Influence of Maternal Toxoplasmosis on the Second-Trimester Aneuploidy Screening Test

Umit GORKEM[1], Ayse Semra GURESER[2], Cihan TOGRUL[1], Djursun KARASARTOVA[2], Tayfun GUNGOR[3] Aysegul TAYLAN OZKAN[2], Ozgur KOCAK[1]

Çorum, Turkey

ABSTRACT

OBJECTIVE: With apoptosis being critical for the development and homeostasis of placental tissues, it is possible to hypothesize that accelerated trophoblastic apoptosis during pregnancy may result in a partial loss of trophoblastic activity or trophoblastic cell mass, and ultimately may alter the second-trimester screening test parameters. Thus, this study was conducted to investigate the influence of maternal toxoplasmosis on second-trimester aneuploidy screening tests.

STUDY DESIGN: This retrospective study was conducted with 552 pregnant women admitted to our University Hospital. The demographic data such as maternal age and weight; and the main parameters of second-trimester aneuploidy screening test including maternal serum alpha-fetoprotein, unconjugated estriol (uE3) and human chorionic gonadotropin were analyzed with the comparison of their Toxoplasma immunoglobulin serology results.

RESULTS: The mean age of the pregnant women was 27.6 (17.0 - 43.0) years, and the mean maternal weight was 65.0 (40.0 - 120.0) kg. The pregnant women with positive Toxoplasma IgG antibody had a higher mean maternal age than those with negative Toxoplasma IgG antibody (p<0.0001). No significant difference for the concentrations and MoM values of second-trimester screening test parameters in women with Toxoplasma IgM and IgG antibodies was observed (p>0.05, for all).

CONCLUSION: Although IgG-seropositivity of toxoplasmosis may lead to an accelerated trophoblastic apoptosis during pregnancy, there is no significant influence on the second-trimester screening test results. There was no data regarding the unaffected population whom used for calculation of MoM, if they had toxoplasmosis in their life span.

Keywords: Toxoplasmosis, Second-trimester, Aneuploidy, Triple test

Gynecol Obstet Reprod Med 2018;24(2):71-75

Introduction

All pregnant women, regardless of their age or probable risk status, are offered screening tests to identify the risk for fetal aneuploidy including trisomy 21 and 18, and fetal open neural tube defects. Serum screening of pregnant women aims to reduce the necessity of invasive diagnostic testing and increase detection of the affected fetuses (1). One such screening, also known as the triple test, involves identifying levels of unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and maternal serum alpha-feto protein (MSAFP).

Although the popularity of triple test has decreased in clinical practice, most clinicians in Turkey still utilize it as an aneuploidy screening test. Maternal age is also taken into consideration during evaluation. When the second trimester DS cut-off risk of 1 in 270 is taken into account, the sensitivity of the triple test is approximately 65% for Down Syndrome (DS) and 70 % for trisomy 18, at a false positive rate of 5%. Whereas, when MSAFP and uE3 levels are 25 - 30% lower, hCG levels are approximately two times higher than that of normal controls in most DS cases (2).

The screening performance of biochemical markers for aneuploidy may be affected by various factors. Gestational age is the most important factor since concentrations of several markers such as hCG and MSAFP change with gestational age. Maternal weight and maternal serum markers show a negative correlation that has been attributed to variations of

[1] *Hitit University Faculty of Medicine, Department of Obstetrics and Gynecology, and* [2]*Microbiology, Çorum, Turkey*
[3] *University of Health Science Zekai Tahir Burak Maternal Health Education and Research Hospital, Ankara, Turkey*
Address of Correspondence: *Ozgur Kocak*
Hitit University Faculty of Medicine, Department of Obstetrics and Gynecology, 19040 Corum Turkey
dr.ozgur@hotmail.com

Submitted for Publication: *17.09.2017*
Accepted for Publication: *28.01.2018*

Access this article online	
Quick Response Code:	Website: www.gorm.com.tr e-mail: info@gorm.com.tr
	DOI:10.201613/GORM.2017.735

How to cite this article: *Gorkem U. Gureser AS. Togrul C. Karasartova D. Gungor T. Taylan Ozkan A. Kocak O. Influence of Maternal Toxoplasmosis on the Second-Trimester Aneuploidy Screening Test. Gynecol Obstet Reprod Med 2018;24(2):71-75*

volume of distribution due to physiologic increase in blood volume (3). Biochemical markers may also show significant variations with maternal ethnicity, insulin-dependent diabetes mellitus, in vitro fertilization, and smoking (4-7).

