that in some patients with high level of

IgG AVIDITY antibody against *T. gondii,* the level of liver transaminases, serum bilirubin, and ferritin increased. For example, in 120 patients with acute toxoplasmosis, the mean serum level of SGOT was 108 IU/L and in 80 patients, the mean serum bilirubin was about 5 mg/dL.

Figure 1 indicates that in 980 toxoplasmosis pregnant women, the mean of ELISA AVIDITY titer was higher in serum sample than in amniotic fluid sample. Moreover, in each abortion, the mean of ELISA AVIDITY titer became high. For example in mothers with fourth abortion, the mean of ELISA AVIDITY titer was higher than in mothers with first abortion.

In Table 1, it is shown that in 980 patients with acute toxoplasmosis, the serum levels of bilirubin with 95% CI of the difference were between 1.1 until 1.7 mg/dL which are normal range.

Figure 2A shows that in 980 patients with acute toxoplasmosis, only 12% of patients had high level of serum SGOT.

In Figure 2B, it is indicated that in 980 patients with acute toxoplasmosis, only 14% of patients had high level of serum SGPT.

Figure 2C shows that in 980 patients with acute toxoplasmosis, only 8% of patients had high level of serum bilirubin.

Figure 2D illustrates that in 980 patients with acute toxoplasmosis, only 14% of patients had high level of serum ferritin.

In Table 2, it is clear that in 980 patients with acute congenital toxoplasmosis, the minimum level of serum Ferritin, for example, was 12 mg/dL and the maximum level of serum Ferritin was 994 mg/dL. The biochemical assay was based on electrochemiluminescence assay.

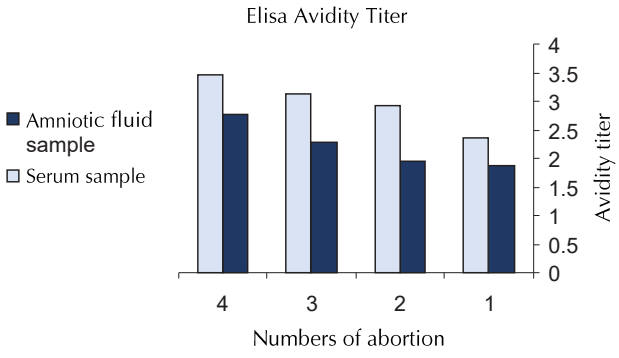

Figure 1. Titer Elisa Avidity From 98 Toxoplasmosis Pregnant Women.

Discussion

Toxoplasmosis, one of the TORCH's infections in pregnant women, is caused by *Toxoplasma gondii*. The seroprevalence of Toxoplasmosis among pregnant women was found to be comparatively high compared with previous reports from Saudi Arabia, for instance, or the prevalence of *Toxoplasma* IgG antibodies in pregnant women was low in Sicilian population. The clinical spectrum of T. gondii infection varies from an asymptomatic state to a severe illness. The preliminary diagnostic utility of 2 mixtures of *T. gondii* recombinant antigens in IgG ELISA and IgG avidity test has been evaluated. In addition, an epidemiological study has reported an association of *T. gondii* infection with liver cirrhosis. Therefore, the high positive results should be treated with some skepticism until additional precise diagnostic tools are developed. My The purpose of the present study was evaluation of biochemical factors using electrochemiluminescence assay and ELISA AVIDITY test in patients with acute congenital toxoplasmosis (asymptomatic IgG avidity positive) in an Iranian population.[16-20]

