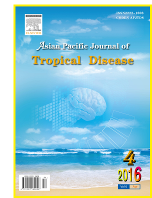
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Parasitological research doi: 10.1016/S2222-1808(15)61027-1 ©2016 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Do we need to screen Egyptian voluntary blood donors for toxoplasmosis?

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ARTICLE INFO

Article history:
Received 14 Feb 2016
Received in revised form 8 Mar 2016
Accepted 3 Apr 2016
Available online 19 Apr 2016

Keywords:
Toxoplasma gondii
Blood donors
ELISA
PCR

ABSTRACT

Objective: To determine the value of voluntary blood donors screening in diagnosing asymptomatic toxoplasmosis in an attempt to reduce the risk of this infection in Egyptian immunocompromised recipients.
Methods: Serum samples from 300 healthy voluntary blood donors were analyzed for anti-*Toxoplasma* antibodies [immunoglobulin G (IgG) and immunoglobulin M (IgM)] using ELISA and detection of *Toxoplasma gondii* (*T. gondii*) parasitemia was done by real-time quantitative PCR (qrtPCR).
Results: Frequency of *T. gondii* infection in 300 healthy blood donors was 101 (33.67%), 10 (3.33%), 18 (6.00%) by ELISA IgG, IgM and qrtPCR, respectively. It was found that 8 of 18 (44.4%) donor samples positive by qrtPCR contained IgM anti-*T. gondii*, conversely 8 of 10 (80%) IgM-positive samples were positive for *T. gondii* DNA. There was a highly significant increase in detection of recent *Toxoplasma* infection using PCR over IgM ELISA by 55.6%. At the same time, *T. gondii* parasitemia was detected in 11 of 101 (10.90%) donor samples positive by IgG ELISA and in 7 of 199 (3.50%) negative donor samples for anti-*T. gondii* IgG antibodies. On the other hand, the negative results obtained by both qrtPCR and ELISA in 192 (64%) subjects ruled out the infection in those donors.
Conclusions: It might be appropriate to include the screening of blood and blood products for *T. gondii* in the pre-transfusion blood testing schedule in Egypt. Also, molecular screening should be carried out on the blood being transfused to immunocompromised patients.

1. Introduction

A number of parasitic agents have been reported to be transmitted by transfusion, including *Toxoplasma gondii* (*T. gondii*), the etiologic agent of toxoplasmosis[1]. *T. gondii* is an obligate intracellular parasite that infects nearly one third of the world's population[2]. Infection can be acquired by ingestion of tissue cysts in raw or undercooked infected meat, ingesting of food or water contaminated with sporulated oocysts shed in the feces of an infected cat, blood transfusion, organ transplantation and congenitally, across the placenta from the mother to the fetus[3]. Most infected immunocompetent individuals are asymptomatic and the prevalence of *T. gondii* as reflected by seropositivity for immunoglobulin G (IgG) anti-*T. gondii* antibodies varies widely among different regions of the globe from a low of 4.1% in Thailand to 79% in Brazil[4,5].

Transfusion-transmitted *T. gondii* infection depends on several factors: asymptomatic parasitemic individual qualified as a blood donor, the ability of the parasite to survive on stored donated blood up to 50 days at 4 °C, thus refrigeration of blood units during storage cannot prevent transmission, and infected blood is transfused in a sufficient dose to a susceptible patient. This poses a great risk to the recipients of blood, especially immunocompromised patients and those who need regular, frequent and multiple transfusions[1].

Screening of voluntary donors has been based mainly on serological tests, including ELISA, indirect immunofluorescence antibody test, indirect heamgglutination test and latex agglutination test for detection of anti-*Toxoplasma* IgG and /or immunoglobulin M (IgM) antibodies in

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The study protocol was performed according to the Helsinki declaration and approved by Research Ethics Committee of the Faculty of Medicine, Benha University, Egypt. All donors included in the study were informed of the study objectives and a written signed consent was taken from each one of them.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

serum[3,6-9]. However, these techniques sometimes fail to detect specific anti-*T. gondii* antibodies during the active phase of infection[10].

Molecular techniques have been used to improve the laboratory diagnosis of toxoplasmosis by amplification of *Toxoplasma* DNA sequences present in various clinical samples including human blood with high sensitivity and specificity[11-14]. The applicability of these assays aimed at detecting the different targets. Among them, two targets are more frequently used because of their high sensitivity and specificity. One is the 529 bp sequence, which has 200–300 copies in the genome of *T. gondii* [15]. The other is the *B1* gene that has 35 copies in the genome and is conserved in different parasite strains (B1)[11]. The number of copies of the DNA target in *T. gondii* genome is an essential factor that affects the sensitivity of many PCR protocols.

