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Evaluation of *Toxoplasma gondii* Molecular Test Results in Patients Admitted to Ankara City Hospital: Three-Year Retrospective Analysis

Ankara Şehir Hastanesi'ne Başvuran Hastalarda *Toxoplasma gondii* Moleküler Test Sonuçlarının Değerlendirilmesi: Üç Yıllık Retrospektif Analiz

Filiz Demirel, Füsun Kırca

Ankara Şehir Hastanesi, Tıbbi Mikrobiyoloji Kliniği, Ankara

Abstract

Aim: *Toxoplasma gondii* infects about 25-30% of the world population. Toxoplasmosis is generally asymptomatic in immunocompetent individuals, but the infection can be life threatening in congenitally infected children and immunocompromised individuals. In this study, it is aimed to analyse the molecular test results of patients suspected with toxoplasmosis, retrospectively.

Material and Method: A total of 647 clinical samples investigated for *T. gondii* DNA with real-time PCR during the three-year period between 2019 and 2022 were evaluated retrospectively. For the qualitative detection of *T. gondii*, DNA isolation and DNA amplification were performed using commercial DNA extraction kit (Qiagen, Germany) and real time PCR kit (Sacace Biotechnologies, Italy), respectively. The data on the demographic and clinical parameters of the patients were obtained from the laboratory information management system.

Results: Out of 647 patients investigated for *T. gondii* DNA with real-time PCR, 51.8% were female and the mean age of the patients was 37.03 years. Among all patients, five were positive for *T. gondii* DNA with real-time PCR and the frequency of a positive PCR result was found 0.8% of all samples analysed. The most frequently positive clinical sample was blood (80%). Among five patients with *T. gondii* DNA positivity, one was diagnosed with congenital toxoplasmosis, four were HIV-infection.

Conclusion: Fast and accurate diagnosis of toxoplasmosis especially in immunosuppressed patients is crucial for rapid and specific treatment. Further studies are needed to understand the importance of molecular tests, in addition to the serological tests, in the diagnosis of toxoplasmosis.

Keywords: *Toxoplasma gondii*, Toxoplasmosis, PCR, HIV

Öz

Amaç: *Toxoplasma gondii* dünya nüfusunun yaklaşık %25-30'unu enfekte eder. Toksoplazmoz bağışıklık sistemi sağlam bireylerde genellikle asemptomatiktir, ancak enfeksiyon konjenital enfeksiyonlu çocuklarda ve immunsupresif bireylerde hayatı tehdit edici olabilir. Bu çalışmada toksoplazmozdan şüphelenilen hastaların moleküler test sonuçlarının geriye dönük olarak incelenmesi amaçlandı.

Gereç ve Yöntem: Hastanemizde 2019-2022 yılları arasındaki üç yıllık dönemde gerçek zamanlı PCR ile *T. gondii* DNA araştırılan toplam 647 klinik örnek geriye dönük olarak değerlendirildi. *T. gondii*'nin kalitatif tespiti için ticari bir DNA ekstraksiyon kiti (Qiagen, Almanya) ve real time PCR kiti (Sacace Biotechnologies, İtalya) kullanılarak DNA izolasyonu ve DNA amplifikasyonu yapıldı. Hastaların demografik ve klinik parametreleri ile ilgili veriler laboratuvar bilgi yönetim sisteminden elde edildi.

Bulgular: Real-time PCR ile *T. gondii* DNA'sı araştırılan 647 hastanın %51,8'i kadındı ve hastaların ortalama yaşı 37,03 idi. Hastalardan beşinde, real-time PCR ile *T. gondii* DNA pozitif tespit edildi ve PCR pozitifliği analiz edilen tüm örnekler içinde %0,8 bulundu. En fazla pozitiflik tespit edilen klinik örnek kandı (%80). *T. gondii* DNA pozitifliği saptanan beş hastadan birine konjenital toksoplazmoz, dördünde HIV enfeksiyonu vardı.

