Lack of association between ABO histo-blood groups, secretor and non-secretor phenotypes, and anti-*Toxoplasma gondii* antibodies among pregnant women from the northwestern region of São Paulo State, Brazil

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Abstract

Introduction: *Toxoplasma gondii* infects humans in several manners including by the gastrointestinal tract where a-2-L-fucosyltransferase (FUTII) coded by the FUT2 gene (19q13.3) controls the expression of the ABH glycoconjugate profile. The presence of functional FUTII defines the secretor phenotype which is associated with ABO erythrocytic phenotypes. Due to the epidemiological and clinical importance of *T. gondii* infection, the aim of this work was to test the hypothesis that the ABH glycoconjugate profile expressed in the gastrointestinal tract is associated with the presence of antibodies against this parasite.
Material and methods: A total of 367 pregnant women from the High-Risk Pregnancy Clinic of the University Hospital de Base in São José do Rio Preto were enrolled in this study. Two blood samples were drawn with only one being mixed with anticoagulant. ABO erythrocytic phenotyping and detection of anti-T. gondii antibodies were achieved by the haemagglutination test. Identification of the secretor status used the PCR-RFLP method.
Results: Differences in the ABO erythrocytic phenotypes (P=0.20) and secretor and non-secretor phenotypes (P=0.41), either in isolation or in association, were not statistically significant with respect to the presence or absence of anti-*T. gondii* antibodies.
Conclusions: These results suggest that the ABH glycoconjugate profile expressed in the gastrointestinal tract under regulation of the *FUT2* gene is not associated with anti-T. gondii antibodies.

Key words: ABO histo-blood groups, secretor status, *FUT2* gene, *Toxoplasma gondii*, *FUT2* G428A.

Introduction

Toxoplasma gondii infects human hosts utilizing the gastrointestinal tract as one route with its adherence to specific receptors being an important

factor in the pathogenesis of toxoplasmosis [1, 2]. Experimental trials have demonstrated that this protozoan expresses sugar-binding proteins on its rhoptries which are able to bind N-acetylglucosamine and galactose [3]. These monosaccharides are present in glycoproteins and glycolipids of the gastrointestinal tract with the expression of these molecules being related, at least in part, to the profile of ABH glycoconjugates secreted by carriers of secretor phenotype. Carriers of non-secretor phenotype are incapable of secreting these glycoconjugates [4].

The secretor and non-secretor phenotypes are controlled by the *FUT2* gene (19q13.3) [4], and consequently the ABH glycoconjugate profile results from the epistatic interactions between this gene and the *ABO* (9q34.1) gene [5]. Therefore, each glycoconjugate of the profile may act as a potential receptor and influence the susceptibility or resistance to infection by micro-organisms [6]. As the entrance of *T. gondii* and the expression of ABH glycoconjugates occur in the gastrointestinal tract, there may be some link between them.

Previous reports have explored possible associations between ABO blood groups and *T. gondii* infection. Four of them reported that individuals of the B and AB blood groups present high susceptibility to infections by this protozoan as seen by the detection of specific IgG class antibodies [7-10]. These studies also indicated that the B antigen may act as a potential candidate receptor for *T. gondii* in the gastrointestinal tract. Two other similar studies did not find an association between ABO blood groups and anti-*T. gondii* antibodies [11, 12].

The results of these reports remain inconclusive as they are based only on the identification of ABO blood group phenotypes and did not include an analysis of the *FUT2* gene as a determinant of the profile of ABH glycoconjugates expressed by secretor and non-secretor phenotype carriers.

The aim of this study was to test the hypothesis that the ABH glycoconjugate profile in the gastrointestinal tract is associated with infection by *T. gondii*.

Material and methods

Selection of pregnant women

A total of 367 pregnant Caucasian and non-Caucasian women, who attended the High-Risk Pregnancy Clinic of Hospital de Base linked to the Medicine School in São José do Rio Preto, were enrolled in this study between October 2005 and February 2007. The number of pregnant women selected is sufficient to demonstrate the difference for the B blood group as reported in the previous paper [7] with power higher than 90%. Self-definition was used as the means of identifying their ethnic groups taking into account two generations of progenitors. Under 18-year olds and individuals suffering from other infectious or parasitic diseases were not included in the study.

