Prevalence of IgG and IgM anti-*Toxoplasma gondii* Antibodies in Blood Donors at Urmia Blood Transfusion Organization, Iran

Iran Urmia Kan Bankası Kan Donorlerinde *Toxoplasma gondii* IgG ve IgM Antikor Prevalansı

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ABSTRACT

Objective: The importance of toxoplasmosis lies in its global spread, opportunistic nature, and causative role in abortion or irreparable adverse effects on infants of infected pregnant women. *Toxoplasma gondii* has different transmission routes to humans, including blood transfusion. The objective of the present study was to evaluate the prevalence of IgG and IgM antibodies specific for *T. gondii* in blood donors at the Urmia Blood Transfusion Organization in west Azerbaijan, Iran.

Methods: The present analytical, descriptive study evaluated the plasma of 270 randomly selected blood bags donated in 2013. The enzyme-linked immunosorbent assay (ELISA) test was employed to measure anti-*T. gondii* IgG and IgM antibodies.

Results: The results of the ELISA test showed that 102 samples (37.8%) from 270 blood bags had IgG antibodies in their plasma and none of them were IgM-positive, whereas 98 were men and four were women.

Conclusion: Any increase in the level of IgM antibodies indicates the presence of an acute disease because the parasite is inside white blood cells and contaminates blood transfusion. Fortunately, all samples were IgM-negative. However, a province-wide seroepidemiological study is required for the Blood Transfusion Organization to consider including screening for anti-*T. gondii* antibodies in its screening programs.

Keywords: Prevalence, *toxoplasma gondii*, Blood donor, Urmia, Iran

Received: 27.08.2016 **Accepted:** 14.12.2016

ÖZ

Amaç: Toksoplazmozun önemi enfekte hamile kadınların bebeklerinde telafisi olanaksız olumsuz etkilerde veya düşük olayındaki nedensel rolüne, global yayılımına ve fırsatçı doğasına dayanmaktadır. *Toxoplasma gondii*'nin kan transfüzyonu dahil, farklı insanlara bulaşma şekli vardır. Bu çalışmanın amacı İran'ın batı Azerbaycan eyaletinde yer alan Urmia Kan Transfüzyon Kurumundaki kan donörlerinde *T.gondii*'ye özgü IgG ve IgM antikorlarının prevalansını değerlendirmektir.

Yöntemler: Mevcut analitik tanımlayıcı çalışmada, 2013 yılında bağışlanan kan torbalarından rastgele seçilmiş 270 plazma değerlendirildi. Anti-T.gondii IgG ve IgM antikorlarını ölçmek amacıyla enzim bağlantılı imünosorbent analizi (ELISA) kullanıldı.

Bulgular: ELISA testi sonuçlarına göre 270 kan torbasından alınan 102 (%37,8) numunenin plazmasında IgG antikorları vardı ve bunların hiçbirisi IgM-pozitif değildi. 98 numune erkek donörden alınmışken, 4'ü kadından alındı.

Sonuç: IgM antikorlarının seviyesindeki herhangi bir artış akut bir hastalığın varlığını göstermektedir, çünkü parazit beyaz kan hücrelerinin içindedir ve kan transfüzyonunu kirletir. Neyse ki, tüm numuneler IgM-negatifti. Ancak, Kan Transfüzyon Örgütünün kendi tarama programlarına anti-*T-Gondii* antikorları taramasını dahil etmeyi düşünmesi için, il genelinde bir seroepidemiyolojik çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Prevalans, *toxoplasma gondii*, kan donörü, Urmia, İran

Geliş Tarihi: 27.08.2016 **Kabul Tarihi:** 14.12.2016

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DOI: 10.5152/tpd.2017.5066
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INTRODUCTION

Toxoplasmosis is an important zoonotic, globally distributed, parasitic disease caused by an obligate intracellular protozoan called *Toxoplasma gondii*. Cats are the major hosts and spread oocysts, as the resistant form of the parasite, through their feces. Consequently, the consumption of contaminated water, fruits, and vegetables would infect humans and other hosts (1, 2). The contamination of humans and other hosts is reported worldwide, but the prevalence of infection depends on several factors, such as eating behavior, age, and geographical location (3). Disease transmission through the placenta is another cause of human infection, and if the mother is infected with toxoplasmosis during pregnancy, there is a risk of fetal infection. This can cause complications such as microcephaly, hydrocephalus, mental retardation, jaundice, abortion, brain calcification, blindness, and fetal death (4). This parasite can also be transmitted from IgM-positive people to negative recipients through whole blood transfusion or other blood products, such as leukocytes (5, 6).

