Seroprevalence of toxoplasmosis in voluntary blood donors in Diyala Province, Iraq

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ARTICLE INFO
Article history:
Received 25 June. 2018
Revised 20 September. 2018
Accepted 14 October. 2018

ABSTRACT
Various parasitic diseases can be transmitted via blood transfusion such as malaria and toxoplasmosis but the screening for malaria is mandatory worldwide but not for toxoplasmosis. The objective of the present study was to investigate the seroprevalence of toxoplasmosis in healthy male blood donors in Diyala province, Iraq during 2016. A total of 103 blood samples from apparently healthy blood donors were obtained from the Blood Transfusion Laboratory in Baquba City, Diyala Province and the sera were tested for specific anti-Toxoplasma gondii IgG and IgM antibodies using Enzyme linked Immunosorbent assay (ELISA). Donors provided informed consent to participate in the study and demographic information was collected by means of a questionnaire. Forty one blood samples were found T. gondii IgG positive out of 103 samples (39.8%). No blood donor was seropositive for anti-T. gondii IgM antibodies. The percentage seropositivity among the blood donors living in the rural areas was significantly higher (P< 0.01) than that in those living in the urban areas. Although the age group of 40-49 showed the highest seropositivity rate, no significant differences were found between the age groups. A positive association has been found between the ABO blood group phenotypes of the blood donors and the seroprevalence of specific anti-T. gondii IgG antibodies and the blood group AB showed the highest seropositivity rate. In conclusion, the results of the present study revealed that seropositivity rate of T. gondii antibodies in healthy blood donors was high (39.8%) in comparison with that reported in previous studies conducted in Iraq and some neighboring countries.

Keywords: Toxoplasmosis; blood donors; blood transfusion, seroprevalence

Introduction
In general, any intracellular microorganism can be transmitted through blood transfusion such as bacteria, viruses, fungi and parasites and thus threatening the safety of the blood transfusion [1, 2]. Although various parasitic diseases can be or suspected to be transmitted via blood transfusion such as malaria, Chaga’s disease, babesiosis, leishmaniasis and toxoplasmosis, only the screening for malaria is mandatory worldwide [3, 4]. However, In Brazil the screening for T. gondii antibodies and ABO blood group system phenotyping are compulsory for pregnant women [5]. More recently, Karimi et al. [2] conducted a review in order to study the prevalence
of *T. gondii* in Iranian blood donors and the effects of toxoplasmosis on blood safety. The authors reviewed and evaluated all studies, which had reported the prevalence of *T. gondii* in Iranian blood donors and found six studies and reported that the percentage of seropositivity of IgG and IgM antibodies varied between 12.3% to 52.8% and 0% to 5.5%, respectively. Although some of the reviewed studies have suggested doing the screening for all blood donors, the authors of this review, based on parasitological and epidemiological evidences, concluded that it is not possible to perform screening of donated blood and instead, they recommended doing leukofiltration method to reduce the risk of parasite transmission.

*Toxoplasma gondii* is an obligate intracellular protozoan parasite which infects a wide range of warm-blooded animals and humans, and establishes a chronic infection in the central nervous system after invasion, although no organ in the body is immune against the infection with this parasite [6, 7]. Worldwide, the prevalence of human infection with *T. gondii* has been increasing due to the increasing number of cats and about one third of the world population are infected with this parasite [8-10]. In a comprehensive review, Pappas et al. [11] evaluated the global status of *T. gondii* seroprevalence and its correlations with risk factors, environmental and socioeconomic parameters and reported that the seropositivity levels ranged from 0.8% to 77.5% and that foci of high prevalence exist in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa.

This high prevalence may be attributed to the various routes of transmission such as the consumption of oocysts in cat feces contaminating drinking water or unwashed vegetables, undercooked meat containing tissue cysts, blood or leukocyte infusion, organ transplants from infected donors containing tissue cysts, and from infected women to her fetus via placenta [3, 4, 8, 12, 13]. Recently, Leukofiltration method has been suggested and now is accessible in the Iranian Blood Transfusion Organization for multi-transfused patients in order to reduce the risk of transmission of infectious agents [2].

In addition to the above mentioned routes of transmission, Flegr et al. [14] hypothesized that toxoplasmosis can be transmitted sexually because *Toxoplasma* tachyzoites were found in the seminal fluid and tissues of the testes of various animals and humans, and in some species, infection of females by artificial insemination with semen from infected males has been observed. Moreover, the huge numbers of oocysts of *T. gondii* shed by the infected cats survive for months or even up to a year in the soil [15]. In addition, seroprevalence of *T. gondii* in free range chickens could also contribute to soil contamination [16].

Screening for *T. gondii* infection in blood and blood products is not mandatory in Iraq. Therefore the objective of the current study was to assess the seroprevalence of *T. gondii* in the blood collected from male healthy voluntary donors at Blood Transfusion Laboratory in Baquba City, Diyala Province, Iraq.

**Materials and Methods**

**Data Collection**

One hundred and three blood donors volunteered to participate in the study when they donated blood to the Blood Transfusion Service of Baquba City, Diyala Province, Iraq during 2016. Additional 5 ml of blood was drawn from each donor by a professional phlebotomist nurse in a plain tube and then the sera were obtained and stored at −20°C until testing. Each volunteered blood donor received a questionnaire form designed specifically for this study describing the
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purpose of the study and to get more information, such as age, blood group and residency. Informed consent was obtained from each donor.

