Clin. Lab. 2022;68:XXX-XXX
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ORIGINAL ARTICLE

Determination of Low IgG Class Antibody Avidity Percentage by IgM Levels Specific to Toxoplasma Gondii

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SUMMARY

Background: **Ig (Immunglobulin) M detection and low avidity index are markers of recent infection in differentiating acute and chronic stages of *Toxoplasma gondii (T. gondii)* infections. In this study, we aimed to evaluate whether the *anti-T. gondii* IgM antibody index threshold value could be a predictive factor in the estimation of low avidity and its use in improving the diagnosis of early toxoplasma infection.**
Methods: **Anti-*T. gondii* IgM, IgG antibody and IgG avidity results were analyzed. *Anti-T. gondii* IgG and IgM antibodies in blood samples were studied with chemiluminescent microparticle immunoassay (CMIA), and IgG avidity test was performed with Enzyme Linked Fluorescent Assay (ELFA) technique.**
Results: **The overall seroprevalence of *anti-T. gondii* antibodies (IgG and/or IgM) was 19.4%. Of the 64 patients whose avidity tests were studied, 47 (73.4%) were female. Twenty seven (57.4%) of the women were pregnant. In the IgG avidity test, 7.8% low avidity was detected. Low avidity was detected in only 4 (15.4%) of 26 IgM positive cases. IgM analysis of a case (6-month-old baby) with low avidity was found to be negative. In the prediction of low avidity, the assay's IgM positivity cutoff value was ≥ 0.6, its sensitivity, specificity, positive predictive value, and negative predictive value were 80%, 62.7%, 2.3%, and 99.7%, respectively. With Architect, 37.3% of samples were false positive. Determining the IgM index cutoff value was unsuccessful in distinguishing low avidity. The area under the ROC curve was 0.574 (p = 0.60).**
Conclusions: **In our study, the positive predictive value of the IgM test kit in estimating low avidity was low and the false positivity rate was 37.3%. It is thought that the index cutoff value of the *anti-T. gondii* IgM antibody test kit cannot be considered as a good predictor of recent infection. Studies with larger patient groups are needed.**
(Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2021.211112)

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Manuscript accepted December 20, 2021

KEY WORDS

Toxoplasma gondii, IgM, serological diagnosis, avidity, primary infection

INTRODUCTION

Toxoplasma gondii (T. gondii) is a worldwide parasite with a wide range of intermediate hosts, including almost all warm-blooded vertebrates and humans, with the only definitive hosts being members of the Felidae family (i.e., domestic cats and other felines) [1,2]. It has been reported that the prevalence is between 0.5% to 87.7% worldwide. African countries have the highest average seroprevalence rate with 61.4%, followed by

Oceania with 38.5%, South America with 31.2%, Europe with 29.6%, USA/Canada with 17.5%, and Asia with 16.4%. Environmental and human factors affect the differences observed in *T. gondii* seroprevalence [3]. The primary infections are usually asymptomatic. However, latent infection will persist throughout life of the host [4,5]. When it develops shortly before pregnancy or in the first trimester of pregnancy, the agent passes through the placental barrier and causes congenital infections and miscarriage [1,6]. As the gestational weeks increase, the vertical transmission rate increases, while the rate of the fetus being affected by congenital toxoplasmosis decreases [1,7].

Diagnosis is usually based on serological methods [8]. Presence of IgM indicates acute *T. gondii* infection and anti-*T. gondii* IgG alone in the absence of IgM is usually indicative of past infection [1]. Although detection of both *T. gondii*-specific IgM and IgG in a single serum sample is suggestive of an acute infection, it is difficult to distinguish between past infections as *T. gondii*-specific IgM antibodies can persist for months or years [9]. The increased *T. gondii* IgG titers can also be used in the diagnosis of new infections, but monitoring requires a long period of time, which can be harmful to the embryo [10]. For these reasons, the *T. gondii* IgG avidity test has been developed [9,11]. By measuring the strength of adherence to *T. gondii* antigen, this test helps to distinguish between primary toxoplasmosis and previous infection [12]. While low avidity may indicate an imminent infection, high avidity excludes a primary infection [13].