Ethical considerations and blood sampling

The study was approved by the Research Ethics Committee of FAMERP (case 089/2005). After receiving written consent forms from each participant, two blood samples were drawn and stored in vacuum tubes, only one of which contained anticoagulant.

ABO phenotyping

The ABO blood groups were determined by the haemagglutination method using commercial antisera for direct typing and commercial standard red blood cells for reverse typing (Biotest, Sao Paulo, Brazil). A drop of a suspension of red blood cells in 5% sterile saline solution (0.9% NaCl) prepared for each blood sample was mixed with a drop of each of the anti-A, anti-B and anti-A/B anti-sera to define the erythrocytic antigens. Two drops of blood plasma from each sample were mixed in a tube to one drop of each of the 5% standard red blood suspensions of groups A and B to identify anti-A and anti-B antibodies. The tubes were centrifuged at 3400 rpm for 1.5 min with interpretation of the results being based on the presence or absence of haemagglutination. In all procedures, the recommendations of the manufacturers of the reagents used were strictly followed.

Extraction of genomic DNA

The genomic DNA was extracted by a salting-out procedure [13]. The isolated leukocytes were rinsed three times in buffered saline solution (PBS) and incubated overnight at 37°C in 20 µl of 10% K-proteinase solution. The DNA was precipitated in 2 ml of 6 M NaCl, rinsed three times in absolute ethanol and three times in 70% ethanol followed by dissolution in 300 µl MilliQ water and stored at –20°C until use.

FUT2 genotyping

The *FUT2* genotypes resulting from homozygosis or heterozygosis of the G428A substitution were identified by PCR-RFLP according to the protocol of Svensson and co-workers [14]. Amplification reactions used the sense, 5' CGC TCC TTC AGC TGG GCA CTG GA 3' and antisense, 5' CGG CCT CTC AGG TGA ACC AAG AAG CT 3', primers to differentiate the G and A alleles at the 428 position of the *FUT2* gene. Each reaction used a final volume of 25 µl containing 10 mM TRIS-HCL, 50 mM KCl, 1.5 mM $MgCl_2$, 20 mM of each dNTP [dATP, dTTP, dCTP, dGTP], 10 pM of each primer, 0.5 U of Taq and 5 ng of genomic DNA. The conditions of amplification involved pre-desnaturation at 94°C for 5 min, followed by 35 cycles (94°C for 1 min, 63°C for 1 min and 72°C for 1 min) and an

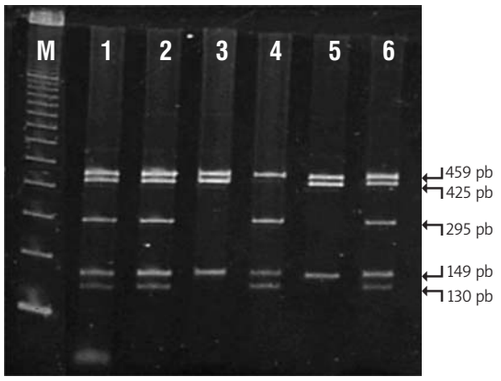

Figure 1. Electrophoretic profile of a 1033 bp fragment amplified by PCR, after *Ava II* restriction enzyme digestion, showing the genotypes *GA* (1, 2, 6), *AA* (3, 5) and *GG* (4), M=100 bp ladder marker

additional extension at 72°C for 5 min. The fragment containing 1033 base pairs after digestion with the *Ava II* enzyme was cleaved in a variable number of fragments according to the alleles: 459, 295, 149 and 130 base pairs for the *G* allele; and 459, 425 and 149 base pairs for the *A* allele. Separation of these fragments was achieved by electrophoresis in 2% agarose gel stained using ethidium bromide and viewed under UV light. Thus, *GG* and *GA* individuals were considered secretors and *AA* individuals non-secretors of ABH glycoconjugates (Figure 1).

