Non-association between anti-Toxoplasma gondii antibodies and ABO blood group system

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Abstract: Toxoplasma gondii infects humans through the gastrointestinal tract (GIT), which elicits humoral immune response with specific antibodies. The expression of the ABO blood group glycoconjugates also occurs in this same system and may influence the human susceptibility of infection by T. gondii. The aim of the present study was to investigate the association between ABO blood group phenotypes and the presence of anti-T. gondii antibodies. Data – including age, results of serology tests for T. gondii infection and ABO blood group phenotypes – were assembled from the medical records of 1,006 pregnant women attended in the Base Hospital of the Medical School of São José do Rio Preto, Brazil, between 2001 and 2004. The chi-square test was used to compare the results with the level of significance set at 5%. Of the studied cases, 64.1% (645/1006) and 35.9% (391/1006) presented respectively positive and negative serology tests for anti-T. gondii antibodies. The mean age of those who tested positive was higher than those with negative serology tests (p = 0.0004). The frequencies of ABO blood group phenotypes were similar in those with and without anti-T. gondii antibodies (p = 0.35). In conclusion, the ABO blood group system is not associated with the presence or absence of anti-T. gondii antibodies.

Key words: human blood groups, Toxoplasma gondii, toxoplasmosis, ABO blood group, pregnant women.

INTRODUCTION

Some microorganisms adhere to glycoconjugates (glycoproteins and glycolipids) expressed in the epithelial cells of the mucous membranes of hosts, since these molecules act as potential receptors (1, 2). The molecules that define ABO blood group phenotypes consist of carbohydrates that are present in the structures of glycoproteins and glycolipids expressed in red blood cells and other tissues (3). The adherence mechanism of microorganisms to mucous membranes of hosts is not totally clear, but it is likely that glycoconjugates of the ABO blood group system are involved in this process (4).

Previous studies investigated possible relationships between the ABO blood group system and the presence of anti-T. gondii antibodies, but their conclusions are conflicting. Four studies reported an association between infection by this parasite and B and AB blood groups (5-8). These studies proposed that the B antigen could act as potential receptor for T. gondii. However, two other similar investigations did not find any evidence of this association (9, 10).

The expression of glycoconjugates of the ABO blood group system occurs in epithelial cells of the gastrointestinal mucosa in approximately 80% of the population (3). T. gondii utilizes the human gastrointestinal tract (GIT) as one of its infection routes (11). So, the occurrence of these two processes in the same tract and the possible contribution of glycoconjugates in infection
by *T. gondii* justify the present research (11, 12). Therefore, this study analyzed a possible association between the ABO blood group system and the presence of anti-*Toxoplasma gondii* antibodies.

**MATERIALS AND METHODS**

**Ethical Considerations**

This study was approved by the Research Ethics Committee of the Medical School of São José do Rio Preto (FAMERP – protocol n. 169/2007). Informed consent was considered unnecessary by the Research Ethics Committee since all data were collected from hospital records.

**Selection of Hospital Records**

For this descriptive study, the hospital records of 1,120 pregnant women, attended in the Gynecology and Obstetrics Outpatient Clinic of the Base Hospital in São José do Rio Preto, São Paulo state, Brazil, between 2001 and 2004, were analyzed. Of these, 1,006 were selected as they contained complete data and no clinical or laboratory evidence of other medical ailments or infections. This number is sufficient to demonstrate whether there is an association between *T. gondii* infection and the ABO blood groups with statistical power of more than 90% as has been previously reported (5).

**Data Collection**

Data, such as age, blood type and the result of serological examinations for anti-*T. gondii* antibodies (only when carried out using the same ELISA technique) were collected from the outpatients' records and noted down on an epidemiological data collection form specifically designed for this study.

**Serology Assay**

The serology tests were performed to determine specific IgG and IgM antibodies for *T. gondii* using a commercial immunoenzymatic assay kit (DiaSorin, Italy). The manufacturers' instructions were precisely followed.

**ABO Phenotyping**

The ABO phenotypes were identified by the standard test tube hemagglutination using commercial monoclonal anti-sera anti-A, anti-B and anti-A,B for direct typing and standard red blood cells A and B for reverse typing (Fresenius Kabi, Brazil).

**Statistical Analysis**

The chi-square test and odds ratio were employed to verify any association between the ABO blood group phenotypes and *T. gondii* infection using the GraphPad Instat® (GraphPad Software Inc., USA) computer program version 3.06. The level of significance was set at 5% (p-value < 0.05).

