ijer.skums.ac.ir

Evaluation of serum concentration of AFP marker in toxoplasmosis pregnant women with high level of IgG and IgM *toxoplasma* antibody by ELISA assay in a population of Tehran, Iran

Javid Sadraie, Ehsan Shariat Bahadory*, Sekhavat Ghaffari Novin
Medical Parasitology Dept., Tarbiat Modaress University, Tehran, I.R. Iran.
Received: 4/Jul/2015 Accepted: 6/Oct/2015

Original article

ABSTRACT

Background and aims: Toxoplasmosis is a parasitic disease which may cause some laboratory symptoms in infected individuals. One of the main ways of transmition this organism is placenta to fetus pathway. If this transmission occurs in the 3th month of pregnancy, the abortion, central nerve system and ocular disorder will happen. Because of this issue, the precise technique for detection of Toxoplasma Antibody such as IgG and IgM is important, that contains ELISA to detect *Toxoplasma* Antibody such as IgG and IgM and AFP.
Methods: This was a cross sectional study. In this study, the main sample was serum that was randomly collected from 255 pregnant women infected with *toxoplasma Gondi* in Avesina center. Then, It was detected the serum concentration of AFP in toxoplasmosis pregnant women with high level of IgG and IgM *toxoplasma* antibody by ELISA assay.
Results: The results of this survey showed that the infection in these pregnant women by *toxoplasma gondii* was occurred and 13% of them (13% of 255 infected mothers) had high levels of AFP in their serum. The statistical analyses was done by SPSS consisted of t-test, case number, comparative study, and Q-Q plot evaluations.
Conclusions: In some pregnant women with high level of IgG and IgM *toxoplasma* antibody was observed in high levels of AFP in their serum and this index correlates with NTD in their fetus.

Keywords: *Toxoplasma gondii*, ELISA, IgG antibody, IgM antibody, AFP, IRAN.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite and an important zoonotic pathogen that causes severe diseases in congenitally infected patients and in immunocompromised patients such as AIDS victims in addition to the parasite infected persons.[1-3] *T. gondii* infects macrophages primarily and is capable of invading and replicating within a wide variety of nucleated host cells. Acute *T. gondii* infection at early pregnancy in women without a history of infection may lead to fetal death in the uterus or severe neurological damage. [4-6] Tran's placental

*Corresponding author: Ehsan Shariat Bahadory, Medical Parasitology Dept., Tarbiat Modaress University, Tehran, I.R. Iran, Tel: 0098912418960, E-mail: ehsanshariat63@gmail.com

infection of the fetus occurs in 12% of cases in which the mothers acquire infection during the first trimester. The incidence of transmission increases thereafter to more than 90% when maternal infection occurs during the last weeks before delivery, which is more likely to be asymptomatic, yet may proceed to choreoretinitis later in childhood or in adolescence. One third of mothers who acquire a primary *T. gondii* infection during the pregnancy transmits the infection to their fetuses.[7-9] Therefore, an antenatal diagnostic method should be employed to screen this fraction.[10,11] Then, the ELISA avidity methods help to detect the time of infection in mothers with *toxoplasma gondii*. This would help the mothers to make an informed decision on either treatment or therapeutic abortion. In Iran, up to present, most of the decisions on infected fetuses were made based on serological findings in their mothers, which might have led to abortion of many uninfected fetuses.[12-14] The low avidity of immunoglobulin G (IgG) has been reported to be a useful marker of recent infection with *Toxoplasma*. Nevertheless, discrepancy results in the maturation of avidity over time have been reported. Alpha fetoprotein (AFP) is a protein normally produced by the liver and yolk sac of a developing baby during pregnancy. AFP levels decrease soon after the birth. AFP probably has no normal function in adults. The normal values in males or no pregnant females are generally less than 40 micrograms/liter. The examples above are common measurements for results of these tests. Normal value ranges may vary slightly among different laboratories.[15-17] Greater than normal levels of AFP may be due to: Birth defections, including: Anencephaly; Duodenal atresia; Gastroschisis; Omphalocele; Spina bifida; Tetralogy of Fallot; Turner syndrome

Controlling the albumin and a-fetoprotein (AFP)' synthesis in the mammalian liver provides a powerful model system for investigating the molecular mechanisms of gene regulation during normal development and oncogenesis. The plasma levels of these two proteins exhibit a reciprocal relationship during development.[15-17] AFP is dominant in the plasma of the developing fetus where it is synthesized by the yolk sac and liver. Its concentration is drastically reduced after the birth in rodents to reach a basal level in adult animals. However, re-expression of this protein occurs in cases of liver neoplastic and germinal tumors.[15-17] The aim of this study was to evaluate of serum concentration of AFP in toxoplasmosis pregnant women with high level of IgG and IgM *toxoplasma* antibody by ELISA assay.

