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Seroprevalence of patients presenting with a pre-diagnosis of *Toxoplasma gondii*, Türkiye

Toxoplasma gondii ön tanısı ile başvuran hastalarda seroprevalans, Türkiye

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Abstract

Purpose: Toxoplasma gondii is a unicellular protozoan parasite. With this study, the aim was to evaluate the *T. gondii* test results that came to the General Directorate of Public Health, National Parasitology Reference Laboratory between January 2019 and December 2020.

Materials and methods: The seroprevalence were studied with the Sabin-Feldman Dye Test which is gold standard, Anti-*T. gondii* IgG and IgM and *T. gondii* IgG avidity with ELISA were evaluated in 1160 patient. The distribution of cases according to gender and age groups was also evaluated statistically.

Results: In this study, SFDT was performed on 589 patients from 1160 patients with suspected Toxoplasmosis samples and Anti-*T. gondii* IgG and IgM tests were studied by ELISA in 478 patients. IgG avidity test by ELISA was performed on 93 cases with positive Anti-*T. gondii* IgG. In addition to these, cases in which Anti-*T. gondii* IgM and IgG were studied together and were positive were also evaluated. The number of samples both positive together was 17 (3.6%). According to Anti-*T. gondii* IgG/IgM results, no significant relationship was found between toxoplasmosis and gender. Moreover, of 93 cases with positive Anti-*T. gondii* IgG values, 71% had high avidity, 16% had cutoff value and 13% had low avidity.

Conclusion: Further studies and surveillance studies should be performed to determine the epidemiology and current prevalence of toxoplasmosis.

Keywords: Avidity, ELISA, Sabin-Feldman Dye Test, *Toxoplasma gondii*.

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Öz

Amaç: *Toxoplasma gondii*, tek hücreli bir protozoon parazittir. Bu çalışma ile Halk Sağlığı Genel Müdürlüğü Ulusal Parazitoloji Referans Laboratuvarına Ocak 2019-Aralık 2020 tarihleri arasında gelen *T. gondii* test sonuçlarının değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: Altın standart olan Sabin-Feldman Boya Testi ile seroprevalans çalışıldı, Anti-*T. gondii* IgG ve IgM ve *T. gondii* IgG aviditesi ELISA ile 1160 hastada değerlendirildi. Vakaların cinsiyet ve yaş gruplarına göre dağılımı da istatistiksel olarak değerlendirildi.

Bulgular: Bu çalışmada Toksoplazmoz şüphesi olan 1160 hastadan 589'una SFDT yapıldı ve 478 hastada ELISA ile Anti-T. gondii IgG ve IgM testleri çalışıldı. Anti-*T. gondii* IgG'si pozitif olan 93 olguya ELISA ile IgG avidite testi uygulandı. Bunlara ek olarak Anti-*T. gondii* IgM ve IgG birlikte çalışıldı ve pozitif olanlar da değerlendirildi. İkisi birlikte pozitif olan örnek sayısı 17 (%3,6) idi. Anti-*T. gondii* IgG/IgM sonuçlarına göre toksoplazmoz ile cinsiyet arasında anlamlı bir ilişki bulunmadı. Ayrıca Anti-*T. gondii* IgG değerleri pozitif olan 93 olgunun %71'inde yüksek avidite, %16'sında cutoff değeri ve %13'ünde düşük avidite vardı.

Sonuç: Toksoplazmozun epidemiyolojisini ve güncel prevalansını belirlemek için ileri çalışmalar ve sürveyans çalışmaları yapılmalıdır.

Anahtar kelimeler: Avidite, ELISA, Sabin-Feldman Boya Testi, *Toxoplasma gondii*.