Patients with aplastic anemia, thalassemia, and immunodeficiency are the recipients of blood transfusion, and considering their weak immune systems and the opportunistic nature of this parasite, the injection of infected blood would cause irreversible complications (4). The prevalence of toxoplasmosis in blood donors is different in different regions of Iran and the world. Approximately 60% of Egyptian blood donors were IgG-positive, and the prevalence of toxoplasmosis among the study population in blood donors in the northern region of Jordan was 35.5%. The prevalence in males and females was 35.8% and 34.3%, respectively. Samples from 385 healthy blood donors from central Turkey were examined for anti-*T. gondii* antibodies using the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). The seroprevalence of the anti-*T. gondii* IgG and IgM antibodies was 19.5% and 2.33% with IFAT, respectively, and 20.25% and 2.33% with ELISA, respectively (7-9). Different studies conducted in Iran have also produced different results. A total of 540 blood samples was randomly collected from healthy blood donors in the Hamadan Blood Transfusion Center. All samples were examined for IgG and IgM antibodies using ELISA. The results were analyzed in relation to epidemiological factors such as age, sex, occupation, and some toxoplasmosis risk factors. Approximately 518 participants in this study were males. In total, 294 (54.4%) of the studied population were IgG-positive and 10 (1.9%) were IgM-positive antibodies, whereas 25% of blood donors in Zahedan in the south of Iran were IgG-positive and all cases were IgM-negative (10, 11).

Unfortunately, blood bags in the Iranian Blood Transfusion Organization are not screened for this parasite. Thus, the present study was conducted to determine if it is necessary to recommend screening for *T. gondii* antibodies. The present study also evaluated *T. gondii* antibodies in donors attending the Urmia Blood Transfusion Organization.

METHODS

This study was approved by the ethics committee of Urmia Medical Sciences University (Code No: 68), and we had no contact with donors. First, the information of blood bags, collected from donors in the Urmia Blood Transmission Organization, was registered. The bags were then centrifuged in Taleghani Hospital (affiliated with Urmia Medical Sciences University), and the plasma bags were frozen at -20°C until the day of the experiment. The present cross-sectional study, with the target population of blood donors attending the Urmia Blood Transfusion Organization in 2013, analyzed 270 blood bags using the ELISA test in the serology section of the Taleghani Hospital laboratory. The ELISA test was conducted using the Pishtaz-Teb ELISA test kit, with which the IgM titer was determined based on antibody capture. The plate wells were covered with anti-human IgM antibodies, and the samples were diluted to 1:100 and were poured into the wells. All IgM-positive serum samples were bound to antibodies at the bottom of the wells. The free antibodies were separated after an initial rinsing with a solution containing phosphate buffer, tween, and distilled water. The conjugation kit containing antigens of *T. gondii* conjugated with horseradish peroxidase was added. The IgG titer was determined based on the binding of *T. gondii* antigens to the wells. If anti-*T. gondii* antibodies exist, they bind to the antigens at the bottom of the wells. They were then added to the conjugated wells. After another rinsing, a dye consisting of tetra-methyl benzidine and hydrogen peroxide was poured into the wells. The intensity of the blue color was proportional to the number of immune complexes formed in the wells. The addition of a 1 N HCl stopping solution turns the blue color to yellow, which was then recorded using light absorption at a wavelength of 450 nm (ELISA Lab system reader, Finland).

According to the kit manufacturer instructions, an IgM antibody titer of ≥1 is positive and <0.9 is negative. In addition, IgG antibody values ≥10 mL/IU were considered positive, and values <10 mL/IU were negative.

The ELISA test results and questionnaires were analyzed using the SPSS 16 software and t-test, respectively. The index for positive/negative answers was calculated using the following formula: cut-off index = optical density of sample/cut-off value.

RESULTS

Of the 270 blood samples, 261 (96.7%) belonged to men and 9 (3.3%) belonged to women. The mean age of the blood donors was 34 years, with the youngest and oldest being 18 and 62 years respectively. The ELISA test results showed that 102 samples (37.8%) were IgG-positive, of which 98 samples belonged to men (37.5%) and four to women (44.5%). ELISA test results were evaluated as positive/negative according to the controls (Table 1 and 2). The complete demographic information is presented in Table 3. IgM antibody was not detected in any of the samples.

DISCUSSION

T. gondii is a globally distributed parasite, and domestic cats and the cat family are its definitive hosts. Considering the large number of cats in urban and rural areas, environmental contamination with oocysts, and the possibility of human infection, it is worth evaluating serum IgM and IgG antibodies. The high anti-*T. gondii* antibody titer is directly related to the population of cats (2). As it is uncommon to have cats as pets in Iranian homes, infection is indirectly transmitted through other routes.