The first aim of this study was to evaluate whether the *T. gondii* IgM index threshold value could be a predictive factor in the estimation of low avidity, the second was to obtain information about the performance characteristics of the IgM test kit (especially positive and negative predictive values) by investigating the *T. gondii* antibody seroprevalence, and finally, to determine the low IgM index value.

MATERIALS AND METHODS

In this cross-sectional retrospective study, the sera sent to Gulhane Training and Research Hospital Microbiology Laboratory between January 1, 2017 and June 30, 2021 for *anti-T. gondii IgG* and *anti-T. gondii IgM* antibody tests and *T. gondii IgG* avidity were included. Demographic information (order date, diagnosis, age, and gender) of the patients were obtained from the hospital data system. The first test results of the patients with duplicate study were included in the study. Patients with insufficient samples and inadequate test results were excluded from the study. *Anti T. gondii* IgG and IgM antibodies in 5,502 serum samples and *T. gondii* IgG avidity results of 64 patients were evaluated retrospectively. *Anti T. gondii* IgM and IgG frequencies were grouped according to gender, age ranges, ethnicity, and years.

Anti T. gondii IgM and IgG antibody levels in serum samples were studied and interpreted by chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT i2000sr (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions. *Anti T. gondii* IgM and IgG antibody values of > 0.60 were accepted as positive, < 0.50 as negative, and 0.50 - 0.60 as cutoff. The study was approved by SBU Gulhane Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (Reference number: 2021/16/357).

T. gondii IgG avidity test

In order to differentiate between primary and non-primary toxoplasmosis, *T. gondii* IgG avidity test was performed by enzyme-linked immunofluorescent assay (ELFA-VIDAS, BioMerieux, France) method in accordance with the manufacturer's recommendations. The principle of the test is a two step enzyme immunoassay sandwich method and a fluorescence readout. It uses a disintegrating agent such as urea. IgG avidity test results of 64 patients who were requested to have *anti-T. gondii* IgG and IgM tests at the same time and whose IgG antibodies were positive and IgM antibodies were negative, borderline, and positive, were evaluated. Data were given with percentages. In the IgG avidity study, > 30% was accepted as high avidity, 20 - 30% as intermediate avidity, and < 20% as low avidity according to the test procedure. Those with an avidity index below 20% were considered to have recently had toxoplasmosis (less than 4 months).

Statistical analysis

Data were analyzed using the SPSS 25 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) package program. Data were expressed as numbers (percentage) and median (interquartile range). Intergroup differences (age groups and genders) were compared using the non-parametric Mann-Whitney U or Kruskal-Wallis test for continuous variables, and Pearson's chi-squared or Fisher's exact tests for categorical variables. For the cutoff value (≥ 0.6) at which the patient's *anti-T. gondii IgM* antibody test result is considered positive, the diagnostic performance of *T. gondii* IgM antibody index levels in predicting low avidity was evaluated. IgM index cutoff values were evaluated using the receiver operating characteristic (ROC), and results were expressed with a 95% confidence interval (95% CI). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the best cutoff value. p-values < 0.05 were considered statistically significant.

RESULTS

Median age of study population (n: 5,502) was 29 (range: 0 - 97) years. General seroprevalence of anti-*T. gondii* antibodies (IgG and/or IgM) was 19.4% (1,069/

Table 1. Seroprevalence of *T. gondii* IgM and IgG antibodies patients according to their characteristics.