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Introduction

Toxoplasma gondii is a single-celled protozoan parasite that needs a host cell to reproduce and is frequently encountered throughout the world. It is an infective agent that can cause infection in all mammals. In its life cycle, the feline is definitive hosts and other warm-blooded vertebrates are intermediate hosts. *T. gondii* has three effective forms. These are tachyzoites [the form that can be found in all cells except rapidly proliferating erythrocytes], the tissue cyst form [the form in which slowly proliferating bradyzoites can be found in the latent and chronic periods], and oocysts [the form found in the intestinal epithelial cells of cats and felines and excreted with their feces]. Transmission of *T. gondii* to humans occurs by ingestion of tissue cysts [with raw/undercooked meat] or by ingestion of contaminated water and food through oocysts dispersed from the feces of infected cats [1, 2]. In cases where the immune system is intact, the infection usually develops in an asymptomatic course. In some infected patients, clinical findings may develop as cervical type lymphadenopathy, ocular findings, central nervous system diseases and abscess within the brain [3]. Asymptomatic infections, especially in pregnant women, constitute an important risk factor for the fetus. In the first trimester of pregnancy, when infected with this parasite, the agent can pass to the fetus. When it causes infection in the fetus, it can cause serious consequences, from miscarriage to stillbirth. Congenital toxoplasmosis can lead to severe symptoms in the newborn such as hydrocephalus, microcephaly, cerebral calcifications, retinal disorders in the eye, advanced hearing problems and mental retardation [2]. Other routes of transmission are blood transfusion, solid organ or stem cell transplantation, and laboratory accidents [4]. In diseases that suppress the immune system (Acquired Immune Deficiency Syndrome (AIDS) or the use of immunosuppressive drugs, etc.), the infection may flare up again in a latent and severe manner and cause case deaths. Therefore, it is known that T. *gondii* infection is an important cause of morbidity and mortality in patients with AIDS [5].

Diagnosis of *Toxoplasma gondii* infection, determination of its acute and chronic stages and genetic characteristics are very important for the treatment, control, prevention, and epidemiology of toxoplasmosis. Indirect diagnostic methods are frequently used to determine the population prevalence in terms of public health [6, 7]. Due to the disease caused by T. *gondii,* which is usually asymptomatic or clinical findings are not specific to the infection, anti-*T. gondii* antibodies should be supported by serological tests. If the infection is detected at an early stage, it can be treated [8]. The ELISA and the Sabin-Feldman Dye Test (SFDT) are the two most frequently used methods in laboratories today. Although SFDT has high sensitivity and specificity, ELISA is more reliable, economic and easier method [9]. While ELISA IgM positivity is interpreted in favor of acute toxoplasmosis, it is important that these antibodies remain positive for months or even years. In addition, the possibility of false positive IgM antibodies can lead to difficulties in diagnosis, and issues such as confirmation of positive values by other methods and understanding the stage of infection require the application of IgG avidity test. Understanding early or late toxoplasmosis is of great clinical importance in pregnant women and immunocompromised cases [10-13]. The aim of this study is to evaluate the frequency of the presence of *T. gondii* antibodies between different sexes and age groups by Sabin Feldman Dye test and ELISA IgG/IgM seroconventional methods in cases sent to the laboratory in 2019-2020.

Materials and methods

This study was carried out with 1160 serum samples sent to the General Directorate of Public Health, Department of Microbiology Reference Laboratories, National Parasitology Reference Laboratories between January 2019, and December 2020 to investigate *Toxoplasma gondii* antibodies. In the study, antibody determination was made with SFDT on 589 serum samples. Anti-*T. gondii* IgG and IgM positivity were investigated by ELISA in the serum of 478 patients, avidity values of 93 patients with Anti-*T. gondii* IgG positive were determined. Data obtained for all patient

and test groups, and gender and age group analyses were performed. ELISA test kits from different manufacturers were studied in accordance with the manufacturer's directives during the study periods. The *T. gondii* strain used in this study was reported by Ekmen et al. (1974) [14], and uploaded to NCBI with the name of *T. gondii* TR01 by defining whole genome studies in UPRL. Pearson Chi-Square was used for statistical analysis of the study data, and the Kappa test was used for univariate logistic regression analysis and fit tests. In the Pearson Chi-Square analysis, $p<0.05$ values were considered statistically significant. Risk assessment tests were also carried out, and all statistical tests were carried out with the SPSS Windows, Version 14.1 package program.