In a recent study in the United States, elevated risk of recent *T. gondii* infection was associated with eating rare lamb. Even frozen lamb has been associated with acute *T. gondii* infection in Brazil. Interestingly, toxoplasmosis with liver involvement has been reported in deer hunters who had eaten undercooked venison. Under normal immune conditions, *Toxoplasma* infection is largely asymptomatic, but in those individuals who are immune-compromised, such as individuals with AIDS, malignant patient under chemotherapy, or organ transplant recipients, the parasite can become widely disseminated, causing severe toxoplasmosis and/or encephalitis. Also the prevalence of acute *T. gondii* infection is high among children with non-specific reactive hyperplasia of the cervical lymph nodes. The diagnosis of toxoplasmosis is most commonly made by detecting the immunoglobulin (IgG and IgG avidity) antibodies in the serum samples of patients using variety methods (ELISA, IFA, IgM-

Table 1. One Sample T-test From Toxoplasmosis Patients

	One-Sample Test					
	Test Value = 0					
	T	*df*	Sig. (2-tailed)	Mean Difference	95% CI	
					Lower	Upper
SGOT	9.078	97	.000	27.64	21.5	33.6
SGPT	8.434	97	.000	31.31	23.9	38.6
Bilirubin	10.451	97	.000	1.44	1.16	1.71
Ferritin	5.884	97	.000	184.35	122.16	246.53

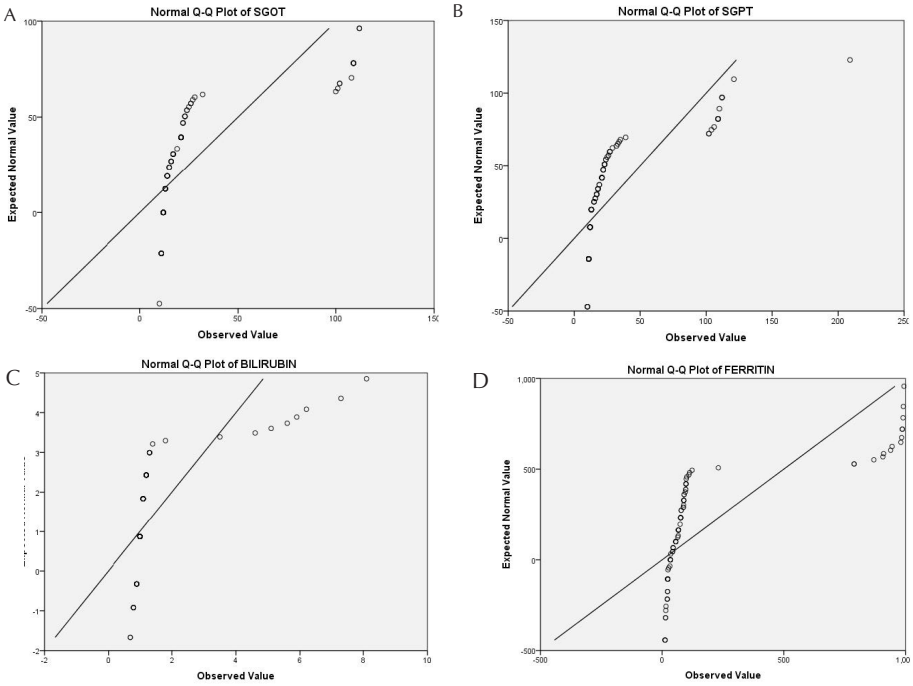

Figure 2. Q-Q Plot of Serum (A) SGOT, (B) SGPT, (C) Serum Bilirubin and (D) Serum Ferritin in Acute Toxoplasmosis Patients.

ISAGA, etc).[21-25]