**RESULTS**

From the total of pregnant women that participated in this study, 79.5% (n = 800) were Caucasians, 9.9% (n = 100) were mulattoes and 10.5% (n = 106) were Blacks. The mean age was

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive serology</th>
<th>Negative serology</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>26.9 ± 6.7</td>
<td>25.6 ± 6.9</td>
<td>1.003</td>
<td>0.855-1.169</td>
<td>0.330</td>
</tr>
<tr>
<td>ABO phenotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n = 397)</td>
<td>261</td>
<td>136</td>
<td>1.124</td>
<td>0.8630-1.465</td>
<td>0.42</td>
</tr>
<tr>
<td>B (n = 113)</td>
<td>76</td>
<td>37</td>
<td>1.170</td>
<td>0.7716-1.773</td>
<td>0.52</td>
</tr>
<tr>
<td>AB (n = 38)</td>
<td>27</td>
<td>11</td>
<td>1.390</td>
<td>0.6811-2.837</td>
<td>0.46</td>
</tr>
<tr>
<td>O (n = 458)</td>
<td>281</td>
<td>177</td>
<td>0.802</td>
<td>0.6197-1.039</td>
<td>0.10</td>
</tr>
<tr>
<td>Total (n = 1006)</td>
<td>645</td>
<td>361</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Mean ages and frequencies of the ABO blood group phenotypes in pregnant women with positive and negative serology for IgG and IgM anti-*Toxoplasma gondii* antibodies, in São José do Rio Preto, Brazil
26.4 ± 6.7 years, ranging from 12 to 44 years old. The frequencies of the four ABO blood group phenotypes, independent of the results of the ELISA, were 39.5% (397/1006) for group A, 11.2% (113/1006) for group B, 3.8% (38/1006) for group AB, and 45.5% (458/1006) for group O.

Of the participants, 64.1% (645/1006) presented positive serological results and 35.9% (361/1006) had negative serological results for anti-\textit{T. gondii} antibodies. Of the 645 positive results, 92.2% (595) were positive only for IgG, 5.0% (32) were IgG and IgM positive and 2.8% (18) were positive only for IgM. The mean age of the pregnant women with positive serology (26.9 ± 6.7) was higher than those with negative serology (25.6 ± 6.9) (p-value = 0.0036).

The frequencies of ABO blood group phenotypes, in respect to results of the serological test (positive [IgG and/or IgM] and negative) for anti-\textit{T. gondii} antibodies as listed in Table 1, do not demonstrate statistically significant differences (p-value = 0.35; $\chi^2$ 3,255; 3 df).

**DISCUSSION**

In this paper we examined the association between the ABO blood group system and infection by \textit{T. gondii} in a large cohort of pregnant women without other infections who received medical attention between 2001 and 2004. Since screening for \textit{T. gondii} antibodies and ABO blood group system phenotyping are compulsory for pregnant women in Brazil, we assembled data from the medical records of women who gave birth in our hospital. The background of the series concerning ethnicity, age and ABO blood groups is representative of the geographic area in which the study was carried out (13, 14).

Although previous studies had demonstrated discordant results, there is a possibility that this parasite utilizes glycoconjugates, which characterize the blood phenotypes of the ABO blood group system, as potential receptors (5-10). These glycosylated molecules are expressed in the GIT, which is also utilized as the main route of \textit{T. gondii} infection (12). Additionally, there is biochemical evidence that several microorganisms utilize glycoconjugates as receptors (1, 2, 4).

Data obtained from hospital records indicate that the group of individuals selected for this study is representative of the geographical area attended by the Gynecology and Obstetrics Outpatient Clinic. Moreover, the frequencies of positive and negative test results for \textit{T. gondii} are similar to those confirmed in recent investigation performed in the northwestern region of São Paulo state (15).

The mean ages of pregnant women with positive serology tests observed in this study were higher than those with negative results. Most humans tend to produce high avidity IgG anti-\textit{T. gondii} antibodies, since contact with the infecting forms of \textit{T. gondii} also stimulates the development of immune memory (16-21). Additionally, older individuals seem to be exposed to a greater number of stimuli as well as reinfections by this parasite, without necessarily manifesting the clinical form of the disease. Directly proportional relationship between the increase in age and positive serology for IgG anti-\textit{T. gondii} antibodies has been reported in Cubans and in Czechoslovakians (6, 8, 22).

It can be assumed that more than 60% of the pregnant women assessed in this study are possibly immunized and without risk of congenital transmission of \textit{T. gondii} as shown by the statistically significant differences in mean ages between individuals with positive and negative serology. These observations agree with previous studies that indicate a percentage of IgG anti-\textit{T. gondii} antibodies in the Brazilian population (20, 23-30).

