

## SEROPREVALANCE OF TOXOPLASMA GONDII ANTIBODIES IN ANTENATAL POPULATION OF TRAKYA REGION

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### SUMMARY

**Objective:** To investigate the distribution of toxoplasma gondii antibodies retrospectively among the first trimester antenatal population in Trakya region.

**Material and methods:** This study retrospectively analyzes seroprevalance of toxoplasma gondii IgM and IgG antibodies in the sera of 1646 first trimester pregnancies attended to the Obstetrics & gynecology Antenatal Care Unit in Trakya University, Faculty of Medicine between January 2000 and June 2009. For this purpose IgM, IgG and if necessary, IgG avidity results were evaluated. Besides, hepatitis B surface antigen (HBsAg), human immunodeficiency virus type 1 and 2 antibodies (Anti HIV), cytomegalovirus (AntiCMV) IgG and IgM antibodies, hepatitis C antibody (AntiHCV), rubella (antirubella) IgM and IgG antibodies and “venereal disease research laboratory” (VDRL) results evaluated in the same period were also investigated.

**Results:** Although the status of seroprevalance is not known in 313 (19%) women, 876 pregnant women (65.71%) were IgG and IgM seronegative, 426 (31.95%) were IgG seropositivite, 13 (0.97%) were IgM positivite and 18 (1.35%) were both IgG and IgM seropositivite. On the other hand, 13 of 18 patients with both IgG and IgM seropositivity showed high avidity but avidity could not be evaluated in 4 patients. HBsAg positivity was found in 3% (47/1526), AntiHCV positivity in 0.8% (12/1474), VDRL in 0.4% (5/1056), rubella IgM antibody in 0.7% (9/1254), rubella IgG antibody in 76.6% (940/1227), according to the other serologic tests. AntiCMV IgM antibody was positive in 17 (17/1284, 1.3%) and IgG was positive in 1027 (1027/1279, 80.3%) patients.

**Conclusion:** As well as toxoplasma exposure rate in the pregnant women from Trakya region of Turkey is high (%30), nearly half of first trimester women were found seronegative. Screening during the first trimester would be appropriate, particularly for women who are under risk of infection.

**Key words:** pregnancy, toxoplasmosis

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### ÖZET

#### TRAKYA YÖRESİNDE ANTENATAL BAKIM ALAN GEBELERDE TOXOPLASMA GONDII ANTİKOR SEROPREVALANSI

**Amaç:** Trakya Bölgesinde antenatal bakım için başvuran ilk trimester gebeliklerinde toxoplasma gondii antikor seroprevalansının retrospektif olarak incelenmesi

**Gereç ve yöntemler:** Bu çalışmada 2000-2009 yılları arasında Trakya Üniversitesi Tıp Fakültesi Kadın Doğum Antenatal Polikliniğine ilk trimesterde başvuran toplam 1646 gebe serumunda toxoplasma gondii IgG ve IgM antikor sonuçları retrospektif olarak değerlendirilmiştir. Bu amaçla IgM, IgG ve gerekli durumlarda IgG aviditesi sonuçlarına bakılmıştır. Ayrıca bu dönemde bakılan hepatit B yüzey antijeni (HBsAg), “human immunodeficiency virus” tip 1

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ve 2 antikor (Anti HIV), sitomegalovirüs antikor (AntiCMV) IgM ve IgG, hepatit C antikor (AntiHCV), rubella antikor (Antirubella) IgG ve IgM ve VDRL ("venereal disease research laboratory") test sonuçları da incelendi. **Bulgular:** Gebelerin 313'de (%19) antikor titresi bilinmemekle beraber, 876'da (%65.71) IgG ve IgM seronegatifliği, 426'da (%31.95) IgG seropozitifliği, 13'de (%0.97) ise IgM seropozitifliği, 18'de (%1.35) IgG ve IgM seropozitifliği görülmüştür. Diğer taraftan avidite testi yapılan IgM ve IgG seropozitifliği saptanan 18 hastanın 13'ünde yüksek avidite bulundu ancak dördüne avidite bakılmadı. Diğer serolojik testlerden ise HBsAg pozitifliği %3 (47/1526), AntiHCV pozitifliği %0.8 (12/1474), VDRL %0.4 (5/1056), rubella IgM antikor %0.7 (9/1254), rubella IgG antikor %76.6 (940/1227) oranında pozitif izlendi. AntiCMV IgM 17 (17/1284, %1.3), IgG ise 1027 (1027/1279, %80.3) olguda pozitif bulundu. **Sonuç:** Trakya Bölgesindeki gebelerde toksoplazma ile gebelik öncesi karşılaşma oranı yüksek (%30) olmakla birlikte ilk trimesterdeki gebe kadınların yarısı seronegatifdir. Gebeliğin ilk trimesterinde toksoplazma taraması yapılması özellikle risk altındaki kişilerde uygun olacaktır.