Toxoplasma gondii (T. gondii) is an obligate intracellular protozoan parasite that has a high capacity of asexual propagation in the nucleated cells of vertebrate hosts, including humans (8). Chronic T. gondii infection is evident in approximately one-third of the human population in the world. The parasites responsible for this infection are considered as one of the most successful to infect humans (9). Pregnant women infected by this parasite may undergo severe symptoms (10). It was reported that congenital toxoplasmosis may cause endocrine disorders (11), imbalance of apoptosis regulation (12,13), and placental structural damage (14). However, the pathophysiological mechanisms remain unclear. It has been estimated that the risk of infection with this pathogen is approximately 0.1 - 1% of all pregnancies (15).

Apoptosis is a normal phenomenon vital for normal placental development and fetal growth, and it physiologically occurs in trophoblastic cells throughout gestation (16). The apoptosis of trophoblasts may be induced or inhibited by a number of stimuli including abortion, preeclampsia, IUGR, and preterm labor (17). In addition, viruses, bacteria, and protozoan parasites that because intrauterine infections may modulate host cell apoptosis (18). T. gondii is able to promote or inhibit apoptosis by altering the apoptotic program of the host cells (19). This dual activity of the parasite may need a balance between pro- and anti-apoptotic signals of the infected host cells (20). It has been shown that apoptotic modulation of T. gondii infection may be associated with the virulence of the parasite (21-22). Promoted apoptosis may be related to decreased serum hCG levels in IgG-seropositive pregnant women.

It has been considered that transplacental transmission of T. gondii causes congenital toxoplasmosis. With apoptosis being critical for the development and homeostasis of placental tissues, it is possible to hypothesize that accelerated trophoblastic apoptosis during pregnancy may result in a partial loss of trophoblastic activity or trophoblastic cell mass, and ultimately may alter the second-trimester screening test parameters. The aim of the present study was to investigate the effects of maternal toxoplasmosis on second-trimester aneuploidy (triple) screening test results.

Material and Method

This case-control retrospective study was conducted between September 2014 and September 2016 at the obstetrics outpatient clinic of our University Hospital. The study was approved by the local institutional review board, which was in accordance with the Declaration of Helsinki (Reference number: 71444544/2063). The factors that regulate the trophoblast apoptosis and complicated pregnancy such as preeclampsia, intrauterine growth retardation, preterm labor, multiple pregnancy, and intrauterine infections were regarded as exclusion criteria. The female Caucasian participants were required to have never smoked, not have achieved pregnancy by assisted reproduction, and not have insulin-dependent diabetes mellitus. The number of women who met the inclusion criteria was 552. Informed consent has been obtained from all participants.

We assessed the parameters of second-trimester aneuploidy screening tests in the laboratory (accredited according to TS EN ISO 15189: 2014 since 2011). The second-trimester screening test was derived from the combination of triple serum markers (hCG, MSAFP and uE3). These marker samples were measured by the electrochemiluminescence immunoassay (ECLIA) method using an auto-analyzer (Beckman Coulter, Beckman Coulter - Unicel DxI 800, US). The measured marker levels were expressed as multiples of the medians (MoM) (23). MoM was calculated by dividing each patient's results of MSAFP, uE3, and hCG levels by their median levels for all of the population at that gestational age in the applied laboratory. The average value corrected according to the weight is 1.0. The serum levels of Toxoplasma IgM and Toxoplasma IgG were analyzed by chemiluminescent microparticle enzyme immunoassay (Cobas E 601, Roche Diagnostics, Switzerland) in the Microbiology Laboratory of the University Hospital. The baseline demographic data such as maternal age and weight, and the main parameters of the triple test including MSAFP, uE3, and hCG were analyzed with the comparison of their Toxoplasma immunoglobulin serology results.

Statistical analysis

The data was analyzed using SPSS (Statistical Packages for The Social Sciences) software, version 22 (SPSS Inc. Chicago. USA). The continuous variables of Toxoplasma IgM serology were compared by using the Mann Whitney U test as a nonparametric test due to the small number of participants in that group (n=13). The continuous variables of Toxoplasma IgG group were analyzed using the independent sample t test as a parametric test. All continuous variables were presented as mean ± standard deviation. A p value smaller than 0.05 was considered statistically significant.