The present results do not demonstrate any association between evidence of infection by \textit{T. gondii} and the ABO blood group system and, thus, does not corroborate those studies that propose that the B antigen, expressed in B and AB blood groups, acts as a potential receptor for this parasite in the GIT (5-8). On the other hand, the results agree with investigations of blood donors in Tanzania and of pregnant French women, which did not report any association between this system and infection by \textit{T. gondii} (9, 10).

The suggestion that the B antigen represents a receptor for \textit{T. gondii} does not appear to be valid for the Brazilian population. The frequencies of the ABO blood group phenotypes did not present statistically significant differences in the presence or absence of anti-\textit{T. gondii} antibodies. The expression of glycoconjugates that define ABO blood group phenotypes also occurs in epithelial cells of the GIT, but their genetic control depends on the presence of at least one functional allele.
of the *FUT2* gene (3). This gene codes the α-2-L-fucosiltransferase enzyme (*FUTII*), which is capable of incorporating a molecule of fucose to carbon 2 of the galactose terminal of type 1 oligosaccharide precursors (Galβ1→3GlcNAcβ1→R) to form type 1 H antigens ([Fucα1→2]Galβ1→3GlcNAcβ1→R). This glycoconjugate, when glycosylated by α-3-D-N-acetylgalactosaminiltransferase or α-3-D-galactosiltransferase enzymes coded by the *A* and *B* alleles of the ABO gene, forms type 1 A (NAcGalα1→3[Fucα1→2]Galβ1→3GlcNAcβ1→R) or type 1 B antigens (Galα1→3[Fucα1→2]Galβ1→3GlcNAcβ1→R) respectively (31, 32).

All these glycoconjugates are derived from a common precursor, but present variations in their chemical and spatial structural compositions which, apart from differing from those expressed by red blood cells, may be recognized by monoclonal antibodies (4, 31-34). However, there is no evidence that these differences influence the susceptibility to *T. gondii* infection, at least when this parasite infects humans by the GIT.

The disagreement among these results and other studies may result from several factors. It is possible that the B antigen exerts a small influence on the adherence of *T. gondii* to the gastrointestinal mucosa and its contribution is obscured by the high prevalence of infection by these parasites in the Brazilian population, as well as by the molecular variability of its strains identified in Brazilian patients (35-38). Moreover, the use of only female patients in this study and in the one performed in France might not be sufficient to reveal possible influences of gender in the association of the ABO blood group system with infection by *T. gondii* (10). In fact, López et al. (6) demonstrated that the AB group is associated with the presence of IgG anti-*T. gondii* antibodies in male Cuban blood donors, but their results conflicted with the findings of Midtvedt and Vaage (5) and Kolbekova et al. (8). Infection by *T. gondii* and the expression of antibodies specific to this parasite did not predominantly occur in any one gender (16). Hence, it can be presumed that if anti-*T. gondii* antibodies are associated with the ABO blood group system in any populations, it does not depend on gender.

Recently, our group carried out a similar study based on a series of high-risk pregnant women but we did not find evidence of any association (15). This proposition is attractive, since it was demonstrated that *in vitro* some microorganisms bind to blood carbohydrates including those belonging to the ABO blood group system (38-41). In this previous study, we included the analysis of the G428A mutation of the *FUT2* gene which controls the expression of ABO blood group antigens in the GIT and defines the secretor and non-secretor phenotypes. We did not find any association even when we analyzed ABO blood group phenotypes and secretor and non-secretor phenotypes isolated and combined in individuals with positive or negative serological tests for IgG and IgM anti-*T. gondii* antibodies.

We believe that pregnancy, whether low- or high-risk, does not affect the relationship between the ABO blood group system and the humoral immune response against *T. gondii* among Brazilian women. Besides, taking into account the biological cycle of *T. gondii*, it does not infect red blood cells of hosts like other parasites belonging to Apicomplex phylum. However, new studies in other Brazilian regions enrolling different ethnic groups of pregnant women aiming to confirm the lack of association reported in this paper would be very valuable.

**CONCLUSIONS**

The ABO blood group system is not associated with the presence or absence of anti-*T. gondii* antibodies in pregnant women in Brazil and, therefore, phenotypic variations resulting from this histo-blood group system do not seem relevant for *T. gondii* infection.

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CONFLICTS OF INTEREST
There is no conflict.

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ETHICS COMMITTEE APPROVAL
The present study was approved by the Research Ethics Committee of the Medical School of São José do Rio Preto (FAMERP), under the protocol number 169/2007.

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