Sonuç: Özellikle bağışıklık sistemi baskılanmış hastalarda toksoplazmozun hızlı ve doğru teşhisi, hızlı ve spesifik tedavi için çok önemlidir. Toksoplazmoz tanısında serolojik testlere ek olarak moleküler testlerin öneminin anlaşılabilmesi için ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Toxoplasma gondii*, Toksoplazmoz, PZR, HIV

Corresponding *(İletişim)*: Dr. Filiz Demirel. Ankara Şehir Hastanesi, Tıbbi Mikrobiyoloji Bölümü, Ankara, Turkey
E-mail *(E-posta)*: dr.filiz.demirel@gmail.com
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INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular protozoan that can infect humans and warm-blooded animals including mammals and birds. *T. gondii* infects nearly a third of the world's population and the prevalence of infection varies among countries.[1,2] Toxoplasmosis may develop through oral ingestion of infective sporulated oocysts in food or water and tissue cysts in undercooked or raw meat, organ transplantation, blood transfusion, and transplasental routes.[3] In Immunocompetent individuals, acute toxoplasmosis is asymptomatic in the majority of the patients. It may occasionally present with flu-like symptoms, fever, cervical lymphadenopathy, myalgia, asthenia and chorioretinitis.[4] Congenital toxoplasmosis occurs mainly after primary infection of pregnant woman and has a broad spectrum of clinical manifestations including central nervous system involvement.[5] *T. gondii* is also a common opportunistic pathogen especially in patients with immunodeficiency such as AIDS, organ transplantation, etc. In immunosuppressed patients, reactivation of a latent infection may result in severe and potentially fatal complications.[6,7] Additionally, in the past few decades, the possible relationship between toxoplasmosis and neuropsychiatric diseases has been the subject of great interest. Alzheimer's disease, schizophrenia, obsessive-compulsive disorder, and multiple sclerosis are some of the disorders that is thought to be related to toxoplasmosis.[8]

Because of the non-specific symptoms of toxoplasmosis, the diagnosis is predominantly depends on the serological tests detecting specific antibodies to *T. gondii*. Enzyme-linked immunosorbent assays (ELISA), indirect fluorescent antibody test (IFAT), and indirect haemagglutination assays (IHA) are some of the serological methods with variable sensitivity and specificity rates for detection of toxoplasmosis. In recent years, DNA-based molecular diagnostic methods have been found beneficial for more effective and accurate detection of toxoplasmosis.[9] Because rapid and definitive diagnoses are needed especially in immunosuppressed patients, molecular techniques are essential.[10]

In this study, it is aimed to analyse the PCR results of patients suspected with toxoplasmosis, retrospectively. To our knowledge, there are a few studies investigating the molecular detection of *T. gondii*, although there are many studies investigating the seroprevalence of toxoplasmosis in our country.

MATERIAL AND METHOD

Ethics committee approval dated 27.04.2022 and numbered E2-22-1770 was obtained from Ankara City Hospital Ethical Committee of Non-Invasive Clinical Research. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Groups

In the study, a total of 647 clinical samples (peripheral blood, cerebrospinal fluid, amniotic fluid, etc.) investigated for *T. gondii* DNA with real-time PCR during the three-year period between 2019 and 2022 were evaluated retrospectively. The data on the demographic and clinical parameters of the patients were obtained from the laboratory information management system.

Molecular Detection of *T. gondii* DNA

For molecular testing, the whole peripheral blood samples were collected to a tube with 6% EDTA solution, cerebrospinal fluid obtained by lumbar puncture and amniotic fluid obtained during amniocentesis by the standard procedure and collected to a sterile Eppendorf tube. DNA isolation was performed using a commercial DNA extraction kit (Qiagen, Germany), according to the manufacturer's instructions. DNA amplification was performed using a commercial real time PCR kit for the qualitative detection of *T. gondii* (Sacace Biotechnologies, Italy. The analytical sensitivity of *Toxoplasma gondii* Real-TM PCR kit is 400 *T. gondii* DNA copies/ml) according to the manufacturer's instructions. The samples were considered positive if Ct values detected in the FAM/Green and Yellow/HEX channel were less than the boundary Ct values (≤38) for these channels.