**Anahtar kelimeler:** gebelik, toksoplazmosis

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## INTRODUCTION

Negative results of toxoplasma gondii infection during pregnancy on fetus and neonate are known. Though it is preventable with various public health measures, diverse clinical manifestations may be seen in 35-40% of the adult population. Infection may be transmitted to humans in cyst form from meat products, via blood transfusion, during organ transplantation and placentally. Highest level of placental transmission is in the third trimester and it can reach to 80 % at the end of pregnancy while lowest level of transmission is seen in the first trimester. In nearly 30-50 % of the infected pregnant women who aren't treated, various complications occur depending on the period when infection begins. Abortus, fetal death, neurological or ocular damage may develop. Although sensory losses such as hearing loss and loss of vision, mental and psychomotor retardation; hematologic problems; hepatosplenomegaly or death may be seen in fetus, most are born asymptotically. Systematic education and serologic researches during pregnancy is the most reliable approach for now in early diagnosis, treatment and prevention of toxoplasma infection. Proper treatment in the early stages of neonate's life improves the clinical results considerably<sup>(1-3)</sup>.

With this retrospective data evaluation, toxoplasma gondii factor exposure among first trimester pregnant women who attended to Obstetrics & Gynecology Department Antenatal Care unit in Trakya University School of Medicine was researched and for this purpose IgM, IgG and if necessary, IgG avidity results were evaluated. Besides, evaluations of this region were compared with those of other regions of Turkey.

## MATERIAL AND METHODS

In our study, 1646 pregnant women between the ages of 16 and 50 who attended Obstetrics & Gynecology Department Antenatal Care unit in Trakya University School of Medicine in the first trimester between the years 2000-2009 were enrolled. Demographic characteristics and serological test results were recorded from the polyclinic follow-up cards of patients. Apart from the routine tests (hemogram, blood group, urine analysis etc.), toxoplasma gondii IgG and IgM antibody tests were also done for the patients who attended to antenatal care. Besides, hepatitis B surface antigen (HBsAg), "human immunodeficiency virus" type 1 and 2 antibodies (Anti HIV), cytomegalovirus IgG and IgM antibodies (AntiCMV), hepatitis C antibody (AntiHCV), rubella (antirubella) IgM and IgG antibodies and VDRL ("venereal disease research laboratory") results were evaluated retrospectively. Toxoplasma gondii IgG avidity was tested by indirect fluorescent antibody test (IFAT) method for toxoplasma gondii IgG and IgM positive patients. No additional test was done for cases in which only IgM (+) was found, in some cases IgM test was repeated. Besides, in 18 IgM and IgG positive cases, neonatal records of infants were investigated. For all the toxoplasma, antirubella and anti CMV IgG and IgM tests (AxSYM, Abbott Lab., IL, USA); for HbsAg, AntiHIV and AntiHCV (Vitros, Ortho-Clinical Diagnostics, Johnson&Johnson CO., Brasil) and for VDRL (Immuntrep VDRL, Omega Diagnostics, UK) kits were used. Analysis of all blood samples was performed using Macro ELISA method and Abbott AxSYM, USA and Vitros Immunodiagnostic ECIQ, UK

devices in Trakya University School of Medicine Microbiology Laboratory whereas VDRL was performed manually.

Fetal anomaly (congenital hip dislocation, pes equinovarus, digital anomaly of the hand) in four of our cases, molar pregnancy in 2 cases and ectopic pregnancy anamnesis in 4 cases were present in their previous pregnancies. In addition, nephrological problems (pyelonephritis, nephrolithiasis, polycystic kidney, glomerulonephritis, nephrotic syndrome) in 8, epilepsy in 5, hematological diseases (thalassemia minor, essential thrombocytopenia) in 27, thyroid diseases (hypo-, hyperthyroidism, goiter, Hashimoto) in 51, cardiac (arrhythmia, congenital cardiac disease, mitral prolapse) in 7, rheumatic or skin diseases (Behcet, lupus, FMF, ankylosing spondylitis, vitiligo, eczema, psoriasis) in 10, psychiatric problems in 6, gastrointestinal diseases (gastritis, hemorrhoid, cholelithiasis, spastic colon) in 10 and Type I diabetes history were present in 2 pregnant women. 2 patients

became pregnant after primary infertility whereas one patient got pregnant with ovulation induction. Of the 19 pregnant women who has a respiratory disease history, chronic sinusitis or bronchitis anamnesis of 3, asthma anamnesis of 12 and tuberculosis anamnesis of 2 were taken.