Results

The comparisons of the baseline demographic data and the main parameters of triple test according to Toxoplasma IgM and IgG serology are demonstrated in table 1. A total of 552 pregnant women were investigated for toxoplasma antibody assessment. The mean age of the pregnant women was 27.6 (17.0 - 43.0) years, and the mean maternal weight was 65.0 (40.0 - 120.0) kg. A positive Toxoplasma IgM serology was found in 13 of the 552 pregnant participants (2.4 %). On the other hand, 22.1% of the pregnant participants constituted the positive Toxoplasma IgG serology. Moreover, no congenital toxoplasmosis was observed in the pregnant women with Toxoplasma positive antibodies.

Table 1: The comparisons of baseline demographic data and the main parameters of triple test according to Toxoplasma IgM and IgG serology

	Toxoplasma IgM			*Toxoplasma IgG*		
	Negative (n=539, 97.6.%)	Positive (n=13, 2.4%)	*p*	Negative (n=430, 77.9%)	Positive (n=122, 22.1%)	*p*
Maternal age (years)	27.5±5.6	28.2±5.4	0.650	27.0±5.4	29.3±5.8	0.000*
Maternal weight (kg)	64.9±12.8	63.6±10.5	0.658	64.6±12.9	66.0±12.3	0.257
Triple test parameters						
MSAFP (IU/mL)	36.8±17.2	40.7±17.5	0.312	36.5±16.0	38.3±20.7	0.290
MSAFP MoM	1.04±0.4	1.1±0.4	0.557	1.0±0.4	1.1±0.6	0.072
uE3 (ng/mL)	0.9±0.5	1.2±0.6	0.092	0.9±0.5	1.0±0.5	0.502
uE3 MoM	1.0±0.4	1.0±0.3	0.545	1.0±0.4	1.0±0.3	0.777
hCG(mIU/mL)	25.3±14.5	23.7±13.1	0.658	25.3±14.3	25.1±15.3	0.905
hCG MoM	1.0±0.5	1.0±0.5	0.584	1.0±0.5	1.0±0.6	0.899

*MSAFP: Maternal serum alpha-feto protein, uE3: Unconjugated estriol, hCG: Human chorionic gonadotropin. *p <0.05 statistically significant*

As calculated in Table 1, the comparisons of maternal age revealed that the pregnant women with positive Toxoplasma IgG antibody had a higher mean maternal age than those with negative Toxoplasma IgG antibody (p <0.0001). However, the pregnant women with negative and positive Toxoplasma IgM antibodies had a similar mean maternal age (p=0.650). There was also no difference in the average weight of the pregnant women with negative and positive Toxoplasma IgM antibodies (p=0.658 and p=0.257, respectively). Moreover, we did not observe a significant difference for the concentrations and MoM values of second-trimester screening test parameters, i.e. MSAFP, uE3, and hCG, in pregnant women with Toxoplasma IgM and IgG antibodies (p> 0.05, for all).

Discussion

Currently, screening for fetal aneuploidy has been recommended in the follow-up of pregnant women. The triple test is one of the most frequently employed screening tests in clinical practice. A number of factors affect the interpretation of the triple screening test results. For example, studies have shown that Black women have relatively higher MSAFP and hCG rates when compared to Caucasian women (4). Pregnancies present with higher hCG and uE3 levels are attributed to the hormonal stimulation and higher progesterone concentrations when compared to spontaneously conceived ones (6). Adjustments of DS screening serum markers for maternal factors slightly affects the overall screening performance (7). Pregnant women with insulin-dependent diabetes mellitus have lower MSAFP and uE3 levels when corrected for maternal weight (5). To avoid these contributing factors, we excluded the pregnant women who had history of one of them. The current study showed that the pregnant women seropositive to Toxoplasma IgG had similar mean MoM values of triple screening test parameters when compared to the pregnant women seronegative to Toxoplasma IgG antibody.