This publication, number 715087, dated 2022, was carried out by BA and approved as a master thesis, and its permission was obtained from the Ministry of Health, Public Health Institution of Türkiye on 05/03/2021.

Results

In this study, a total of 1160 toxoplasmosis suspected patients who applied to UPRL were studied. In the study, SFDT was studied in 589 serum samples to investigate Anti-*T. gondii* antibodies and the results were evaluated in terms of demographic findings in Table 1. Of the 589 cases, 348 (59.1%) of the total 589 cases were found to be positive for SFDT, of which 211 (60.6%) were female and 137 (39.4%) were male. According to gender groups, this difference was found to be statistically significant. When the SFDT seropositivity rates are analyzed by age groups, the lowest is in the 2-14 age group (3.7%), and the highest in the 0-1 age group (51.1%) (Table 1). A statistically significant correlation was found between patient age groups and the presence of *T. gondii* infection in SFDT ($p<0.05$). The high seropositive rates seen in the infant patient group in the SFDT study results indicate that the presence of mother-to-infant antibody titers was detected.

In this study, Anti-*T. gondii* IgG and IgM tests were studied together and Anti-*T. gondii* IgG avidity test was performed on 93 cases with positive Anti *T. gondii* IgG. Anti-*T. gondii* IgG values are presented in Table 2. Anti-*T. gondii* IgG positive result was detected in 45% of all samples, 44.7% of men and 55.3% of women. The lowest anti-*T. gondii* IgG results were found in the 2-14 (4.7%) age group and the highest in the 15-45 (58.1%) age group (Table 2).

In this study, Anti-*T. gondii* IgM values were evaluated with demographic findings (Table 3). Anti-*T. gondii* IgM result was found positive in 4.2% of the samples and in 30% of the men and 70% of the women. Anti-*T. gondii* IgM positivity was observed most frequently in the 15-45 age group with 75%, while the lowest frequency was detected at the same rate with 5% in the 2-14 and >45 age group (Table 3). In this respect, it has been observed that the 15-45 age group is riskier in terms of acute toxoplasmosis infection. According to the anti-Toxo- IgM/IgG results, no statistically significant difference was found between the sex and age groups in positive patients ($p>0.05$).

In this study, Anti-*T. gondii* IgG was positive in 215 samples and Anti-*T. gondii* IgM was positive in 20 samples of 478 patients aged 0-88 years. During this study, there were 17 patients in whom IgG and IgM were positive together (common positives). IgG and IgM were both positive in 17 patients (3.6%), IgG positive in 198 patients (41.4%), and IgM positive in 3 (0.6%). *T. gondii* seropositivity of the total patients in whom ELISA IgG and IgM were studied in 2019-2020 was 45.6% with the number of 218 patients. Of these 218 positive patients, 120 (55%) were female and 98 (45%) were male (Table 4).

In the study, the results of 93 patients with positive ELISA IgG results and *T. gondii* IgG avidity requests were evaluated. Of the 12 (13%) patients whose avidity test was low (Avidity index value <40%), 7 were female, 5 were male, and 15 (16%) patients had a cutoff value (Avidity index value of 40-50%). Of the 66 (71%) patients, 8 were female, 7 were male, with a high value (Avidity index value >50%), 44 were female and 22 were male (Table 5).