Variables	Categories	Number analysed	Anti-*T. gondii* IgG			Anti-*T. gondii* IgM		
			Number positive	%	p-value	Number positive	%	p-value
Gender	Male	1,667	410	24.6	< 0.001	19	1.1	0.95
	Female	3,835	653	17		43	1.1	
	Total	5,502	1,063	1.1		62	1.1	
Age groups (years)	0 - 18	749	178	23.8	0.007	10	1.3	0.27
	19 - 30	2,212	390	17.6		32	1.4	
	31 - 40	1,378	276	20.0		12	0.9	
	41 - 50	493	93	18.9		3	0.6	
	≥ 50	670	126	18.8		5	0.7	
Ethnicity	Turkish	5,333	1,034	19.4	0.47	59	1.1	0.42
	Others	169	29	17.2		3	1.8	
Study group	Malignancy	319	78	24.5	< 0.001	5	1.6	0.50
	Lymphadenitis	281	46	16.4		6	2.1	
	Bone marrow donor	175	41	23.4		3	1.7	
	Hematological diseases	586	129	22.0		4	0.7	
	Female infertility	37	3	8.1		1	2.7	
	Immunosuppressive state *	60	14	23.3		0	0	
	Eye diseases	90	27	30.0		1	1.1	
	Pregnancy status	1,409	213	15.1		16	1.1	
	Threatened abortion	20	4	20.0		0	0	
	Others	2,525	508	20.1		26	1.0	
Region	Central Anatolia	4,671	868	18.6	< 0.001	50	1.1	0.10
	Black Sea	243	65	26.7		5	2.1	
	Marmara	154	24	15.6		1	0.6	
	Aegean	94	24	25.5		2	2.1	
	Mediterranean	90	27	30.0		0	0	
	Eastern Anatolia	183	33	18.0		1	0.5	
	Southeastern Anatolia	67	22	32.8		3	4.5	
Years	2017	834	156	18.7	0.003	6	0.7	0.27
	2018	1,184	216	18.2		10	0.8	
	2019	1,590	312	19.6		19	1.2	
	2020	1,172	203	17.3		14	1.2	
	2021	722	176	24.4		13	1.8	

*** - Immunosuppressive state: HIV infection, kidney transplant, multiple sclerosis, systemic lupus, Hematological diseases - anemia, thrombocytopenia, splenomegaly, etc., Other - General medical examination.**

5,502) [95% CI = 18.4 - 20.5]. Prevalences of anti-*T. gondii* IgG and IgM antibodies were 19.3% (1,063/5,502) and 1.1% (62/5,502), respectively. Six out of 62 IgM positive samples were (9.7%) IgG negative.

In this study, 69.7% of 5,502 patients were female. *Anti-T. gondii* IgG antibodies were positive in 24.6% of males and 17% of females ($p < 0.001$). IgM positivity (1.1%) was found to be similar in both male and female patients ($p = 0.95$).

When the *anti-Toxo* IgG antibody positivity was evaluated according to the diagnostic status of the patients, this rate was the highest with 30% in the serum samples sent from the eye outpatient clinics. Malignancy was the second in line with 25.4% and bone marrow donor status was the third with 23.4% ($p < 0.001$), while in pregnant women *T. gondii* IgG and IgM antibody positivity

Table 2. Correlation between anti-*T. gondii* antibody IgM index levels and percentage IgG-class antibody avidity to *T. gondii* (< 20% and > 30%).

IgM range	Avidity < 20	Avidity 20 - 30	Avidity > 30	p-value
	n (%)	n (%)	n (%)	
Negative < 0.5	1 (2.7)	3 (8.1)	33 (89.2)	
Gray zone 0.5 - 0.6	0	0	1 (100)	
Positive				
> 0.6 - 1.0	2 (18.2)	4 (36.4)	5 (45.5)	
> 1.0 - 2.0	1 (12.5)	0	7 (87.5)	**0.01**
> 2.0 - 3.0	1 (50)	0	1 (50)	
> 3.0	0	2 (40)	3 (60)	
Total	5 (7.8)	9 (14.1)	50 (78.1)	

Table 3. Performance of the Architect *T. gondii* IgM assay according to index ratios (n = 64).