Sometimes, congenital toxoplasmosis received from undercooked meat. In spite of the fact that antibody to *Toxoplasma* is widely prevalent in human throughout the world, clinical toxoplasmosis is less common. The occurrence of congenital toxoplasmosis is still a problem. It is clear that most infections are asymptomatic or mild. Several factors influence this phenomenon: the virulence of the strain of *Toxoplasma*, the susceptibility of the individual host species, the age of the host, and the degree of the acquired immunity of the host. Occasionally, circumstances conspire to make a mild case important. For example, Elahain et al suggested a significant relationship between the age of the youngest child and the infection rate. Since there seems to be an age resistance, influence of adults or weaned juveniles are asymptomatic, although some exceptions occur. Asymptomatic infections can suddenly become fulminating if immunosuppressive drugs such as corticosteroids are employed for other conditions. The first extra intestinal sites to be infected in both cats and other hosts (humans) are the mesenteric lymph nodes and the parenchyma of the liver. These sites, experience rapid regeneration of cells too and perform an effective preliminary screening of the parasites. The most common symptom of acute toxoplasmosis is painful, swollen lymph glands in the cervical and abnormality in the RES system. In chronic phase, the cyst of *Toxoplasma* can remain intact for years and produce no obvious clinical effect. The larger number of acute toxoplasmosis (about 70%) is asymptomatic. Mumcuoglu et al,[24] suggested *T. gondii* IgM, IgG, and IgG avidity tests were performed by VIDAS automated analyzer using TOXO IgM, TOXO IgG II, and TOXO IgG avidity kits. In this study, we showed that in most asymptomatic acute toxoplasmosis patients with good immune system, the biochemical factors such as serum SGOT, serum SGPT, serum bilirubin, and serum Ferritin had different levels and required further study such as ELISA AVIDITY test. In acute congenital toxoplasmosis, the evaluation of

Table 2. Descriptive Statistics of Biochemical Index in Acute Toxoplasmosis Patients

Descriptive Statistics					
	N	Minimum	Maximum	Mean	SD
SGOT	980	10.00	112.00	27.6	30.1
SGPT	980	10.00	209.00	31.3	36.7
Bilirubin	980	.70	8.10	1.44	1.36
Ferritin	980	12.00	994.00	184.34	310.17
Valid N (listwise)	980				

IgG AVIDITY was first step and then the measurement of biochemical factors such as serum transaminases, serum bilirubin and serum ferritin was important.[26-33] In Iran there are no study about biochemical factors in discrimination of acute and chronic toxoplasmosis but in this study, we found many differences in biochemical factors of acute and chronic toxoplasmosis.

Conflict of Interest Disclosures

None.

Acknowledgements

This work has been supported by Tarbiat Modares University, Faculty of Medicine, Department of Parasitology.