METHODS

This research was a cross sectional study. In this study, it was randomly collected 255 serum samples from toxoplasmosis pregnant women from Avecina Center, Tehran, Iran. They had high levels of serum IgG and IgM antibody against *toxoplasma gondii* parasite. This study was based on comparative study. The mothers that were chosen were patients who had one or more abortion in their life. It was followed up these mothers' disease process to understand the cause of their children abortion. All of mothers had high levels of serum IgG and IgM antibody against *toxoplasma gondii* parasite and about 13% of these mothers had high serum levels of AFP marker and the child' of these patients had suffered from encephalitis disorders with attention of their radiography experiments. All of 255 toxoplasmosis mothers were selected from Avesina laboratory center and were sent to radiography test and other experiments on NTD disorders. It was divided all of infected pregnant women to 13 groups that in groups 3 and 10 these mothers had high levels of AFP markers. The main procedure was

ELISA assay to detect AFP marker in toxoplasmosis pregnant women. Because of sampling in equal conditions at the same time and devoting aborted 20 mothers in each group we divided all of the infected pregnant women to 13 groups. Materials used in this study were: Two hundred and fifty five serum samples from toxoplasmosis pregnant women; ELISA kit to detect IgG and IgM antibody against *toxoplasma gondii* parasite (This method was gold standard test); ELISA kit to detect AFP marker in toxoplasmosis pregnant women; ELISA washer device to wash ELISA plates to discard unwanted proteins and antibodies from ELISA wells; ELISA reader devices with 450 nm wavelength to measure OD of each ELISA well. With measurement of OD from each ELISA well, it was evaluated the precise concentration of each antibody or marker such as IgM or IgG or AFP marker; SPSS software for analyzing data.

ELISA kit contents: 1. Six standard of IgG, IgM or AFP marker to delineated curve of quantitative ELISA assay; 2. Assay buffer or diluents buffer to dilute 125 serum samples; 3. Wash buffer to wash ELISA wells; 4. Enzyme conjugates solution to trace IgG, IgM or AFP marker; 5. TMB solution as substrate of ELISA assay; 6. Stop solution to halt total ELISA reaction.

Method to detect IgG and IgM *toxoplasma* antibody: 1. We added 100λ of serum or amniotic fluid to ELISA avidity wells; 2. Incubated what? For 30 minutes in 37^0C; 3. We washed plates for 3 times with PBS (ELISA Washer); 4. We added 100λ enzyme conjugate to these plates; 5. Incubated for 30 minutes in 37^0C; 6. We added 100λ substrate (TMB); 7. After 15 minutes we added stop solution and read it at 450nm ELISA reader.

Method to detect AFP marker: 1. To select appropriate wells; 2. To pour 50 λ standards, control serum and samples into wells; 3. To pour 100 λ assay buffer into wells; 4. To shake plate gently and incubate it in room temperature until 30 minutes; 5. To discard every content of plate and wash 5 times by washing buffer; 6. To add 100 λ enzyme conjugate to each wells and incubate 30 minutes in room temperature; 7. To discard every content of plate and wash 5 times by washing buffer; 8. To add 100 λ TMB into each wells; 9. To incubate plate 15 minutes in room temperature at dark condition; 10. To add 100 λ stop solution into each wells and read on 450nm wavelength by ELISA reader device.

It was suggested to read this plate on 630 nm wavelength such as reference filter. Before starting this procedure, it was reached any sample and standards to room temperature and shakes them gently. Total experiment should be done without stopping. It was used disposable sampler tip to achieved good results. After adding stop solution, it should be read OD each well until 30 minutes. In each time of washing procedure it was added about 300 λ of washing buffer to achieve good results. The important factor to achieve proper results is incubation time.