In Egypt, donated blood samples were screened for HIV, hepatitis B and C and syphilis and were not screened for other potentially pathogenic organisms such as *Toxoplasma*. So, the present study was conducted to determine the value of voluntary blood donors screening for diagnosing asymptomatic toxoplasmosis in an attempt to reduce the risk of this infection among Egyptian immunocompromised recipients.

2. Materials and methods

2.1. Subjects

This cross-sectional study was performed on 300 healthy voluntary male blood donors from the blood bank in Faculty of Medicine, Benha University in the period from September 2014 to January 2015. Their ages ranged from 18 to 40 years (Mean ± SD = 22.8 ± 4.3). They satisfied the following criteria: normal full blood count, normal blood pressure, no acute infection, negative hepatitis B surface antigen, negative anti-hepatitis C virus antibodies, negative anti-HIV antibodies, and a negative *Treponema pallidum* haemagglutination test. Blood samples were collected from all donors and sera were obtained by centrifugation, frozen down and kept stored at -20 °C until analyzed.

2.2. Serological techniques

All serum samples were analyzed for both anti-*T. gondii* IgG and IgM antibodies by the commercially available enzyme immunoassays "*Toxoplasma* IgG & *Toxoplasma* IgM" kits (DRG International, Inc., USA) following the manufacturer's instructions. A positive IgG test with a negative IgM test in a donor was interpreted as a chronic infection. A positive IgM test with or without a positive IgG test in a donor was interpreted as the probability of recent infection.

2.3. T. gondii DNA extraction

T. gondii DNA was extracted from serum specimens using QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The extracted DNA concentration was confirmed through measurement by NanoDrop 2000c spectrophotometer. Readings were taken at wave lengths of 260 and 280 nm. The concentration of DNA sample was measured = 50 µg/mL × A260.

2.4. Detection of T. gondii B1 gene by real-time quantitative PCR (qrtPCR)

Amplification was performed using two primers with the following sequences; 5'-AACGGGCGAGTAGCACCTGAGGAGA-3' and 5'-TGGGTCTACGTCGATGGCATGACAAC-3' which specifically amplified 115 bp sequence of *T. gondii B1* gene[11]. The master mix used in amplification was SuperReal PreMix Plus (SYBR Green) TIANGENE BIOTECH. DNA extract (2 µL) were used in 20 µL reaction volume.

In ABI7900 fast real time machine (Applied Biosystem, USA) the design of the plates was to detect the *T. gondii* in each sample using its specific primers in addition to using the glyceraldehyde phosphate dehydrogenase gene as an internal positive control with the following sequences; 5'-TGATGACATCAAGAAGGTGGTGAAG-3' and 5'-TCCTTGGAGGCCATGTGGGCC AT-3'. The program conditions were 95 °C, 15 min for initial denaturation followed by 35 cycles of 95 °C, 30 s; 54 °C, 1 min; 72 °C, 30 s. Positive and negative controls were used for each run. The positive control was *T. gondii* DNA extracted from RH strain tachyzoites, kindly provided by the Department of Zoonotic Diseases, Veterinary Research Division, National Research Center, Giza, Egypt, while negative control was blank containing all PCR reagents without DNA.

The cycle threshold value, indicative of the quantity of target gene at which the fluorescence exceeds a preset threshold, was determined. This threshold was defined as 20 times the standard deviation of the baseline fluorescent signal. After reaching the threshold, the sample was considered positive.

2.5. Statistical analysis

The positive findings were expressed as a percentage, and the statistical analysis was carried out using *Chi*-square test (χ^2) to clarify statistically significant differences. $P < 0.05$ was considered statistically significant and $P < 0.001$ was considered statistically highly significant.

2.6. Ethical considerations

The study protocol was approved by Research Ethics Committee of the Faculty of Medicine, Benha University, Egypt. All donors included in the study were informed of the study objectives and a written signed consent was taken from each one of them.

3. Results

Out of 300 asymptomatic blood donors, 93 had anti-*Toxoplasma* IgG antibodies, 2 had anti-*Toxoplasma* IgM antibodies and 8 had both IgG and IgM. The results revealed that anti-*Toxoplasma* IgG antibodies were prevalent among those donors (101/300) with values of

33.67% reflecting latent toxoplasmosis. On the other hand, the presence of IgM (10/300) in donors with values of 3.33% reveals a recent toxoplasmic infection. *T. gondii* specific DNA amplification using qrtPCR revealed that 18 donors' samples (6.00%) were positive.