Serological Diagnoses

The presence of anti-*T. gondii* IgM ve IgG antibodies were detected by enzyme linked immunosorbent assay using a commercial kit (Atellica IM Toxoplasma M/G, Siemens Healthcare Diagnostic, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS 20 (IBM Inc, New York, USA). Chi-square test was used to compare the gender, age group and clinical distributions between positive and negative cases, and $p<0.05$ was considered statistically significant. Descriptive statistics was given as percentage and frequency.

RESULTS

Out of 647 patients investigated for *T. gondii* DNA with real-time PCR, 335 (51.8%) were female, 312 (48.2%) were male. The mean age of the patients was 37.03 years. The distribution of clinical samples was as 545 (84.2%) blood, 52 (8.1%) CSF and 50 (7.7%) amniotic fluid.

Among all patients, only five were positive for *T. gondii* DNA with real-time PCR and the frequency of a positive PCR result was found 0.8% of all samples analysed. The most frequently positive clinical sample was blood (80%), followed by CSF (20%). PCR positivity was not detected in any of the amniotic fluid samples. Among these five patients, four (80%) were male with a mean age of 45.25 years. All of male patients had HIV infection; three of them had encephalitis; one had pneumonia. Toxoplasmosis prophylaxis status of these immunosuppressed patients with positive PCR results were unknown. Among five patients with *T. gondii* DNA positivity, one (20%) was a refugee female child with an age of two and diagnosed with congenital toxoplasmosis.

Demographic and serological characteristics of the patients with *T. gondii* DNA positivity were given in **Table 1**. According to serologic test results for *T. gondii*, anti-Toxoplasma IgM test was negative and anti-Toxoplasma IgG test was positive in all patients with PCR positivity. At the same time, Sabin Feldman Dye test was found positive and high Toxoplasma IgG avidity was detected in the patients undergoing these tests.

DISCUSSION

Toxoplasmosis is an important parasitic disease in which prevalence rates vary with differences such as geographical factors, socio-cultural status, dietary habits, etc. Epidemiological data indicate that approximately 30% of the world's population is infected with this protozoon.[11]

In Turkey, many seroprevalence studies reporting antibody levels against *T. gondii* have been conducted. Esenkaya Taşbent et al. reported that anti-Toxoplasma IgM and IgG seropositivities in different patient groups were 2.4% and 24.1%, respectively.[11] Similarly, Maçin et al. reported the seropositivity rates of anti-*T. gondii* IgM and IgG antibodies as 2.4% and 29.5%, respectively.[12] Malatyalı et al. found that the overall rate of anti-Toxoplasma IgG positivity was 31.5% and anti-Toxoplasma IgM positivity was 1.6%. The authors emphasized that it is important to use more than one method together in the laboratory diagnosis of toxoplasmosis.[13] Similarly, Aydın Turkoğlu et al. detected anti-Toxoplasma IgM and IgG seropositivities as 1.2% and 21%, respectively.[14] In another study, Alver et al. found anti-Toxoplasma IgM and IgG seropositivities as 1.7% and 37.9%, respectively. It was also reported in the same study that the seropositivity of *T. gondii* IgG was higher in women belonging to the childbearing age group, suggesting that screening and diagnosis of *T. gondii* serology in women at childbearing age are important.[15] It is well known that the acute *T. gondii* infection during pregnancy is one of the most important causes of perinatal mortality and morbidity. Primary toxoplasmosis in pregnancy may cause congenital toxoplasmosis, which is characterized with central nervous system involvement (CNS).[5,16,17] In a study conducted by Hansu et al, *T. gondii* seropositivity was detected more common in the refugee pregnant women (in Turkey) than in the local residents in all age groups, and the difference was found statistically significant.[17] In our study, one of the patients whose molecular testing was positive for *T. gondii* DNA was a refugee child at the age of two and the child was diagnosed with congenital toxoplasmosis with CNS involvement. Probably, the child's mother was not followed up for *T. gondii* serology during pregnancy.