RESULTS

It was divided total toxoplasmosis mothers into 13 groups (AFP1 to AFP 13). The statistical surveillances were done by SPSS software consisted of t-test, case number, comparative study, and Q-Q plot evaluations. In this table it was showed that in the group 3 and 10 the toxoplasmosis pregnant women had high level of AFP marker and in these mothers, their fetus had any kind of encephalitis in radiography experiments. The normal range of AFP in serum was 0.2 to 8.5 ng/ml. It was detected *toxoplasma gondii* IgG and IgM antibody by fresh designed ELISA method and it was detected acute toxoplasmosis by ELISA avidity IgG antibody assay.

Table 1: T-test analyses infected pregnant women for AFP serum levels

Groups of infected mothers	t	Df	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
AFP1	9.153	19	2.69400	2.0780	3.3100
AFP2	16.448	19	2.70000	2.3564	3.0436
AFP3	25.192	19	101.60000	93.1588	110.0412
AFP4	9.159	19	2.99400	2.3098	3.6782
AFP5	13.199	19	3.37550	2.8402	3.9108
AFP6	10.443	19	3.49100	2.7913	4.1907
AFP7	11.453	19	3.25300	2.6585	3.8475
AFP8	12.203	19	2.46500	2.0422	2.8878
AFP9	12.260	19	2.74500	2.2764	3.2136
AFP10	20.642	14	86.37333	77.3988	95.3479
AFP11	11.917	19	3.03500	2.5020	3.5680
AFP12	14.498	19	3.40150	2.9104	3.8926
AFP13	13.552	19	2.90000	2.4521	3.3479

In the table 1, it was showed that the t-test analyzes the serum levels of AFP for toxoplasmosis pregnant women. In this table, it was showed that in the group 3 with 95% Confidence Interval of the difference the serum levels of AFP were between 93 to 110 ng/ml and in the group 10 with 95% Confidence Interval of the difference the serum levels of AFP were between 77 to 95 ng/ml and in these toxoplasmosis pregnant women the encephalitis symptoms in the fetus were detected by radiology tests.

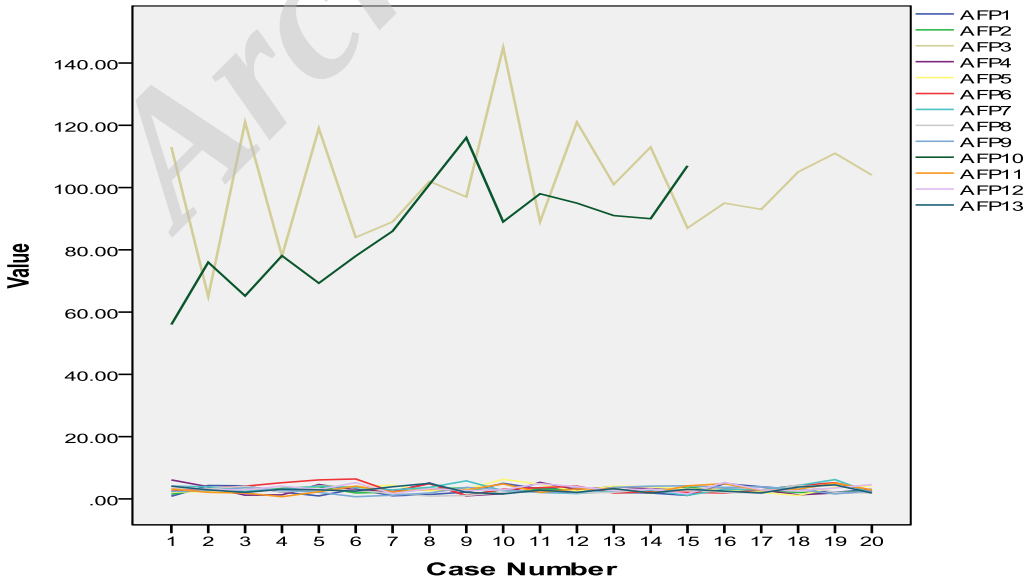

Figure 1: Case number histogram to show serum concentration of AFP IN group 3 infected mothers

In the figure 1 (two lines that are above of other lines), it was showed that in the groups 3 and 10 (toxoplasmosis pregnant women), the serum levels of AFP were in a higher levels than in the other groups and in these groups the encephalitis symptoms in the fetus were detected by radiology tests.

