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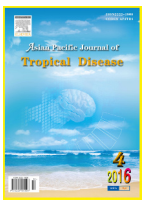

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Do we need to screen Egyptian voluntary blood donors for toxoplasmosis?

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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.

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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 ℃ 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 ℃, 15 min for initial denaturation followed by 35 cycles of 95 ℃, 30 s; 54 ℃, 1 min; 72 ℃, 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