**RESULTS**

The mean age of 1646 cases was calculated as 27.8±2.12. Among the pregnant women 1330 (79%) stated that they are housewives while 346 (21%) are working. Numbers of median gravidity (minimum-maximum), parity and abortus of our cases are indicated in Table I. HIV tests done in this period were found negative in all the pregnant women (n=1510).

For 313 (%19) of the pregnant women toxoplasma tests weren't done. On the other hand, of 1333 (%81) patients who had test results; for 876 (%65.71) IgG and IgM seronegativity; for 426 (%31.95) IgG

**Table I:** Distribution of other serological tests by demographic characteristics and toxoplasma serological results of the cases

	IgM(+) IgG(+) (n=18)	IgM(+) IgG(-) (n=13)	IgM(-) IgG(+) (n=426)	IgM(-) IgG(-) (n=876)	Indefinite Serology (n=313)	Total (n=1646)
Age (years, mean ±SD)	27.2±1.41	25.9±6.36	28.5±1.41	27.3±0.71	28.6±3.54	27.8±2.12
Working	3 (%16.6)	3 (%23)	94 (%22)	194 (%22.1)	52 (%16.6)	346 (%21)
Gravidity [median (min.-max.)]	2 (1-5)	1 (1-3)	2 (1-7)	1 (1-7)	2 (1-8)	2 (1-8)
Parity [median (min.-max.)]	1 (0-4)	0 (0-2)	1 (0-4)	0 (0-5)	1 (0-5)	0 (0-5)
Abortus [median (min.-max.)]	0 (0-1)	0 (0-1)	0 (0-4)	0 (0-4)	0 (0-4)	0 (0-4)
HBsAg (n=1526)	1	0	14	23	9	47 (%3)*
AntiHCV (n=1474)	0	0	3	6	3	12 (%0.8)*
VDRL (n=1056)	0	0	4	1	0	5 (%0.4)*
Antirubella Ig M (n=1254)	2	0	2	5	0	9 (%0.7)*
Antirubella Ig G (n=1227)	14	6	322	573	25	940 (%76.6)*
AntiCMV Ig M (n=1284)	0	0	5	12	0	17 (%1.3)*
AntiCMV Ig G (n=1279)	12	8	355	627	25	1027 (%80.3)*

\*: Percentages were calculated according to the number of patients who had serological tests.

IgM: Immunoglobulin M, IgG: Immunoglobulin G, HBsAg: Hepatitis B surface antigen, AntiHCV: Hepatitis C antibody, VDRL: "Venereal disease research laboratory", AntiCMV: Cytomegalovirus antibody.

**Table II:** Distribution of toxoplasma serological results in pregnant woman by years.

	IgG(-) IgM(-)	IgG(+) IgM(-)	IgG(-) IgM(+)	IgG(+) IgM(+)	Indefinite Serology	Total
2000	8	1	0	0	2	11
2001	7	1	0	0	1	9
2002	74	45	0	1	78	198
2003	203	94	3	2	84	386
2004	176	87	5	4	59	331
2005	145	52	5	6	17	225
2006	81	33	0	2	9	125
2007	59	41	0	2	30	132
2008	102	60	0	1	28	191
2009	21	12	0	0	5	38
Total	876	426	13	18	313	1 646

IgM: Immunoglobulin M, IgG: Immunoglobulin G.

seropositivity; and for 13 (%0.97) IgM seropositivity (seronegativity was found in repeated controls of 4 pregnant women) were determined. IgG and IgM seropositivity was seen in eighteen pregnant women (1.35%). Distribution of test results by years in our cases was also analyzed (Table II).

Of the 18 patients whose toxoplasma IgG and IgM were found positive, IgM of 1 patient was negative in control, 13 patients showed high IgG avidity and avidity of 4 patients couldn't be checked because they weren't reached (Table III).

**Table III:** Distribution of "Avidity" test results in real seropositive patients.

	High Avidity	Low Avidity	Control Negative	Avidity not checked
IgG(+)				
IgM(+)				
n=18	13	0	1	4

*IgM: Immunoglobulin M, IgG: Immunoglobulin G.*

Among the other serological tests evaluated HBsAg positivity and AntiHCV positivity were determined in %3 (47/1526) and %0.8 (12/1474) of the patients, respectively. VDRL test, rubella IgM antibody, rubella IgG antibody were seen positive at the percentages of 0.4% (5/1056), 0.7% (9/1254), 76.6% (940/1227) respectively. Of the 9 people who had rubella IgM positivity, coexisting rubella IgG positivity was seen in only 3. Similarly, number of antiCMV IgM positive cases was 17 (17/1284, 1.3%) and IgG positive was 1027 (1027/1279, %80.3). AntiCMV IgG (+) was found in 14 of the AntiCMV IgM positive patients.