Statistics	Index value
	≥ 0.6
False Positives Rate	22/59 (37.3%)
True Positive Rate	4/5 (80%)
Sensitivity	80% (28.4% - 99.5%)
Specificity	62.7% (49.2% - 75.0%)
Positive Likelihood Ratio *	2.2 (1.2 - 3.7)
Negative Likelihood Ratio *	0.3 (0.1 - 1.9)
Positive Predictive Value	2.3% (1.4% - 4%)
Negative Predictive Value	99.7% (98% - 99.9%)
Accuracy *	62.9% (49.9% - 74.7%)

*** - These values are dependent on disease prevalence (*T. gondii* IgM prevalence 1.1%). The index value recommended by the kit manufacturer for positive IgM is ≥ 0.6.**

were 15.1% and 1.1%, respectively. IgG positivity was detected in 20% of the cases with the risk of threatened abortion, while IgM positivity was not detected. In the study group, the distribution of *anti-T gondii* antibodies is presented in Table 1.

In our study, *anti-T. gondii* IgG positivity was found to be significantly higher (23.8%) in the 0 - 18 year age group compared to other age groups (p = 0.007), and the highest IgG positivity was found in 2021 with 24.4% (p = 0.003).

Foreign patients were 3.1% (169/5,502) of all patients. Turkish and foreigner's *anti-T. gondii* IgG seroprevalences were 19.4% (1,034/5,333) and 17.2% (29/169), respectively (p = 0.47).

The study population consisted of patients from 7 different regions of Turkey. *Anti-T. gondii* IgG seroprevalence rates were the highest in patients residing in the Southeastern Anatolia Region (32.8%), the Mediterranean region (30%), and the Black Sea region (26.7%) (p < 0.001).

T. gondii IgG avidity test

Of the 64 patients whose avidity test was studied, 47 (73.4%) were female. Twenty seven (57.4%) of the women were pregnant. *Anti-T. gondii* IgG antibody was positive in 64 serum samples for whom the avidity test was requested. IgM tests were positive in 26 (40.6%), borderline (suspected) in 1 (1.6%) and negative in 37 (57.8%) patients. The results of 64 cases of *toxoplasma* IgG avidity test were as follows: 50 (78.1%) high, 5 (7.8%) low, and 9 (14.1%) moderate avidity. Low avidity was detected in only 4 (15.4%) of 26 IgM positive cases. Interestingly, in a case with low avidity (6 months old baby), *anti-T. gondii* IgM antibody analysis was negative.

Anti-T. gondii IgM antibody index values (median) at

low, medium, and high avidity were 0.79 (range: 0.07 - 2.9), 0.67 (range: 0.08 - 11.20), 0.22 (range: 0.06 - 8.40), respectively (p = 0.34). The distribution of avidity rates in the ranges of IgM index < 0.5, 0.5 - 0.6, 0.6 - 1.0, 1.0 - 2.0, 2.0 - 3.0, and > 3.0 is presented in Table 2.

Serum anti-*T gondii* index cutoff value recommended by the manufacturer and low avidity diagnostic performance of anti-*T gondii* IgM

When the index value recommended by the manufacturer was higher than 0.6, *T. gondii* IgM antibody was considered positive. In this study, the same index value was first used to analyze our data. In 64 cases for whom *T. gondii* IgG avidity test was requested, IgM positivity cutoff value of the test kit was 0.6, sensitivity was 80% (95% CI: 28.4 - 99.5), specificity was 62.7% (95% CI: 49.2 - 75.0), PPV was 2.3% (95% CI: 1.4 - 4), and NPV was 99.7% (95% CI: 98 - 99.9) in the prediction of low avidity (recent infection). Of the samples, 37.3% were identified as false positive in the diagnosis of acute infection with Architect (Table 3).

ROC (Receiver Operating Characteristic) curve analysis of serum positive anti-*T. gondii* IgM index cutoff value for the diagnosis of low avidity

Significant index value and performance were evaluated by ROC analysis in *T. gondii* IgM antibody positive cases to predict low avidity. ROC analysis for anti-*T. gondii* IgM antibody revealed sensitivity of 100% (95% CI: 39.8 - 100), specificity of 9.1% (95% CI: 1.1 - 29.2), PPV of 20.8% (95% CI: 18.7 - 23.1), and NPV of 100%, when index value was set to be 0.66. The area under the ROC curve was 0.574 (95% CI: 0.292 - 0.856, p = 0.60) and was not significant.