**Serological Technique**
The presence and/or absence of anti- *Toxoplasma gondii* specific IgG and IgM antibodies were determined by using the enzyme linked immunosorbent assay (ELISA). The procedure was done according to the instructions of the manufacturer and as described by Molan and Rasheed [7]. The ELISA kit was provided by a commercial manufacturer (Acon, U.S.A).

**Statistical Analysis**
The data were analyzed using Chi-square test and the Statistical Programme for the Social Sciences (SPSS) version 22 (IBM SPSS, Chicago, Illinois, USA). The results were considered significantly different when the P value is equal to or less than 0.05 (P ≤ 0.05).

**Results**
The results of the present study showed that 41 apparently healthy male blood donors were found *Toxoplasma gondii* IgG positive out of 103 (39.8%). No blood donor was seropositive for anti-*T. gondii* IgM antibodies (Table 1). It can be seen from Table 1 that the % seropositivity among the blood donors living in the rural areas was significantly higher (P< 0.01) than that in those living in the urban areas.

Concerning the effect of age on the seropositivity rate (Table 1), the results of the current study revealed that the age group of 40-49 showed the highest seropositivity rate (41.2%) followed by the age group 21-29 (36.1%) and then the age group 30-39 (34.6%) but no significant differences were found between the age groups.

The results of the present study revealed the presence of an association between the ABO blood group phenotypes of the blood donors and the seroprevalence of specific anti-*T. gondii* IgG antibodies and the blood group AB showed the highest seopositivity rate (45%) followed by the blood group A (40.0%), O (39.1%) and B (33.3%) (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Percentage seropositivity of anti- <em>Toxoplasma gondii</em> IgG antibodies in apparently healthy male blood donors (n= 103) based on the gender, residency and ages.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Residence</td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>Age group (years)</td>
</tr>
<tr>
<td>21-29</td>
</tr>
<tr>
<td>30-39</td>
</tr>
<tr>
<td>40-49</td>
</tr>
</tbody>
</table>

<p>| Table 2. Distribution of seropositive cases for <em>Toxoplasma gondii</em> IgG among blood donors according to ABO blood group phenotypes. |</p>
<table>
<thead>
<tr>
<th>ABO phenotypes</th>
<th>No. tested</th>
<th>Number positive</th>
<th>% seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>18</td>
<td>40.0</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>AB</td>
<td>10</td>
<td>4</td>
<td>45.0</td>
</tr>
<tr>
<td>O</td>
<td>23</td>
<td>9</td>
<td>39.1</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>41</td>
<td>39.8</td>
</tr>
</tbody>
</table>

**Discussion**

The principal finding of the present study was that seropositivity rate of *T. gondii* antibodies in apparently healthy blood donors was high (39.8%) in comparison with that reported in previous studies conducted in Iraq and some neighboring countries. Many previous studies have evaluated the seropositivity rate of *T. gondii* antibodies in healthy blood donors in different countries and the rate ranged between 0.961% and 40% [3, 17-21]. Sundar et al. [3] evaluated the seroprevalence of *T. gondii* in 1000 healthy blood donors in Karnataka, south India by ELISA and found that 20.3% were positive for *T. gondii* IgG antibody. The authors concluded that a high prevalence of *T. gondii* antibodies in healthy voluntary blood population and recommended to include screening for *T. gondii* in the pretransfusion blood testing schedule.

It is well known that *T. gondii* can be transmitted via blood transfusion as it has been found that the tachyzoites of this parasite can survive for several days in blood and blood products, especially leukocytes [22, 23]. Moreover, the parasite can survive in citrated blood stored at 4°C for 50 days [24]. Only second to malaria, toxoplasmosis is the most important protozoan disease that is transmitted by blood transfusion in India [3]. Currently, toxoplasmosis is not part of donor blood screening in many countries including Iraq, but it has been proposed in some countries such as India, where high seropositivity rates have been reported in the general population [3]. It is important to mention that the evaluation of the seropositivity of toxoplasmosis among volunteer blood donors is a valuable indicator of the epidemiology of this disease in any community [9]. Accordingly, the high seropositivity rate of toxoplasmosis in Diyala Province, Central Iraq indicates that the general population in this Province probably at a high risk of getting toxoplasmosis.

The results of the present study revealed the presence of an association between the ABO blood group phenotypes of the blood donors and the seroprevalence of specific anti-*T. gondii* IgG antibodies in that the blood donors with AB blood group showed the highest seropositivity rate. Conflicting results regarding the association between the ABO blood group phenotypes and the infection with *T. gondii* have been found in the literature. For example, five studies investigated the possible relationships between the ABO blood group phenotypes and the seropositivity of anti-*T. gondii* antibodies and reported that there was an association between infection with this parasite and B and AB blood groups and suggested that the B antigen could act as potential receptor for *T. gondii* [25-29]. On the other hand, three studies conducted in Tanzania, France and Brazil and the results of these studies did not find any evidence for the association between ABO blood group phenotypes and presence or absence of anti-*T. gondii* antibodies [5, 30, 31].

**Conclusion**

The results of the present study revealed that seropositivity rate of *Toxoplasma gondii* antibodies in apparently healthy blood donors was high (39.8%) in comparison with that reported in previous studies conducted in Iraq and some neighboring countries.

**References**