It was found that 8 of 18 (44.4%) donors' samples positive by PCR contained IgM anti-*T. gondii*, conversely 8 of 10 (80%) IgM-positive samples were positive for parasite DNA. There was a highly significant increase in detection of recent *Toxoplasma* infection using PCR over IgM ELISA by 55.6% ($P < 0.001$). At the same time, *T. gondii* parasitemia was detected in 11 of 101 (10.9%) donor samples positive by IgG ELISA and in 7 of 199 (3.5%) negative donor samples for anti-*T. gondii* IgG antibodies. On the other hand, the negative results obtained by both qrtPCR and ELISA in 192 (64%) subjects ruled out the infection in those donors (Tables 1 and 2).

Table 1

Efficacy of qrtPCR in the detection of recent *T. gondii* infection in 300 asymptomatic blood donors compared to IgG ELISA.

IgG ELISA	qrtPCR				Total		*P*-value
	Positive		Negative				
	No.	%	No.	%	No.	%	
Positive	11	3.67	90	30.00	101	33.67	0.01*
Negative	7	2.33	192	64.00	199	66.33	
Total	18	6.00	282	94.00	300	100.00	

*: Significant detection of recent *Toxoplasma* infection using PCR.

Table 2

Efficacy of qrtPCR in the detection of recent *T. gondii* infection in 300 asymptomatic blood donors compared to IgM ELISA.

IgM ELISA	qrtPCR				Total		*P*-value
	Positive		Negative				
	No.	%	No.	%	No.	%	
Positive	8	2.67	2	0.66	10	3.33	0.000 1**
Negative	10	3.33	280	93.34	290	96.67	
Total	18	6.00	282	94.00	300	100.00	

**: Significant increase in detection of recent *Toxoplasma* infection using PCR.

4. Discussion

Toxoplasmosis is usually asymptomatic in the immunocompetent subjects while, it is the most frequent protozoal opportunistic infection in immunocompromised individuals resulting in the dissemination of the infection that causing serious complications in the form of encephalitis, myocarditis, and pneumonitis with higher mortality rates[16]. In Egypt with the availability of advanced medical facilities for bone marrow, renal and hepatic transplantation, cardiac surgery with extracorporeal circulation and neurosurgical procedures, children with thalassemia, sickle cell and aplastic anemia, and the increased prevalence of HIV positive population, the need for *Toxoplasma*-seronegative blood from voluntary donors will be crucial for reducing the impact of this infection.

In this study, screening of blood donor for *Toxoplasma* infection was achieved by ELISA for detection of the specific anti-*T. gondii* IgM and IgG antibodies (3.33% and 33.67%) and qrtPCR for detection of its DNA (6.00%). These results were compatible with the results of Zainodini *et al*.[14] who found that out of 235 Iranian blood donors, 80 (34.04%) and 4 (1.71%) were positive regarding anti-*T. gondii* IgG and IgM antibodies, respectively. Also, real-time PCR results showed that 14 out of 200 (6.97%) of their blood donor had mRNA molecules of *SAG1* gene. However, in Taiwan blood donors, 9.3%, 0.28% were tested positive for anti-*Toxoplasma* IgG, IgM respectively, and no active parasitemia was detected by real-time PCR assays[17]. It was found that the seroprevalence of *T. gondii* in blood donors varies greatly among different countries using different detection methods. Regarding the seropositivity of anti-*T. gondii* IgG and IgM, they were 20.25% and 2.33% in Turkey[3], 20.3% and 3.6% in India[6], 7.4% and 1.9% in Mexico[18], and 4.1% and 4.3% in Thailand[4]. The higher estimate of anti-*T. gondii* IgG was detected among blood donors in Eastern Saudi Arabia (40%), Northeastern Egypt (59.6%) and Northeast Brazil (79%)[5,7,19]. These variations in *T. gondii* positivity might be attributed to the differences in the characteristics of the blood donors, sociocultural habits, geographic and environmental factors, the state of hygiene in the society and routes of transmission in the population studied, host immune function, host genetic factors and the differences in the virulence of *T. gondii* strains that prevalent in different areas of the world[20,21].