DISCUSSION

The prevalence of *T. gondii* infection varies according to geographical location. The reason for this variation is different risk factors and the different sources of transmission [15,16]. In this study, the overall seroprevalence of *T. gondii* infection (IgG and/or IgM) was 19.4%, and the prevalence of IgG and IgM antibodies was 19.3% and 1.1%, respectively. However, IgG positivity showed regional differences. The highest seropositivity was found in patients residing in the Southeastern Anatolia region with 32.8%. This was followed by patients from the Mediterranean (30%) and Black Sea region (26.7%). It has been evaluated that this variability in *T. gondii* seropositivity may be related to the changes in climate and dietary habits (raw meatball eating culture), as well as the difference in the size of the groups included in the study.

Numerous studies have been conducted in our country investigating the seropositivity of *T. gondii*. Considering the data in recent years, reports published on *T. gondii* infection in Turkey stated that the prevalence of anti-*T. gondii* IgG antibodies varies between 17.5 - 69.5% and the prevalence of anti-*T. gondii* IgM antibodies varies between 0 - 5.4% [16]. Variation in the seroprevalence of *T. gondii* infection is also observed worldwide [3]. The rate of IgG seropositivity was significantly higher in males. This may be related to the eating habits of men.

In this study, anti-*T. gondii* IgG antibody seropositivity was higher in the pediatric age group. *T. gondii* seroprevalence increases with age, but the age-related infection rate varies by country and socioeconomic status. However, it has been reported that seroprevalence increases in childhood in populations living under inadequate hygiene conditions, especially consuming oocyst-contaminated water, which is an important source of human infection [17,18].

Infection during pregnancy is important because of the risk of transmission to the fetus. Most of the acute infections acquired during pregnancy cannot be diagnosed clinically because there are no significant symptoms or signs [19]. In these cases, *T. gondii* IgG avidity, which has the ability to distinguish between new and previous infections, is beneficial [20-23]. Of the 64 patients whose avidity tests were studied, 26 were IgM positive and only 5 (7.8%) had low-avidity IgG antibodies suggestive of a recent *T. gondii* infection. Thus, this indicates the continued presence of *T. gondii* IgM antibodies in the chronic phase of infection. This is consistent with previous reports of persistent IgM positivity in chronic toxoplasmosis [24,25]. Interestingly, one of the 5 cases with low avidity index was a 6-month-old baby and IgM antibody was negative. Although negative toxoplasma IgM and IgA test results and positive neonatal toxoplasmosis IgG test results are rarely observed in newborns, if other etiologies are excluded for clinical signs (eye disease, intracranial calcifications, hydrocephalus, etc.), newborn infants are considered infected and treatment is recommended until proven otherwise. In neonatal toxoplasmosis, IgM and/or IgA antibodies may not be detected in 20% to 50% of congenital toxoplasmosis cases [26]. This situation may be related to antepartum therapy or may occur in the case of fetal infection in the early stages of gestation that does not receive any antepartum therapy. Positive *T. gondii* IgM and/or IgA antibody responses may be lost during delivery [27,28]. It can also be seen in some patients who exhibit a rapid decrease in IgM titer [29].

In this study, the IgM index positivity cutoff value of the test kit specified by the manufacturer was 0.6, 37.3% of the samples were false positive at the diagnosis of acute infection with Architect. False positive IgM antibody test results have been reported previously [30]. The false positive rate of the *T. gondii* IgM test can be as high as 60% [31]. The false-positive rate of any diagnostic test is a function of the specificity of the test and the prevalence of the disease in the population [32]. False-positive *T. gondii* may be caused by autoimmune antibodies, including IgM, rheumatoid factor, and antinuclear antibodies, acute viral infection, and non-specif-

ic *in vitro* binding [33]. However, IgM antibodies specific to *T. gondii* can persist for months or years and false positive tests make it difficult to distinguish from past infections [9].