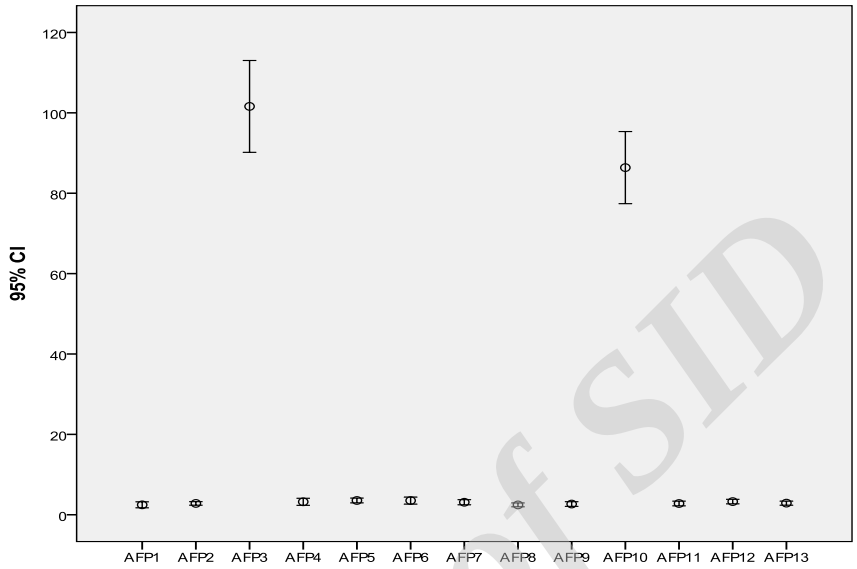

Figure 2: Comparative study between 13 groups of toxoplasmosis pregnant women by AFP evaluation

In the figure 2, it was showed that (with 95% Confidence Interval) the groups that had high levels of AFP were groups 3 and 10 that in these groups of toxoplasmosis pregnant women the NTD symptoms in their fetus were detected by radiology tests.

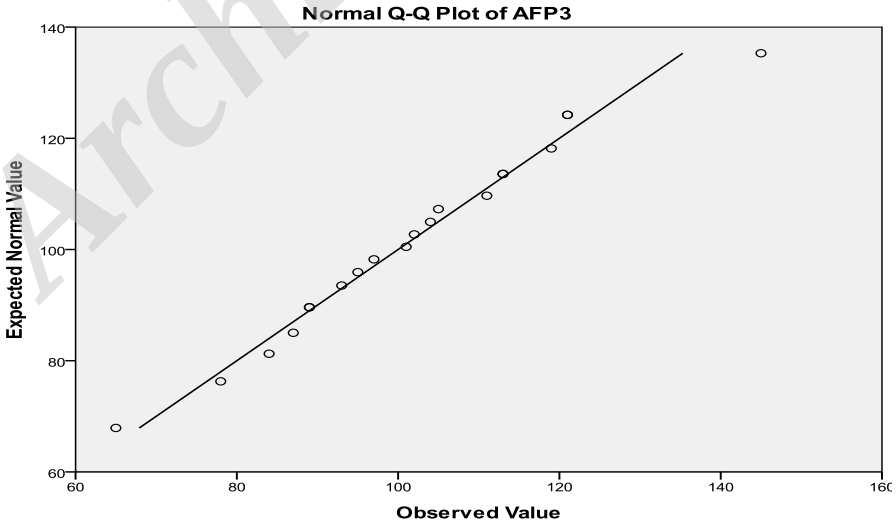

Figure 3: Q-Q plot of AFP3

In the figure 3 it was showed that in AFP3 toxoplasmosis pregnant women the mean range of serum AFP was between 80 to 120 ng/ml.