Table 1. Statistical indicators of light absorption: IgM

Negative control	Positive control	Cut-off value	SD	Min	Max	Average
0.058	1.438	0.28	0.0076	0.053	0.089	0.069

Table 2. Statistical indicators of light absorption: IgG

Negative control	Positive control	Cut-off value	SD	Min	Max	Average
0.023	0.185	2.86	0.321	0.19	1.52	0.47

Table 3. Demographic data of blood donors

Variable	Personal characteristics	Frequency	Percentage
Sex	Female	9	3.3%
	Male	261	96.7%
Household location	Rural	54	20%
	Urban	216	80%
Literacy	Illiterate	4	1.5%
	Below high school diploma	70	26%
	High school diploma	89	33%
	University	109	39.5%

Furthermore, toxoplasmosis is also transmitted through blood transfusion (1). Considering the fact that blood recipients are often people with suppressed immune systems, those who receive dialysis, and children with aplastic anemia or thalassemia, it is essential to screen donated blood. The findings of the present study showed that from the total 270 collected samples, 102 were IgG-positive (37.8%) and none were IgM-positive. Other studies, such as Abdolgani's in Jordan, reported 35.8% IgG-positive cases in north Jordan, whereas no samples were IgM-positive (8). Blood samples were collected from 493 non-pregnant women between 2009 and 2012. The presence of antibodies to *T. gondii* was determined using the latex agglutination test. Thirteen of 493 (2.6%) samples were found to be seropositive for *T. gondii* infection. There was no age dependence in the prevalence (12). Another seroepidemiological study in Karnataka, India, reported 3.20% and 6.3% of samples to be positive for *T. gondii* IgG and IgM, respectively (13). The results of this study revealed that 114 (45.2%) cases were anti-*T. gondii* IgG-positive, 26 (10.3%) cases were anti-*T. gondii* IgM-positive, and 17 (6.7%) cases were anti-*T. gondii* IgG- and IgM-positive. In the control group, 92 (36.5%) cases and 15 (6%) cases were revealed to be seropositive for IgG and IgM, respectively (14). The cross-sectional study conducted by Ormazdi et al. (15) on 250 samples taken from the Iranian Blood Transfusion Organization reported IgG and IgM in 52.8% and 3.6% cases, respectively. Siegel et al. (5) reported toxoplasmosis in 40 patients with leukemia after receiving contaminated packed white blood cells (leukopheresis) in 1971. The recipients of heart, lungs, and bone marrow transplants can also be at the risk of toxoplasmosis through blood transfusion. Caner et al. (16) conducted another study in Turkey on 40 patients with kidney transplants, and the serum test results in 67.5% cases were positive. In another study in pregnant women in Urmia, the seroprevalence of anti-*T. gondii* antibody IgG and IgM was 28.32% and 1.44%, respectively, had the highest toxoplasmosis rate increased by age. This may be because of the increased risk of exposure to infection source with age (17). In another study by Sarkari et al. (18), the prevalence of anti-*T. gondii* antibodies among blood donors from five blood centers in Fars province were analyzed. Anti-*T. gondii* antibodies were detected in the sera of 286 out of 1480 blood donors, indicating a seroprevalence of 19.3% in this population. From these, 182 (12.3%) were only IgG-positive, 81 (5.47%) were only IgM-positive, and 23 (1.6%) were both IgG- and IgM-positive (18).

CONCLUSION

The presence of anti-*T. gondii* antibodies in the blood donors of the present study highlights the importance of further investigations into this area. The Blood Transfusion Organization in Iran does not screen donated blood for anti-*T. gondii* antibodies. Therefore, similar studies can provide invaluable information to the Blood Transfusion Organization in preparing appropriate blood screening programs for anti-*T. gondii* antibodies. The present study evaluated donated blood after examining the donors by a physician and performing routine blood tests.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Urmia Medical Sciences University (Decision No: 68).

Informed Consent: Not required in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - H.T., H.A.; Design - H.T.; Supervision - H.A.; Resource - Urmia University Department of Parasitology, Taleghani Hospital; Materials - Urmia University Department of Parasitology, Taleghani Hospital; Data Collection and/or Processing - Urmia University Department of Parasitology, Taleghani Hospital; Analysis and /or Interpretation - H.A.; Literature Search - H.T.; Writing – H.A., J.M.; Critical Reviews - H.G., H.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study financially supported by Research Deputy of Urmia University of Medical Sciences (Project No: 2012/897).

Etik Komite Onayı: Bu çalışma için etik komite onayı Urmia Tıbbi Bilimler Üniversitesi'nin etik kurulundan alınmıştır (Karar No: 68).

Hasta Onamı: Bu çalışma için hasta onamına gerek yoktur.

Hakem Değerlendirmesi: Dış Bağımsız.

Yazar Katkıları: Fikir - K.H.T., H.A.; Tasarım - K.H.T.; Denetleme - H.A.; Kaynaklar - Urmia Universitesi Parazitoloji Anabilim Dalı, Taleghani Hastanesi; Malzemeler - Urmia Universitesi Parazitoloji Anabilim Dalı, Taleghani Hastanesi; Veri Toplanması ve/veya işlemesi - Urmia Universitesi Parazitoloji Anabilim Dalı, Taleghani Hastanesi; Analiz ve/veya Yorum - H.A.; Literatür Taraması - K.H.T.; Yazıyı Yazan - H.A., J.M.; Eleştirel İnceleme - H.G., H.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma Urmia Tıbbi Bilimler Üniversitesi Araştırma Kurulu tarafından desteklenmiştir (Proje No: 2012/897).

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