Table 1. Distribution of Anti-*T. gondii* antibody results detected by SFDT by gender and age group (n=589)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	211	60.6	120	49.8	331	56.2
	Male	137	39.4	121	50.2	258	43.8
	Total	348	59.1	241	40.9	589	100
Age	**0-1**	178	51.1	92	38.2	270	45.8
	2-14	13	3.7	26	10.8	39	6.6
	15-45	139	39.9	93	38.6	232	39.4
	>45	18	5.2	30	12.4	48	8.1
	Total	348	100.0	241	100.0	589	100.0

Table 2. Distribution of seropositivity by gender and age groups according to Anti- *T. gondii* IgG (n=478)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	119	55.3	124	47.1	243	50.8
	Male	96	44.7	139	52.9	235	49.2
	Total	215	45	263	55	478	100
Age	**0-1 Age**	50	23.3	13	4.9	63	13.2
	2-14 Age	10	4.7	29	11.0	39	8.2
	15-45 Age	125	58.1	201	76.4	326	68.2
	>45 Age	30	14.0	20	7.6	50	10.5
	Total	215	100.0	263	100.0	478	100.0

Table 3. Distribution of Anti-*T. gondii* IgM seropositivity by gender and age group (n=478)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	14	70	229	50	243	50.8
	Male	6	30	229	50	235	49.2
	Total						
Age	**0-1 Age**	3	15.0	60	13.1	63	13.2
	2-14 Age	1	5.0	38	8.3	39	8.2
	15-45 Age	15	75.0	311	67.9	326	68.2
	>45 Age	1	5.0	49	10.7	50	10.5
	Total	20	100.0	458	100.0	478	100

Table 4. Combination and distribution of Anti-*T. gondii* IgG and IgM between 2019-2020 (n=218)

IgM(+)/IgG(-)		IgM(+)/IgG(+)		IgM(-)/IgG(+)		Total	
n	%	n	%	n	%	n	%
3	0.6	17	3.6	198	41.4	218	45.6

Table 5. IgG Avidity test results (n=93)

			Avidity			
		Genes	Low Value	Breakdown	High Value	Total
IgG	Positive	Female	7	8	44	59
		Male	5	7	22	34
		Total	12	15	66	93

Discussion

Toxoplasmosis is an infection caused by *T. gondii* protozoa. According to the results of this study's investigation of the seroprevalence of *T. gondii,* it was stated that although the prevalence is high globally, it changes in conjunction with socio-economic conditions, increases with age, and is more common in hot climates [10]. Poor socio-economic conditions in countries, cause the prevalence of the disease to rise [5]. While the seropositivity of the disease was found to be below 3% in Australia and North America, rates exceeding 50% were detected in Europe and Africa [15]we describe the effects of global climate change for one specific pathogen: the parasite Toxoplasma gondii. It is postulated that an increase of T. gondii prevalence in humans can occur in some regions of North-Western Europe as a result of changing environmental conditions. Such a change can be predicted by using Global Climate Change models. We have elaborated such a prediction for one scenario (SRES A1. Due to the parasite's asymptomatic feature, clinical findings are rare [16].

Toxoplasma gondii-IgG antibodies appear within two weeks of contracting toxoplasmosis and these antibodies reach their highest levels within 3 months. IgM antibodies specific to *T. gondii*, which are accepted as an indicator of acute infection, can be detected by ELISA in the first weeks, since they are the first antibodies [17]. IgM-type antibodies disappear earlier than expected in some acute infections, making it difficult to distinguish between acute infection and chronic infection [18]. In such cases, the IgG avidity ELISA test helps us to obtain information about the time of detection by calculating the avidity of *T. gondii*-specific IgG in the acute and chronic phases of the infection [19]. In order to increase the reliability of the diagnosis, as Western Blot and PCR should be used [20].

Considering the recent studies in Türkiye, Anti-*T. gondii* IgG positivity is between 17.5% and 69.5% and Anti-*T. gondii* IgM has been reported between 0-5.4% [21]. In a retrospective study conducted in the same laboratory before, SFDT was found to be 52%, Anti-*T. gondii* IgG 47.1% and Anti-*T. gondii* IgM positive 10.2% [10].

In this study, SFDT 59.1%, Anti-*T. gondii* IgG 45% and Anti-*T. gondii* IgM 4.2% seropositivity was detected. These results are similar to *T. gondii* seropositivity studies conducted in Türkiye so far.