DISCUSSION

The AFP test is done to: Check the developing baby (fetus) of a pregnant woman for brain or spinal problems (called neural tube defects). Such defects occur in about 2 out of every 1,000 pregnancies. The chance of a neural tube defect in a baby is not related to the mother's age. Most women whose babies have neural tube defects had no family history of these problems such as toxoplasmosis.[18-20] AFP plus IgM and IgG detection in toxoplasmosis mothers are very important factors in cerebral toxoplasmosis. [21-23]

Our objective was evaluation of serum concentration of AFP in toxoplasmosis pregnant women with high level of IgG and IgM *toxoplasma* antibody by ELISA assay. Toxoplasmosis is a very common disease in the world. Two types of acquired toxoplasmosis are detected; 1) In the chronic toxoplasmosis, the abnormality of liver function is little but, 2) In acute congenital toxoplasmosis liver function became suppressed and the assessment of liver transaminases in this stage was very important.[24-26]

Prevention of congenital toxoplasmosis in pregnant women has been mainly based on serological tests for anti-*Toxoplasma* antibodies. Many serological tests, including the haemagglutination test, latex agglutination test (LAT), ELISA, and indirect fluorescence antibody test, have been utilized in the detection of antibodies against *T. gondii*. There have been several reports regarding the screening of anti-*T. gondii* antibodies among Koreans. Ryu et al. (1996) demonstrated 4.3% and 0.94% of positive rates, using ELISA and LAT kits among pregnant women who visited medical institutes in Yangpyong-gun and Kwangju-gun of Kyonggi-do. Distinguishing of acute and chronic phases of toxoplasmosis has critical importance in pregnant women and immuno-compromised patients. Some assays have been used to measure *Toxoplasma* specific IgM antibodies as indicators of the acute phase infection. *Toxoplasma* IgM antibodies can be detected for 1 year or longer in some cases. So, in asymptomatic individuals with stable titers of *Toxoplasma* IgG antibodies, positive IgM results are not easy to interpret and in acute phase of toxoplasmosis NK cells also increased that is specific factor.[27-28]

Han M and et al. showed that under the situation of *T. gondii*-infected dNK cells co-cultured with trophoblast cells, the up-regulation of sHLA-G could induce dNK cells apoptosis which ultimately may contribute to the abnormal pregnancy outcomes with *T. gondii* infection. Fan CK and et al. showed that Parents' educational level and cats kept indoors seemed to be the high risk factors for PSC in acquisition of *T. gondii* infection. While, ocular manifestation and/or headache of PSC should be checked for the possibility of being *T. gondii* elicited. Measures such as improving environmental hygiene and intensive educational intervention to both PSC and their parents should be performed immediately so as to reduce *T. gondii* infection of DRSTP inhabitants including PSC and adults. Rojas Y and etc showed that using serial serum AFP levels as the preferred method of surveillance in children with AFP-positive hepatoblastoma, reserving imaging for the early postoperative period, for children at high risk of relapse, and for determination of the anatomical site of clinically suspected recurrence. Given the small size of this preliminary study, validation in a larger patient population is

warranted. Carr BI and et al. showed that Patients with low AFP were the majority in this cohort, and patients with elevated GGTP had worse prognosis than those with low GGTP. GGTP may be a useful tumor and prognosis marker in low-AFP patients. AFP-negative patients are important to identify due to their enhanced survival.[27-30]

AFP is a glycoprotein molecule with 70000 D molecular weight that resembles to human albumin that both of them code on chromosome 4. AFP is the major protein in blood circulation of fetus that synthesizes primarily by yolk sac and then by liver. AFP concentration reaches to maximum level at 9th weeks of pregnancy in blood circulation of fetus (3000000 ng per ml). Then, gradually it decreases and becomes 20000 ng per ml in final period of pregnancy. AFP transmits from pregnant woman to her fetus and its level becomes high in serum of pregnant woman at 3th months of pregnancy.[29.30]. AFP concentration reaches to 5 ng per ml in pregnant woman at 10th weeks of pregnancy and these concentration increases 15% for each week that its level becomes 180 ng per ml at 25th weeks of pregnancy and its level reaches to 2ng per ml after delivery. AFP penetrates to amniotic fluid and its concentration reaches to 15000 ng per ml at 16th weeks of pregnancy. AFP levels increase in many disorders such as hepatocellular carcinoma, testis tumors and in adults. Its levels increase in hepatoblastoma and nephroblastoma tumors in children. Its level increases in metastatic malignancies from intestine to liver. Nowadays the evaluation of AFP serum levels in pregnant women are used to detect NTD disorders such as congenital toxoplasmosis in fetus.[29,30]

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT

This study was financially supported by Tarbiat Modares University. The authors wish to thank Dr Marsusi from Medical University of Tehran.