The risk of acquiring transfusion-transmitted toxoplasmosis depends on the presence of the parasite in blood at the time of donation. *T. gondii* is found in the blood during the phase of parasitemia early in the acute phase of infection and also in reactivating disseminated cases. Diagnosis of acute toxoplasmosis is based on the demonstration of a significant increase in specific IgG antibody levels and or the presence of specific IgM[22]. However, in the blood donors, the conventional single serum assays do not make a clear distinction between an acute and a chronic infection[23]. Also, the prevalence of high *Toxoplasma* IgG antibody titers among normal individuals in most population and the sustained persistence of specific IgM antibodies in some persons for months or even years pose another problem in the timing of infection[24,25]. Moreover, these antibodies may not be produced until after several weeks of parasitemia[26]. Therefore, the risk of transfusion-transmitted *T. gondii* infection may be undetected because the donor might test negative during the active phase of *T. gondii* infection. For these reasons, there is an increasing demand for more accurate diagnostic assays to distinguish the active phase of *T. gondii* infection from past, especially in those blood donors. In this study, detection of *T. gondii* parasitemia using qrtPCR was proven to be valuable in this respect.

In the present study, it was found that 8 of 18 (44.40%) donor samples were positive by qrtPCR contained IgM anti-*T. gondii*, conversely 8 of 10 (80.00%) IgM-positive samples were positive for parasite DNA. There was a significant increase in detection of recent *Toxoplasma* infection using PCR over IgM ELISA by 55.6%. In agreement with Hussein *et al*.[27] who evaluated IgM ELISA *vs.* PCR in diagnosis of recent *T. gondii* infection and found that 14 of 29 (52%) patient samples were positive by PCR contained IgM anti-*T. gondii*, conversely 14 of 18 (78%) IgM-positive samples were positive for parasite DNA. Moreover, Labalette *et al*.[28] found that PCR test was positive in 44% of cases while IgM ELISA was positive only in 11% of cases. These results were confirmed by Hafid *et al*.[12] who demonstrated that amplification of *B1* gene by PCR was more sensitive and faster than ELISA. The sensitivity of PCR was found to be high as it was able to detect the specific gene from purified DNA samples containing as few as 0.25 *T. gondii* tachyzoites per 100 000 human leukocytes[29]. This is attributed to the fact that, PCR is able to amplify a fragment of the *B1* gene, which is present in 35 copies and is conserved in the *T. gondii* genome. This region has been reported to be a very sensitive and specific target for toxoplasmosis diagnosis[11].

Also in this study, it was found that 7 subjects had *Toxoplasma* DNA in their serum samples with no detected antibodies which can be explained by after infection, *T. gondii* DNA appears earlier than the immunological response[26]. At the same time, *T. gondii* parasitemia was detected in 11 of 101 (10.9%) donor samples positive by IgG ELISA. There is a possible explanation that a small number of parasites might have been released from tissues into the blood at a subclinical level, and can be detected by PCR. The positive PCR results indicated that persisting parasitemia in those asymptomatic donors is not the reason to produce symptoms[30]. Based on this, the presence of *Toxoplasma* DNA in those asymptomatic donors whether positive or negative by ELISA probably indicated a recent infection or an apparent parasitemia that may reflect a significant risk of *Toxoplasma* transmission via donated blood. In this study, the negative results obtained by both PCR and ELISA in 192 (64%) subjects ruled out the infection in those donors. The remaining 90 subjects that are negative by PCR and positive by ELISA IgG could be explained by the clearance time for *Toxoplasma* DNA from the blood of patients with acute toxoplasmosis estimated to be 5.5–13 weeks[31] and the presence of long-standing immunity to toxoplasmosis up to years or the presence of the cross-reactive antibodies[26,32]. Moreover, the false positive results of *T. gondii* IgM and IgG have been reported previously that may mislead in the interpretation of the test results[22,33].

In the current study, using serum samples from donated blood to detect *T. gondii* DNA as an alternative to total blood samples gives a minor concentration of probable agents that interfere with PCR, facilitating the obtainment of higher-quality DNA. Hemin, a hemoglobin derivative, and its breakdown products, bilirubin and bile salts, were found to be PCR inhibitors. It was reported that hemoglobin can inhibit PCR activity because the connection between the groups' heme and/or perforin and *Taq* DNA polymerase inactivates the enzyme[34]. Al-Soud and Radstrom[35] suggested that heme regulates DNA polymerase activity and coordinates the synthesis of hemoglobin components in erythroid cells by feedback inhibition. In addition, using serum samples avoided the use of ethylene diamine tetraacetic acid, which is known as a PCR inhibitor by depletion of free Mg^{2+}[36].

In conclusion, it might be appropriate to include the screening of blood and blood products for *T. gondii* in the pre-transfusion blood testing schedule in Egypt. Also, molecular screening might offer a valuable aid for rapid and specific detection of active *T. gondii* infection in asymptomatic blood donors and should be carried out on the blood being transfused to immunocompromised patients, pregnant women and intrauterine and neonatal transfusion.

Conflict of interest statement

We declare that we have no conflict of interest.

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