An accurate assessment of the risk of Down syndrome is dependent on the correct assessment of the triple aneuploidy screening parameters. It has been demonstrated that several different diseases paired with certain maternal factors can affect the levels of these parameters and lead to misestimating of Down syndrome risk. In the existing literature, there are a few studies of auto inflammatory or autoimmune diseases associated with vasculitis and serum screening markers for Down syndrome. Maymon et al. found elevated hCG levels in the second trimester in systemic lupus erythematosus patients (24). Turkcapar et al. from Turkey also reported low levels of PAPP-A in pregnant women with Familial Mediterranean fever in the first trimester (25). In a study by Wiener, it was reported that the triple test serum markers might be altered in thrombophilia patients treated with low molecular weight heparin (26).

Apoptosis has been regarded as a crucial physiological process for normal development of the human embryo as it eliminates abnormal embryonic cells and promotes the immune tolerance to pregnancy (27). The apoptotic phenomenon consists of the normal cell turnover in trophoblastic cells that leads to elimination of those cells without a local inflammatory reaction (28). Moreover, pregnancy loss may result from alterations of apoptosis in trophoblastic cells or embryonic damage, particularly in the early stages of pregnancy (29). Maternal T. gondii infection induces trophoblast apoptosis through oxidative stress-initiated mitochondrial dysfunction, which is followed by activation of the downstream signal pathway (30). However, the findings of the present study do not support that cytokine secretions by trophoblasts may alter hCG production.

To the best of our knowledge, this is the first conducted study in the literature investigating the influence of IgG-seropositivity of toxoplasmosis on parameters of triple aneuploidy screening test. Hence, limited information is available on the relationship between IgG-seropositivity and serum

hCG level triple test results in the current literature. The retrospective design and small sample size are the main limitations of the current study. Additionally, we could not obtain detailed data on the maternal demographics.

In conclusion, although IgG-seropositivity of toxoplasmosis may lead to an accelerated trophoblastic apoptosis during pregnancy, there is no significant influence on the second-trimester screening test results. However, there was no data regarding if the unaffected populations used for the calculation of MoM had toxoplasmosis sometime during their life span. Further prospective studies are warranted to reveal whether IgG-seropositivity of toxoplasmosis may be an influencing factor on results of triple screening test during pregnancy.

✍: *Acknowledgements: The authors would like to acknowledge the staff of the Hitit University Hospital for the data collection and their assistance.*
Conflicting Interests: The authors declare that they have no conflicting interests.