REFERENCES

1. Park YH, Nam HW. Clinical features and treatment of ocular toxoplasmosis. Korean J Parasitol. 2013; 51(4): 393-9.
2. Glor SB, Edelhofer R, Grimm F, Deplazes P, Basso W. Evaluation of a commercial ELISA kit for detection of antibodies against *Toxoplasma gondii* in serum, plasma and meat juice from experimentally and naturally infected sheep. Parasit Vectors. 2013; 6(85):1-2.
3. Zhao G, Zhou A, Lu G, Meng M, Sun M, Bai Y, et al. Identification and characterization of *Toxoplasma gondii* aspartic protease 1 as a novel vaccine candidate against toxoplasmosis. Parasit Vectors. 2013; 6: 175.
4. Mwambe B, Mshana SE, Kidenya BR, Massinde AN, Mazigo HD, Michael D, et al. Sero-prevalence and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in Mwanza, Tanzania. Parasit Vectors. 2013; 6(222): 2-3.
5. Lilly EL, Wortham CD. High prevalence of *Toxoplasma gondii* oocyst shedding in stray and pet cats (Felis catus) in Virginia, United States. Parasit Vectors. 2013; 6: 266.
6. Wang L, Cheng HW, Huang KQ, Xu YH, Li YN, Du J, et al. *Toxoplasma gondii* prevalence in food animals and rodents in different regions of China: isolation, genotyping and mouse pathogenicity. Parasite vectors. 2013; 6: 273.
7. Zhang XX, Lou ZZ, Huang SY, Zhou DH, Jia WZ, Su C, et al. Genetic characterization of *Toxoplasma gondii* from Qinghai vole, Plateau pika and Tibetan ground-tit on the Qinghai-Tibet Plateau, China. Parasite vectors. 2013; 6: 291.

8. Galvan-Ramirez Mde L, Sanchez-Orozco LV, Rodriguez LR, Rodriguez S, Roig-Melo E, Troyo Sanroman R, et al. Seroepidemiology of *Toxoplasma gondii* infection in drivers involved in road traffic accidents in the metropolitan area of Guadalajara, Jalisco, Mexico. Parasit Vectors. 2013; 6(1): 294.
9. Hajsoleimani F, Ataeian A, Nourian A, Mazloomzadeh S. Seroprevalence of *Toxoplasma gondii* in Pregnant Women and Bioassay of IgM Positive Cases in Zanjan, Northwest of Iran. Iran J Parasitol. 2012; 7(2): 82-6.
10. Selseleh MM, Keshavarz H, Mohebali M, Shojaee S, Modarressi M, Eshragian M, et al. Production and Evaluation of *Toxoplasma gondii* Recombinant Surface Antigen 1 (SAG1) for Serodiagnosis of Acute and Chronic Toxoplasma Infection in Human Sera. Iran J Parasitol. 2012; 7(3): 1-9.
11. Zhu C, Cui L, Zhang L. Comparison of a Commercial ELISA with the Modified Agglutination Test for Detection of *Toxoplasma gondii* Antibodies in Sera of Naturally Infected Dogs and Cats. Iran J Parasitol. 2012; 7(3): 89-95.
12. Muluye D, Wondimeneh Y, Belyhun Y, Moges F, Endris M, Ferede G, et al. Prevalence of *Toxoplasma gondii* and associated risk factors among people living with HIV at Gondar University Hospital, Northwest Ethiopia. ISRN Trop Med. 2013; 1-5.
13. Galvan-Ramírez MdlL, Troyo-Sanroman R, Roman S, Bernal-Redondo R, Vázquez Castellanos JL. Prevalence of toxoplasma infection in Mexican newborns and children: a systematic review from 1954 to 2009. ISRN Pediatr. 2012; 1-2.
14. Kamerkar S, Davis PH. Toxoplasma on the brain: understanding host-pathogen interactions in chronic CNS infection. J Parasitol Res. 2012: 5(8): 92-5.
15. Schumacher A, Costa SD, Zenclussen AC. Endocrine factors modulating immune responses in pregnancy. Front Immunol. 2014; 5: 196.
16. Emir S, Karakurt N, Karakus E, Senel E, Kirsaclioglu C, Demir HA, et al. Alpha-fetoprotein-producing hepatoid gastric adenocarcinoma in a child presenting with spontaneous gastric perforation. Turk J Pediatr. 2014; 56(1): 88-91.
17. Albany C, Einhorn L. Pitfalls in management of patients with germ cell tumors and slight elevation of serum alpha-fetoprotein. J Clin Oncol. 2014; 32(19): 2114-5.
18. Elfahal AM, Elhassan AM, Hussien MO, Enan KA, Musa AB, El Hussein AM. Seroprevalence of *Toxoplasma gondii* in Dairy Cattle with Reproductive Problems in Sudan. ISRN Vet Sci. 2013; 89: 51-65.
19. Rahbari AH, Keshavarz H, Shojaee S, Mohebali M, Rezaeian M. IgG avidity ELISA test for diagnosis of acute toxoplasmosis in humans. Korean J Parasitol. 2012; 50(2): 99-102.
20. Yang Z, Cho PY, Ahn SK, Ahn HJ, Kim TS, Chong CK, et al. A surge in the seroprevalence of toxoplasmosis among the residents of islands in Gangwha-gun, Incheon, Korea. Korean J Parasitol. 2012; 50(3): 191-7.
21. Lim H, Lee SE, Jung BK, Kim MK, Lee MY, Nam HW, et al. Serologic survey of toxoplasmosis in Seoul and Jeju-do, and a brief review of its seroprevalence in Korea. Korean J Parasitol. 2012; 50(4): 287-93.
22. Amin A, Mazloomzadeh S, Haniloo A, Mohammadian F, Fazaeli A. Evaluation of anti-toxoplasma IgG, IgM, and IgA in mothers with spontaneous abortion in Zanjan, Northwest Iran. Korean J Parasitol. 2012; 50(4): 371-4.
23. Bayani M, Mostafazadeh A, Oliaee F, Kalantari N. The Prevalence of *Toxoplasma gondii* in Hemodialysis Patients. Iran Red Crescent Med J. 2013; 15(10): e5225.
24. Shirbazou S, Delpisheh A, Mokhetari R, Tavakoli G. Serologic Detection of Anti *Toxoplasma gondii* Infection in Diabetic Patients. Iran Red Crescent Med J. 2013; 15(8): 701-3.