T. gondii can cause severe and life-threatening opportunistic infections such as encephalitis and pneumonia in immunosuppressed patients especially in HIV-positive individuals while the parasite causes asymptomatic chronic persistent infections in healthy immunocompetent individuals. In HIV-infected patients, reactivation of latent infection leads to symptomatic disease.[18] In a study conducted by Şenoğlu et al., anti-Toxoplasma IgG positivity was detected in 43.5% of HIV-infected patients, whereas anti-Toxoplasma IgM positivity was not detected. The authors emphasized that in these patient groups, it is important to apply the prevention measures from toxoplasmosis and to give a prophylactic treatment in necessary conditions.[19] In a retrospective study determining the incidence and laboratory characteristics of primary *T. gondii* infection in HIV-infected individuals, seroconversion was observed in 1.2% of the patients.[20] Nissataporn et al. reported that toxoplasmic encephalitis incidence is 33% in HIV-infected patients who were seropositive for *T. gondii* and did not use prophylaxis for toxoplasmosis in the pre-antiretroviral period.[21] In our study, four of the patients whose molecular testing were positive for *T. gondii* DNA were HIV-infected individuals. In all these patients, anti-Toxoplasma IgG was positive whereas anti-Toxoplasma IgM was negative. In three of them, Toxoplasma IgG avidity test were also high reactive. All of the patients were symptomatic; three of them had encephalitis, and one had pneumonia.

Although there are many studies investigating the seroprevalence of *T. gondii*, the number of studies based on the molecular detection of the parasite is very few. In a multicentric retrospective study conducted by Robert-Gangneux et al. emphasized the importance of molecular diagnosis of toxoplasmosis in immunosuppressed patients (IP), *T. gondii* detected higher in non-HIV IP patients than in HIV-infected patients. Regular PCR follow-up of IP patients (especially allogeneic hematopoietic stem cell transplant patients) was recommended to guide prevention measures.[22] As Filisetti et al. reported, the detection of *T. gondii* DNA in amniotic fluid by using PCR is crucial for the prenatal diagnosis of congenital toxoplasmosis. Another multicentric study by Filisetti et al. showed the diagnostic sensitivity and specificity of PCR assays for amniotic fluid samples were to be 86% and 100%, respectively.[23] In our study, *T. gondii* DNA was not detected

Table 1. Characteristics of the patients with *T. gondii* DNA positivity.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Male	Male	Female
Age	34	40	51	56	2
Diagnose	HIV+ Pneumonia	HIV+Encephalitis	HIV+Encephalitis	HIV+Encephalitis	Congenital toxoplasmosis/ epilepsy
Anti-Toxoplasma IgM	0.81 (Neg)	0.21 (Neg)	0.10 (Neg)	0.10 (Neg)	4.21 (Neg)
Anti-Toxoplasma IgG	>700 (Pos)	>700 (Pos)	16.80 (Pos)	16.80 (Pos)	>700 (Pos)
Toxoplasma IgG Avidity Test	0.582 (High)	-	0.458 (High)	0.461 (High)	-
Sabin Feldman Dye Test	-	Positive (1/4)	Positive (1/16)	Positive (1/16)	Positive (1/16)
Positive PCR Sample	Blood	CSF	Blood	Blood	Blood

in amniotic fluid samples investigated. In another study on the molecular diagnosis of *T. gondii*, among 807 samples analysed 26.9% were found to be positive and in the patients symptomatic toxoplasmosis confirmed by clinical diagnosis.[24] In a study on the evaluation of serologic and molecular test results of toxoplasmosis suspected patients, anti-*T. gondii* DNA was found to be positive in patients with and without positive anti-*T. gondii* IgM and IgG results.[25] Similarly, in our study, five patients with *T. gondii* DNA positivity, anti-*T. gondii* IgG tests were positive while anti-*T. gondii* IgM tests were negative.

CONCLUSION

Fast and accurate diagnosis of toxoplasmosis in immunosuppressed patients is crucial for rapid and specific treatment. Molecular detection of *T. gondii* is an important diagnostic method especially in symptomatic HIV-infected patients because of the low antibody response. In these patients, Toxoplasma DNA should be investigated besides the presence of anti-Toxoplasma IgG, even if anti-Toxoplasma IgM is negative. Further studies are needed to understand the importance of molecular tests, in addition to the serological tests, in the diagnosis of toxoplasmosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval dated 27.04.2022 and numbered E2-22-1770 was obtained from Ankara City Hospital Ethical Committee of Non-Invasive Clinical Research.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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