References

1. Chiang TY, Kuo MC, Chen CH, Yang JY, Kao CF, Ji DD, et al. Risk factors for acute Toxoplasma gondii diseases in Taiwan: a population-based case-control study. PLoS One. 2014;9(3):e90880. doi: 10.1371/journal.pone.0090880.
2. Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis. 2009;49(6):878-84. doi: 10.1086/605433.
3. Alvarado-Esquivel C, Torres-Berumen JL, Estrada-Martinez S, Liesenfeld O, Mercado-Suarez MF. *Toxoplasma gondii* infection and liver disease: a case-control study in a northern Mexican population. Parasit Vectors. 2011;4:75. doi: 10.1186/1756-3305-4-75.
4. McAuley JB. Congenital Toxoplasmosis. J Pediatric Infect Dis Soc. 2014;3(suppl 1):S30-S5. doi: 10.1093/jpids/piu077.
5. Prusa AR, Kasper DC, Pollak A, Gleiss A, Waldhoer T, Hayde M. The Austrian Toxoplasmosis Register, 1992-2008. Clin Infect Dis. 2015;60(2):e4-e10. doi: 10.1093/cid/ciu724.
6. Tomasoni LR, Meroni V, Bonfanti C, Bollani L, Lanzarini P, Frusca T, et al. Multidisciplinary approach to congenital Toxoplasma infection: an Italian nationwide survey. New Microbiol. 2014;37(3):347-54.
7. Sato S, Nishida M, Nasu K, Narahara H, Norose K, Aosai F. Congenital toxoplasmosis from a mother with type 2 diabetes mellitus: a case report. J Obstet Gynaecol Res. 2014;40(11):2158-61. doi: 10.1111/jog.12477.
8. Kavari A, Nowzari N, Moazeni Jula G, Moazeni Jula F, Hashemzadeh H. A Serological and Molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. Archives of Razi Institute. 2013;68(1):29-35. doi: 10.7508/ari.2013.01.005.
9. Yad Yad MJ, Jomehzadeh N, Jafar Sameri M, Noorshahi N. Seroprevalence of Anti-*Toxoplasma gondii* Antibodies Among Pregnant Woman in South Khuzestan, Iran. Jundishapur J Microbiol. 2014;7(5):e9998. doi: 10.5812/jjm.9998.
10. Zhang YP, Song RH. [Investigation on pregnancy outcomes and risk factors in pregnant women infected with *Toxoplasma gondii*]. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2014;26(2):221-3.
11. Davami MH, Pourahamd M, Jahromi AR, Tadayon SM. Toxoplasma seroepidemiology in women who intend to marry in Jahrom, Islamic Republic of Iran. East Mediterr Health J. 2014;19 Suppl 3:S71-5.
12. Park YH, Nam HW. Clinical features and treatment of ocular toxoplasmosis. Korean J Parasitol. 2013;51(4):393-9. doi: 10.3347/kjp.2013.51.4.393.
13. Andiappan H, Nissapatorn V, Sawangjaroen N, Khaing SL, Salibay CC, Cheung MM, et al. Knowledge and practice on Toxoplasma infection in pregnant women from Malaysia, Philippines, and Thailand. Front Microbiol. 2014;5:291. doi: 10.3389/fmicb.2014.00291.
14. Rodrigues IM, Costa TL, Avelar JB, Amaral WN, Castro AM, Avelino MM. Assessment of laboratory methods used in the diagnosis of congenital toxoplasmosis after maternal treatment with spiramycin in pregnancy. BMC Infect Dis. 2014;14:349. doi: 10.1186/1471-2334-14-349.
15. Marangoni A, Capretti MG, De Angelis M, Nardini P, Compri M, Foschi C, et al. Evaluation of a new protocol for retrospective diagnosis of congenital toxoplasmosis by use of Guthrie cards. J Clin Microbiol. 2014;52(8):2963-70. doi: 10.1128/jcm.00106-14.
16. Andiappan H, Nissapatorn V, Sawangjaroen N, Chemoh W, Lau YL, Kumar T, et al. Toxoplasma infection in pregnant women: a current status in Songklanagarind hospital, southern Thailand. Parasit Vectors. 2014;7:239. doi: 10.1186/1756-3305-7-239.
17. El-Shahawy IS, Khalil MI, Bahnass MM. Seroprevalence of *Toxoplasma gondii* in women in Najran City, Saudi Arabia. Saudi Med J. 2014;35(9):1143-6.
18. Drapała D, Holec-Gasior L, Kur J, Ferra B, Hiszczynska-Sawicka E, Lautenbach D. A new human IgG avidity test, using mixtures of recombinant antigens (rROP1, rSAG2, rGRA6), for the diagnosis of difficult-to-identify phases of toxoplasmosis. Diagn Microbiol Infect Dis. 2014;79(3):342-6. doi: 10.1016/j.diagmicrobio.2014.03.005.
19. Puccio G, Cajozzo C, Canduscio LA, Cino L, Romano A, Schimmenti MG, et al. Epidemiology of Toxoplasma and CMV serology and of GBS colonization in pregnancy and neonatal outcome in a Sicilian population. Ital J Pediatr. 2014;40:23. doi: 10.1186/1824-7288-40-23.
20. Chemoh W, Sawangjaroen N, Nissapatorn V, Suwanrath C, Chandeying V, Hortiwakul T, et al. *Toxoplasma gondii* infection: What is the real situation? Exp Parasitol. 2013;135(4):685-9. doi: 10.1016/j.exppara.2013.10.001.
21. Dlugonska H. *Toxoplasma gondii* and the host cells. Ann Parasitol. 2014;60(2):83-8.
22. Krueger WS, Hilborn ED, Converse RR, Wade TJ. Drinking water source and human *Toxoplasma gondii* infection in the United States: a cross-sectional analysis of NHANES data. BMC Public Health. 2014;14:711. doi: 10.1186/1471-2458-14-711.
23. Tomasoni LR, Meroni V, Bonfanti C, Bollani L, Lanzarini P, Frusca T, et al. Multidisciplinary approach to congenital Toxoplasma infection: an Italian nationwide survey. New Microbiol. 2014;37(3):347-54.
24. Li ZH, Guo FY, Wang ZQ, Cui J. Intracranial inflammatory granuloma caused by toxoplasmosis. Pathog Glob Health. 2014;108(5):255-9. doi: 10.1179/2047773214y.0000000147.
25. Bilal JA, Alsammani MA, Ahmed MI. Acute *Toxoplasma gondii* infection in children with reactive hyperplasia of the cervical lymph nodes. Saudi Med J. 2014;35(7):699-703.
26. Andiappan H, Nissapatorn V, Sawangjaroen N, Khaing SL, Salibay CC, Cheung MM, et al. Knowledge and practice on Toxoplasma infection in pregnant women from Malaysia, Philippines, and Thailand. Front Microbiol. 2014;5:291. doi: 10.3389/fmicb.2014.00291.
27. Elahian Firouz Z, Kaboosi H, Faghih Nasiri A, Tabatabaie SS, Golhasani-Keshtan F, Zaboli F. A Comparative Serological Study of Toxoplasmosis in Pregnant Women by CLIA and ELISA Methods in Chalus City Iran. Iran Red Crescent Med J. 2014;16(4):e15115. doi: 10.5812/ircmj.15115.
28. Mumcuoglu I, Toyran A, Cetin F, Coskun FA, Baran I, Aksu N, et al. [Evaluation of the toxoplasmosis seroprevalence in pregnant women and creating a diagnostic algorithm].