References

1. Screening for fetal chromosomal abnormalities. ACOG Practice Bulletin No.77. American College of Obstet Gynecol 2007;109(1):217-7.
2. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012; 367(23):2175-84
3. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. J Med Screen 1997;4(4):181-246.
4. Watt HC, Wald NJ, Smith D, Kennard A, Densem J. Effect of allowing for ethnic group in prenatal screening for Down's syndrome. Prenat Diagn 1996;16(8):691-8
5. Huttly W, Rudnicka A, Wald NJ. Second trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. Prenat Diagn 2004; 24(10):804-7.
6. Wald NJ, White N, Morris JK, Huttly WJ, Canick JA. Serum markers for Down's syndrome in women who have had in vitro fertilization: implications for antenatal screening. Br J Obstet Gynaecol 1999;106(12):1304-6
7. Palomaki GE, Knight GJ, Haddow JE, Canick JA, Wald NJ, Kennard A. Cigarette smoking and levels of MSAFP, uEe3 and hCG impact on Down syndrome screening. Obstet Gynecol 1993;81(5 (Pt 1):675-8.
8. Peng H-J, Chen X-G, Lindsay DS. A Review: Competence, compromise, and concomitance-reaction of the host cell to Toxoplasma gondii infection and development. J Parasitol 2011;97(4):620-8
9. Lindsay DS, Dubey JP. Toxoplasma gondii: the changing paradigm of congenital toxoplasmosis. Parasitology 2011; 138(14):1829-31
10. Wong SY, Remington JS. Toxoplasmosis in pregnancy. Clin Infect Dis 1994;18(6):853-61
11. Geva E, Gingirzer DG, Zaloudek CJ, Moore DH, Byme A, Jaffe RR. Human placental vascular development: vasculogenic angiogenic (branching and nonbrancing) transformation is regulated by vascular endothelial growth factor-A, angiopoetin-1 and angiopoetin-2. J Clin Endocrinol Metab 2002;87(9):4213-24.
12. Angeloni MB, Silva NM, Castro AS, Gomes AO, Silva DA, Mineo JR, et al. Apoptosis and S phase of the cell cycle in BeWo trophoblastic HeLa cells are diffentially modulated by Toxoplasma gondii strain types. Placenta 2009;30(9):785-91
13. Senegas A, Villard O, Neuville A, Marcellin L, Pfaff AW, Steinmetz T, et al. Toxoplasma gondii-induced foetal resorption in mice involves interferon-gamma induced apoptosis and spiral artery dilatation at the maternofoetal interface. Int J Parasitol 2009;39(4):481-7
14. Yavuz H, Aydın F, Seyhan A, Topuz S, Karagenc Y, Tuzlali S, et al. Granulomatous villitis formed by inflamatory cells with maternal origin: a rare manifestation type of placental toxoplasmosis. Placenta. 2006;27(6-7):780-2
15. Abbasi M, Kowelewska-Grochowska K, Bahar MA, Kilani RT, Winkler-Lowen B, Guilbert LJ. Infection of trophoblasts by Toxoplasmosis gondii. J Infect Dis 2003: 15;188(4):608-16.
16. Huppertz B, Kadyrov M, Kingdom JC. Apoptosis and its role in the trophoblasts. Am J Obstet Gynecol 2006; 195(1):29-39.
17. Straszewski-Chavez SL, Abrahams VM, Mor G. The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. Endocr Rev 2005; 26(7):877-97.
18. Luder CGK, Gross U, Lopes MF. Intracellular protozoan parasites and apoptosis: diverse strategies to modulate parasite–host interactions. Trends Parasitol. 2001;17 (10):480-6.
19. James ER, Green DR. Modulation of apoptosis in the host-parasite interaction. Trends Parasitol 2004;20(6): 280-7
20. Heussler VT, Küenzi P, Rottemberg S. Inhibition of apoptosis by intracellular protozoan parasites. Int J Parasitol 2001;31(11):1166-76.
21. Saeij JP, Boyle JP, Boothroyd JC. Differences among the three major strains of Toxoplasma gondii and their specific interactions with the infected host. Trends Parasitol 2005;21(10):476-81.
22. Liu T, Zhang Q, Liu L, Xu X, Chen H, Wang H, et al. Trophoblast apoptosis through polarization of macrophages induced by Chinese Toxoplasma gondii isolates with different virulence in pregnant mice. Parasitol Res 2013;112(8):3019-27

23. Wald NJ, Cuckle H, Brock JH, Peto R, Polani PE, Woodford FP. Maternal Serum-Alpha-Fetoprotein Measurement in Antenatal Screening for Anencephaly and Spina Bifida in Early Pregnancy: Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. Lancet 1977;1(8026):1323-32

24. Maymon R, Cuckle H, Sehmi IK, Herman A, Sherman D. Maternal serum human chorionic gonadotrophin levels in systemic lupus erythematosus and antiphospholipid syndrome. Prenat Diagn 2001;21(2):143-5.

25. Turkcapar F, Engin-Üstün Y, Sımsek ÖY, Deveer R, Danisman N, Dilmen U, et al. First and second trimester biochemical markers in familial mediterranean fever. Eur Rev Med Pharmacol Sci 2013;17(13):1820-3.

26. Wiener Y, Frank M, Neeman O, Kurzweil Y, Bar J, Maymon R . Does low molecular weight heparin influence the triple test results in pregnant women with thrombophilia? Isr Med Assoc J 2012;14(4):247-50

27. Crocker IP, Cooper S, Ong SC, Baker P. Differences in apoptotic susceptibility of cytotrophoblasts and syncytiotrophoblast in normal pregnancy to those complicated with preeclampsia and intrauterine growth restriction. Am J Pathol 2003;162(2):637-43

28. Huppertz B, Kadyrov M, Kingdom JCP. Apoptosis and its role in the trophoblast. Am J Obstet Gynecol 2006; 195(1):29-39

29. Levy R, Nelson DM. To be or not to be, that is the question. Apoptosis in human trophoblast. Placenta 2000; 21(1):1-13

30. Xu X, He L, Zhang A, Li Q, Hu W, Chen H, et al. Toxoplasma gondii isolate with genotype Chinese 1 triggers trophoblast apoptosis through oxidative stress and mitochondrial dysfunction in mice. Exp Parasitol 2015; 154:51-61