According to the ELISA results performed in this study, only IgG positivity was found in 198 (41.4%) of the patients, only IgM positivity was found in 3 patients (0.6%), and both were positive in 17 (3.6%) patients. 17 samples form a common group that were evaluated as positive according to both IgG and IgM. It has been determined that the results of the study show similarities with other studies conducted in Türkiye, including the studies conducted in the same laboratory before, and have consistent results.

In this study, all three tests (SFDT, IgG, IgM) were evaluated statistically and it was found that women had a higher rate of contracting *T. gondii*. It is thought that the reason for this is that women are more likely to feed cats at home and therefore have a higher number of interactions with feces in the cleaning of cat feces and the contact with both the parasite-contaminated food in the kitchen and the garden. Studies carried out in Malatya in Türkiye, and in the Netherlands are compatible with this study [22, 23]. In the large surveillance study in which they compared the data collected from the National Health and Research Survey (NHANES) system in the USA between 1988/1994 and 1999/2000, it was stated that no significant differences were observed between the sexes [24]. In the studies conducted in Kayseri and Manisa in Türkiye, as well as in Korea and Israel, gender differences were not found either [25-28]. In a study conducted in 2008 using the National Inpatient (NIS) data in the USA, seropositivity was found to be higher in HIV-positive patients and in another study in France, males [29, 30].

In many studies, it has been shown that a significant relationship between seroprevalence and age is frequently observed [31-33]. In this study, the difference between the high Anti-*T. gondii* IgG seroprevalence in the 15-45 age group (58.1%) and the 0-1 age group (23.3%) was found to be statistically significant by univariate regression analysis (p<0.05). However, when we evaluated Anti-*T. gondii* IgM seroprevalence, it was seen that the results were not statistically significant in age groups (p>0.05). Moreover, in the univariate logistic regression analysis performed between SFDT and age groups, the difference was found to be significant (p<0.05).

The avidity test is used to diagnose infections acutely or chronically. In the test evaluated according to antibody and antigen binding concentration, weak binding detects low avidity indicating infection within 3-4 months, strong binding detects high avidity indicating exposure to the agent 6 months or before and chronic infection [34]. In this study, *T. gondii* IgG avidity test was performed in 93 patients with *T. gondii* IgG positive, 12 (13%) patients with low avidity, 15 (16%) patients with cutoff avidity, and 66 (71%) patients with high avidity. In other studies, conducted in Türkiye, a high avidity of 30% was found in Akdeniz University hospital [35], and 70.8% in the study of Yazar et al. [36] (2005). In the study conducted in Iran, 92.7% high avidity values were determined. Values seen in this study are similar to other avidity values.

In conclusion, with this study, SFDT, ELISA Anti *T. gondii* IgG - Anti *T. gondii* IgM and Avidity tests were performed on patients who applied with the suspicion of toxoplasmosis and were evaluated prospectively with demographic data. The high rates detected in Türkiye and in our study show that the disease can be overlooked, as toxoplasmosis generally continues with an asymptomatic course. At the same time, this study emphasizes the importance of screening for *T. gondii* before and during pregnancy in order to protect the population from parasites and especially to prevent the risk of congenital toxoplasmosis. The fact that there are some difficulties in the detection of the disease from a serological point of view has shown that the detection of acute infections should not be satisfied with a single test. In these cases, ELISA IgG/IgM, as well as avidity tests are required. We believe that the application of molecular tests (PCR and qPCR) will be reliable in order to prevent interventional approaches and unnecessary drug treatments. Providing treatment for this disease, which is of great importance for public health all over the world in the future and cannot be eradicated yet, will prevent the emergence of dangerous dimensions such as congenital toxoplasmosis.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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Authors' contributions to the article

B.A. and A.S.N. constructed the main idea and hypothesis of the study. B.A., A.S.N. and B.Y. developed the theory and arranged/edited the material and method section. B.A., A.S.N. and B.Y. have done the evaluation of the data in the Results section. Discussion section of the article written by B.A., A.S.N. and B.Y.

A.S.N. and B.Y. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.