In our study, the prevalence of IgM was 1.1%, and the specificity of the test for detecting recent infection was found to be low (62.7%). The high sensitivity and high NPV of the IgM test make it a good test to exclude toxoplasmosis when the result is negative [33]. In our study, this test kit gave 80% sensitivity and 92.9% NPV in the diagnosis of acute infection (low avidity). Regarding low IgG avidity, the PPV of IgM was 2.3%. These results show that a negative test may be a good index to exclude recent infection, but low PPV shows that IgM index value equal to 0.6 is not a good index for the diagnosis of recent infection, that is, acute infection. In a similar study, De Paschale et al. reported that IgM PPV was 45.98% for a recent infection related to IgG avidity [33].

Especially if the case is pregnant and early pregnancy is in question, it may cause misdiagnosis and unnecessary termination of pregnancy [34]. It has been reported that the best index limit for *T. gondii* IgM tests can be defined in the diagnosis of acute infection, together with avidity tests for evidence of recent toxoplasma infection in clinical routine [6]. Thus, false positive results could be reduced. Leite et al. investigated the threshold value for *T. gondii* IgM test in the diagnosis of acute infection. In their study, they analyzed anti-toxoplasma antibodies by microparticle enzymatic immunoassay (MEIA) method using Axsym® automatic analyzer. While no cases with a low avidity index were detected with an IgM value below 2, they found a low avidity index of 88.2% in cases with an IgM value above 6. However, they stated that avidity test results and kits from different manufacturers may be different when used, and each laboratory should establish its own values according to the most suitable reagents and kits for their own routines [6]. ROC analysis, together with evaluating the clinical accuracy of laboratory tests, can also be useful for classifying the infection status of the IgM immunoassay with the data obtained with the ROC curve [35]. In this study, ROC curve analysis was performed to select the most appropriate cutoff to determine the potential discrimination of acute toxoplasma infection (low avidity) for positive Architect IgM values. The area under the ROC curve was 0.574, $p = 0.60$. This result indicates that the cutoff IgM shows that determining the value may not be useful in the discrimination of low avidity (acute infection).

The study was originally designed to establish the place and importance of avidity tests in the serological diagnosis of toxoplasmosis. However, the most sensitive method in the diagnosis of toxoplasmosis is the molecular method. In some studies, its specificity is close to 100% and sensitivity around 75 - 90%. Especially in this regard, since real-time PCR determines both the detection of the parasite and parasite load, it allows the evaluation of treatment response and prognosis [36,37].

Our study has some limitations. Because of its retrospective design, our study did not provide enough information about the clinial status of patients. Moreover, the number of patients who were tested for avidity is limited.

In conclusion, the general prevalence of *T. gondii* in our study was 19.3%. The PPV of the IgM test kit in the diagnosis of acute infection was low, and the false-positive rate was 37.3% in the diagnosis of acute infection. The index cutoff value of the IgM test kit suggests that it does not have a good performance in predicting recent infection. Studies with larger patient groups are needed.

Source of Funds:

There is no grant, equipment, or drug support in the study.

Declaration of Interest:

There are no conflicts of interest to declare. All authors have not real or perceived conflicts of interest. This study has not been supported by any organization.

References:

1. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz H. Role of Toxoplasma gondii IgG Avidity Testing in Discriminating between Acute and Chronic Toxoplasmosis in Pregnancy. J Clin Microbiol 2020 Aug 24;58(9):e00505-20. (PMID: 32321784)

2. Montazeri M, Mikaeili Galeh T, Moosazadeh M, et al. The global serological prevalence of Toxoplasma gondii in felids during the last five decades (1967 - 2017): a systematic review and meta-analysis. Parasit Vectors 2020 Feb 17;13(1):82. (PMID: 32066495)

3. Molan A, Nosaka K, Hunter M, Wang W. Global status of Toxoplasma gondii infection: systematic review and prevalence snapshots. Trop Biomed 2019 Dec 1;36(4):898-925. (PMID: 33597463)

4. Gay-Andrieu F, Fricker-Hidalgo H, Sickinger E, et al. Comparative evaluation of the ARCHITECT Toxo IgG, IgM, and IgG Avidity assays for anti-Toxoplasma antibodies detection in pregnant women sera. Diagn Microbiol Infect Dis 2009 Nov;65(3): 279-87. (PMID: 19822270)