25. Shirbazou S, Delpisheh A, Mokhetari R, Tavakoli G. Serologic Detection of Anti *Toxoplasma gondii* Infection in Diabetic Patients. Iran Red Crescent Med J. 2013; 15(8): 701-3.
26. Davarpanah M, Mehrabani D, Neirami R, Ghahremanpoori M, Darvishi M. Toxoplasmosis in HIV/AIDS patients in Shiraz, southern Iran. Iran Red Crescent Med J. 2007; 200(1): 22-7.
27. Han M, Jiang Y, Lao K, Xu X, Zhan S, Wang Y, et al. sHLA-G involved in the apoptosis of decidual natural killer cells following *Toxoplasma gondii* infection. Inflammation. 2014; 37(5): 1718-27.
28. Fan CK, Lee LW, Liao CW, Huang YC, Lee YL, Chang YT, et al. *Toxoplasma gondii* infection: relationship between seroprevalence and risk factors among primary schoolchildren in the capital areas of Democratic Republic of Sao Tome and Principe, West Africa. Parasit Vectors. 2012; 5: 141.
29. Rojas Y, Guillerman RP, Zhang W, Vasudevan SA, Nuchtern JG, Thompson PA. Relapse surveillance in AFP-positive hepatoblastoma: re-evaluating the role of imaging. Pediatr Radiol. 2014; 44(10): 1275-80.
30. Carr BI, Guerra V, Giannini EG, Farinati F, Ciccarese F, Rapaccini GL, et al. Low alpha-fetoprotein HCC and the role of GGTP. Int J Biol Markers. 2014; 29(4): e395-402.

How to cite the article: Sadraie J, Shariat Bahadory E, Ghaffari Novin S. Evaluation of serum concentration of AFP marker in toxoplasmosis pregnant women with high level of IgG and IgM *toxoplasma* antibody by ELISA assay in a population of Tehran, Iran. Int J Epidemiol Res. 2016; 3(1): 3-11.