Mikrobiyol Bul. 2014;48(2):283-91.
29. Capobiango JD, Bregano RM, Navarro IT, Rezende Neto CP, Casella AM, Mori FM, et al. Congenital toxoplasmosis in a reference center of Parana, Southern Brazil. Braz J Infect Dis. 2014;18(4):364-71. doi: 10.1016/j.bjid.2013.11.009.
30. Jerant PV, Milosevic V, Hrnjakovic Cvjetkovic I, Patic A, Stefan Mikic S, Ristic M. [*Toxoplasma gondii* infection in pregnant women]. Med Pregl. 2013;66(11-12):459-63.
31. Murat JB, Hidalgo HF, Brenier-Pinchart MP, Pelloux H. Human toxoplasmosis: which biological diagnostic tests are best suited to which clinical situations? Expert Rev Anti Infect Ther. 2013;11(9):943-56. doi: 10.1586/14787210.2013.825441.
32. Geleneky M. [Toxoplasmosis in pregnancy - questions in clinical practice]. Klin Mikrobiol Infekc Lek. 2013;19(2):48-51.
33. Schmidt M, Sonneville R, Schnell D, Bige N, Hamidfar R, Mongardon N, et al. Clinical features and outcomes in patients with disseminated toxoplasmosis admitted to intensive care: a multicenter study. Clin Infect Dis. 2013;57(11):1535-41. doi: 10.1093/cid/cit557.

How to cite the article: Bahadory ES, Ghaffari AD, Namrood S, Mosavipour SS, Sadraei J. Electrochemiluminesence epidemiologic detection of Toxoplasma Gondii infection in pregnant women with direct and indirect diagnostic techniques (ELISA avidity plus biochemical assay). Int J Epidemiol Res. 2018;5(1):24-29. doi: 10.15171/ijer.2018.06.