No pathology was found in the early and late neonatal periods of the infants of pregnant women who were found toxoplasma IgM seropositive during their follow-up in our clinic.

## DISCUSSION

Congenital toxoplasmosis generally occurs concomitantly with primary infection. Very rarely, in the course of immunodeficiency, AIDS and corticosteroid usage, clinical manifestation appears as a result of reactivation of the chronic disease. Clinical manifestation of the disease is generally seen with non-specific symptoms

such as fatigue, medium fever and vision problems related to chorioretinitis. Maternal infection is transmitted to fetus by vertical transmission in direct proportion to the week of pregnancy. While congenital toxoplasmosis clinic that occurs in the early weeks of pregnancy progresses slowly, possibility of sequel increases. But disease causes mortality and morbidity in inverse proportion to pregnancy week<sup>(1)</sup>.

4 groups are found regarding serology in the case of infection during pregnancy. First group naive to toxoplasma antigen with negative IgM and IgG is at risk regarding toxoplasma during pregnancy. Public health measures are fundamental for these people who are in risk in case they are infected with toxoplasma infection during pregnancy. If the person is living in a risky society in terms of toxoplasmosis, in order to see the seroconversion that may occur during pregnancy, it might be necessary to repeat the tests. 65.71% of our patient group was found seronegative in terms of toxoplasmosis and is at risk of having toxoplasmosis during pregnancy but having toxoplasmosis tests in each trimester brings costs. Therefore, it was not recommended to take these tests in each trimester. But for the cases that are suspicious regarding infection in ultrasonography, repeating the toxoplasmosis tests for early diagnosis and treatment may be recommended.

Second group is the one that is toxoplasma IgG (+) IgM (-) in the first trimester and regarded as immune. This group has the lowest risk in terms of congenital toxoplasmosis but there may be reactivation of chronic infections in the patients whose immune system is impaired. Besides, it is recommended that these tests are obtained in the first trimester and serum samples taken are kept during pregnancy<sup>(1)</sup>. In the research we did in Trakya region, ratio of people in this group in the pregnant society of reproductive age was 31.95%. Gunes et al. found this ratio for 18-49 age group in Isparta and its surrounding high, approximately 75.6%<sup>(4)</sup>. In various studies, it was determined that depending on age 20-60% of our society, is infected with toxoplasma gondii<sup>(4-11)</sup>. While seropositivity ratio in Urfa region was given in a wide range between 21% and 59%; it was determined as 41.9% in Erzurum region, 36% in Van region, 39.6% in Malatya region, 31% in Ankara according to 2 different studies, 30.7% in Afyon region, 30.1% in Aydin region and 43% in İstanbul<sup>(12-21)</sup>. According to the toxoplasma seroprevalance study done by Saracoglu and Sahin

among 231 pregnant women in Ankara region: IgM and IgG (-) was determined as 58.4%; Ig M (-) IgG (+) as 38.1%; IgM (+) and IgG (+) as 3.46% and IgM (+) IgG (-) as 1.48%<sup>(9)</sup>. Similarly, in a study conducted in Kocaeli where 1972 pregnant women were scanned,, IgG (+) was found 48.3%, only IgM(+) was 0.4%, IgM and IgG (+) was 1.6<sup>(22)</sup> %. Parallel to the previous studies, in Hatay and its surroundings, toxoplasma IgG (+) and IgM (+) were found 52.1% and 0.54% respectively<sup>(23)</sup>. In the years 2003-2004 in İzmir among a woman population of 1274 (82%), 25% IgG seropositivity, 0.7% IgM seropositivity, 2.64% IgG and IgM seropositivity were determined<sup>(10)</sup>. These numbers are similar to the ratios we found in our study. It is reported that 20-80% seropositivity was found in America, Europe and Asia <sup>(11,24)</sup>.