5. Zhang K, Lin G, Han Y, Li J. Serological diagnosis of toxoplasmosis and standardization. Clin Chim Acta 2016 Oct 1;461:83-9. (PMID: 27470936)

6. Leite M, Siciliano S, Rocha LS, Justa MT, Cesar KR, Granato CF. Correlation between specific IgM levels and percentage IgG-class antibody avidity to Toxoplasma gondii. Rev Inst Med Trop Sao Paulo 2008 Jul-Aug;50(4):237-42. (PMID: 18813765)

7. Shojaee S, Teimouri A, Keshavarz H, Azami SJ, Nouri S. The relation of secondary sex ratio and miscarriage history with Toxoplasma gondii infection. BMC Infect Dis 2018 Jul 5;18(1):307. (PMID: 29976155)

8. 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 Jun;50(2):99-102. (PMID: 22711919)

9. Garnaud C, Fricker-Hidalgo H, Evengard B, et al. Toxoplasma gondii-specific IgG avidity testing in pregnant women. Clin Microbiol Infect 2020 Sep;26(9):1155-60. (PMID: 32334096)

10. Pour Abolghasem S, Bonyadi MR, Babaloo Z, et al. IgG avidity test for the diagnosis of acute Toxoplasma gondii infection in early pregnancy. Iran J Immunol 2011 Dec;8(4):251-5. (PMID: 22201623)

11. Lappalainen M, Hedman K. Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity. Ann Ist Super Sanita 2004;40(1):81-8. (PMID: 15269456)

12. Aynali A, Cicioglu-Aridogan B, Tola EN, Onal S, Cetin ES. Evaluation of anti-Toxoplasma IgM-IgG Seropositivity among Women in Reproductive Period, Who Amitted to Suleyman Demirel University Hospital. Turk Hij Den Biyol Derg 2016; 73(1):33-8. https://www.turkhijyen.org/eng/jvi.aspx?pdir=turkhijyen&plng=eng&un=THDBD-35683

13. Curdt I, Praast G, Sickinger E, et al. Development of fully automated determination of marker-specific immunoglobulin G (IgG) avidity based on the avidity competition assay format: application for Abbott Architect cytomegalovirus and Toxo IgG Avidity assays. J Clin Microbiol 2009 Mar;47(3):603-13. (PMID: 19129411)

14. Wang S, Yao Z, Li H, et al. Seroprevalence and risk factors of Toxoplasma gondii infection in primary school children in Henan province, central China. Parasite 2020;27:23. (PMID: 32254019)

15. Mumcuoglu I, Toyran A, Cetin F, et al. [Evaluation of the toxoplasmosis seroprevalence in pregnant women and creating a diagnostic algorithm]. Mikrobiyol Bul 2014 Apr;48(2):283-91. (PMID: 24819265)

16. Malatyali E, Yildiz I, Tileklioglu E, Ertabaklar H, Ertug S. Retrospective Analysis of *Toxoplasma gondii* Serology Results from Adnan Menderes University Faculty of Medicine Parasitology Laboratory from 2007 to 2017. Turkiye Parazitol Derg 2019 Mar 28;43(1):1-4. (PMID: 30938117)

17. Ertug S, Okyay P, Turkmen M, Yuksel H. Seroprevalence and risk factors for toxoplasma infection among pregnant women in Aydin province, Turkey. BMC Public Health 2005 Jun 15;5:66. (PMID: 15958156)

18. Jones JL, Dubey JP. Waterborne toxoplasmosis--recent developments. Exp Parasitol 2010 Jan;124(1):10-25. (PMID: 19324041)

19. Boyer KM, Holfels E, Roizen N, et al. Risk factors for Toxoplasma gondii infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. Am J Obstet Gynecol. 2005 Feb;192(2):564-71. (PMID: 15696004)

20. Cozon GJ, Ferrandiz J, Nebhi H, Wallon M, Peyron F. Estimation of the avidity of immunoglobulin G for routine diagnosis of chronic Toxoplasma gondii infection in pregnant women. Eur J Clin Microbiol Infect Dis 1998 Jan;17(1):32-6. (PMID: 9512179)