Third group is the group that is toxoplasma IgM (+) and still found to be IgG (-) during pregnancy. In this group seroconversion may be seen by repeating the IgG/IgM tests at intervals of 1-3 weeks. If seroconversion has not occurred, IgM positivity is not clinically significant<sup>(1)</sup>. These results are followed as the IgM (-) and IgG (-) ones. On the other hand, if IgM (+) IgG (+) seroconversion has occurred, then infection has developed in pregnancy and fetus is at the risk of congenital toxoplasmosis. These cases should be followed in terms of congenital toxoplasmosis for nearly 18 weeks by doing a PCR (polimerase chain reaction) in amniotic fluid with amniocentesis and ultrasound. In the studies conducted in Europe and America, it was reported that infants with congenital toxoplasmosis were born at the ratios of 1/1000 - 0.1/1000<sup>(25-27)</sup>. It has been determined that 500-5000 neonates are infected in America each year<sup>(28)</sup>. In the countries like France where toxoplasma is commonly seen, congenital toxoplasma prevalence has been determined as 3.3 in 10.000 live births whereas symptomatic infection has been determined as 0.34/10.000<sup>(2)</sup>. It is alleged that "Toll-like" receptors, adhesins, cytokines, chemokines or chemokines receptors, immunoglobulines or gene polymorphism of Fc receptors may be responsible for the tendency of toxoplasma towards vertical, inherited, abortus and fetal infection<sup>(3)</sup>.

In the forth group, IgG positivity accompany with IgM positivity. But there is a problem about when IgG positivity occurs. In this case IgG avidity tests are demanded. 12-16 weeks after the infection, IgG antibodies showing high avidity develop. So, if IgG

showing high avidity has developed, then infection must have occurred at least 12-16 weeks ago and there is no congenital toxoplasmosis risk<sup>(1)</sup>. Of the 18 cases in which IgM and IgG (+) were determined in our study, avidity was determined as high in all 13 cases for which avidity test was done and they indicated the old infection. In a research made in Brasil, another country with a high toxoplasma seropositivity, the fact that avidity test presented the new infection at a ratio of 28% pointed out the significance of national screening<sup>(29)</sup>.

Toxoplasmosis screening is stil an up-to-date and controversial subject about prenatal care. Nowadays, in our country toxoplasmosis screening is not done at all or is performed by unstandardized methods<sup>(30)</sup>. Systematic serological screening in terms of toxoplasma antibodies is required by law in countries like France and Austria. While there is no such obligation in our country, it may be recommended in the first trimester in places like Southeastern and Eastern Anatolia where raw meat is consumed. Reevaluation of the toxoplasma IgM(+) and IgG(+) samples in laboratories that are regarded as reference may be appropriate<sup>(1)</sup>. Serological screenings should be obtained in the first trimester as far as possible. Toxoplasma IgG, IgM and IgG avidity tests cost below 30TL dating from 2008.

In our country frequency of hepatitis B surface antigen was researched among 5366 pregnant women and positive results at the ratio of 4.2% were found<sup>(31)</sup>. HBsAg ratio in our region was also found as 3%, compatible with this number. Although VDRL is a diagnosis test for syphilis and its seroactivity was stated as 3-18% among the pregnant women in African countries, in more recent studies VDRL seroreactivity has been found in lower ratios as 0.98%<sup>(32,33)</sup>. In our society, on the other hand, as expected from these countries, it has been determined in lower ratios as 0.4%. On the west of our country, antirubella IgG positivity has been determined as 94.3-96.1%, IgM positivity as 0.2-1.7%, antiCMV IgG (+) as 96.4-97.3%, IgM(+) 0.7-1%<sup>(22,34)</sup>. In Middle Anatolia, antirubella IgG positivity has been found as 96.2% <sup>(35)</sup>, in Hatay and its surroundings as 95%, IgM (+) as 0.54%; antiCMV IgG (+) as 94.9%, IgM (+) as 0.4%<sup>(23)</sup>. Rubella IgG seroprevalance was determined at the ratio of 93.1% among school-age children in Edirne and its surroundings and it was calculated that only 6.9% of the adolescents may be at risk during pregnancy<sup>(36)</sup>. Rubella IgG

seroprevalance was determined as %93.8 in Malatya and its surroundings<sup>(37)</sup>. On the other hand, in a research made in Mersin, rubella seroprevalance was determined as 55%, as 92.5% in Adana and as 82.1% in Ankara<sup>(38-40)</sup>. As is seen, even in the regions that are close to each other these ratios don't show a homogeneous distribution. Results that are similar to these stated serological results in our country have been found in our research. Consequently, incidence of toxoplasmosis before pregnancy in Trakya region is about 30%. About 65% of the pregnant women are seronegative and toxoplasmosis screening may be appropriate. In addition to this, numbers are not clear about seroconversion of acute maternal toxoplasmosis in pregnancy and polycentric wide screening programs and studies that will represent all Turkish society are needed.

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