21. Buffolano W, Lappalainen M, Hedman L, et al. Delayed maturation of IgG avidity in congenital toxoplasmosis. Eur J Clin Microbiol Infect Dis 2004 Nov;23(11):825-30. (PMID: 15558340)

22. Meroni V, Genco F, Tinelli C, Lanzarini P, Bollani L, Stronati M, Petersen E. Spiramycin treatment of Toxoplasma gondii infection in pregnant women impairs the production and the avidity maturation of T. gondii-specific immunoglobulin G antibodies. Clin Vaccine Immunol 2009 Oct;16(10):1517-20. (PMID: 19692628)

23. Lefevre-Pettazzoni M, Bissery A, Wallon M, Cozon G, Peyron F, Rabilloud M. Impact of spiramycin treatment and gestational age on maturation of Toxoplasma gondii immunoglobulin G avidity in pregnant women. Clin Vaccine Immunol 2007 Mar;14(3):239-43. (PMID: 17202303)

24. Garry DJ, Elimian A, Wiencek V, Baker DA. Commercial laboratory IgM testing for Toxoplasma gondii in pregnancy: a 20-year experience. Infect Dis Obstet Gynecol 2005 Sep;13(3):151-3. (PMID: 16126500)

25. Dhakal R, Gajurel K, Pomares C, Talucod J, Press CJ, Montoya JG. Significance of a Positive Toxoplasma Immunoglobulin M Test Result in the United States. J Clin Microbiol 2015 Nov; 53(11):3601-5. (PMID: 26354818)

26. Maldonado YA, Read JS, COMMITTEE ON INFECTIOUS DISEASES. Diagnosis, Treatment, and Prevention of Congenital Toxoplasmosis in the United States. Pediatrics 2017 Feb;139(2): e20163860. (PMID: 28138010)

27. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. Pediatr Infect Dis J 2011 Dec;30(12):1056-61. (PMID: 21956696)

28. Gilbert RE, Thalib L, Tan HK, Paul M, Wallon M, Petersen E; European Multicentre Study on Congenital Toxoplasmosis. Screening for congenital toxoplasmosis: accuracy of immunoglobulin M and immunoglobulin A tests after birth. J Med Screen 2007;14(1):8-13. (PMID: 17362565)

29. Sensini A. Toxoplasma gondii infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clin Microbiol Infect 2006 Jun;12(6):504-12. (PMID: 16700697)

30. Reischl U, Bretagne S, Kruger D, Ernault P, Costa JM. Comparison of two DNA targets for the diagnosis of Toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infect Dis 2003 May 2;3:7. (PMID: 12729464)

31. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004 Jun 12; 363(9425):1965-76. (PMID: 15194258)

32. Woods CR. False-Positive Results for Immunoglobulin M Serologic Results: Explanations and Examples. J Pediatric Infect Dis Soc 2013 Mar;2(1):87-90. (PMID: 26619450)

33. De Paschale M, Agrappi C, Belvisi L, et al. Revision of the positive predictive value of IgM anti-Toxoplasma antibodies as an index of recent infection. New Microbiol 2008 Jan;31(1):105-11. (PMID: 18437848)

34. Kaul R, Chen P, Binder SR. Detection of immunoglobulin M antibodies specific for Toxoplasma gondii with increased selectivity for recently acquired infections. J Clin Microbiol 2004 Dec; 42(12):5705-9. (PMID: 15583302)

35. Doyle S, Kerr S, O'Keeffe G, O'Carroll D, Daly P, Kilty C. Detection of parvovirus B19 IgM by antibody capture enzyme immunoassay: receiver operating characteristic analysis. J Virol Methods 2000 Nov;90(2):143-52. (PMID: 11064115)

36. Saadatnia G, Golkar M. A review on human toxoplasmosis. Scand J Infect Dis. 2012 Nov;44(11):805-14. (PMID: 22831461)

37. Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasit Vectors 2015 May; 28;8:292. (PMID: 26017718)