Diagnosis of *Toxoplasma gondii* infection

Anti-*T. gondii* antibodies were detected by commercial haemagglutination serodiagnosis test kits (IMUNO-HAI-TOXO – Wama Diagnóstica, São Carlos, Brazil). The instructions of the manufacturer were strictly followed. The results are expressed as "seropositive" and "seronegative" for the presence and absence of anti-*T. gondii* antibodies, respectively.

Statistical analysis

The data were entered into the Graphpad Instat computer program and analyzed using Pearson's χ^2 test and Fisher's exact test. The level of significance adopted was 5%.

Results

Of the 367 participating pregnant women, 155 (42.2%) were Caucasian, 170 (46.3%) were half-castes, 39 (10.6%) were Afro-descendents, two (0.54%) were Native Indians and one (0.27%) was Oriental. The average age of the participants was 26.4 years (range 18 to 44 years). The overall distribution of ABO blood group phenotypes was 34.3% (A), 12.8% (B), 3.8% (AB) and 48.7% (O). The overall frequencies of genotypes that define the secretor phenotype (*GG* and *GA*) and non-secretor phenotype (*AA*) were 76.3% (280/367) and 23.7% (87/367), respectively. Overall, 49.6% (182/367) were seropositive and 50.4% (185/367) were seronegative for anti-*T. gondii* antibodies.

The participants were divided into two groups according to the presence or absence of anti-*T. gondii* antibodies but did not reveal statistically significant differences in relation to the frequencies of the ABO blood groups (P value =0.20, χ^2=4.567) or the secretor and non-secretor phenotypes (P value =0.41, χ^2 =0.6786), when considered in isolation or in combination (Table I).

Discussion

Investigations of *T. gondii* infection have paid special attention to the prevalence of positive serological tests in pregnant women because of the risk of congenital transmission and the resulting sequelae in newborn babies [15-18]. Parallel to this, there has been growing interest in the biology of this protozoan, especially in its ability to utilize glycosylated molecules expressed in the gastrointestinal tract as receptors [1, 2]. The previous demonstration that the rhoptries of tachyzoites of *T. gondii* express sugar-binding proteins which bind N-acetylglucosamine and galactose [3], the use of the gastrointestinal tract as a route of infection [1, 2] and the action of the *FUT2* gene controlling the secretor and non-secretor phenotypes [4] prompted us to test the hypothesis

Table I. Frequencies of ABO blood groups, secretor and non-secretor phenotypes of pregnant women who are seropositive or seronegative for *T. gondii* infection

ABO*	Seropositive (N=182, 49.6%)				Seronegative (N=185, 50.4%)				P***
	Secretor** (*GG* + *GA*)		Non-Secretor ** (*AA*)		Secretor** (*GG* + *GA*)		Non-Secretor ** (*AA*)		
	N	%	N	%	N	%	N	%	
A	53	39.2	16	34.0	43	29.6	14	35.0	1.00
B	17	12.6	1	2.1	24	16.6	5	12.5	0.38
AB	5	3.7	4	8.5	5	3.5	1	2.5	0.58
O	60	44.5	26	55.3	73	50.3	20	50.0	0.23
Total	135	100.0	47	100.0	145	100.0	40	100.0	

**P=0.20 for ABO blood groups only, **P=0.41 for secretor and non-secretor phenotypes only, ***By Fisher's exact test*

that the ABH glycoconjugate profile in the gastrointestinal tract is associated with infection by *T. gondii*.

The background of the casuistic approach used in this study is representative of the northwestern region of Sao Paulo State, in Brazil, concerning the ethnicity, ABO blood group phenotypes, secretor and non-secretor phenotypes, and prevalence of infection by *T. gondii* [19-22].

According to the results of this study all the pregnant women seem to be equally susceptible to *T. gondii* infection, this fact being confirmed by the presence of specific anti-*T. gondii* antibodies in both secretor and non-secretor individuals of all the ABO blood groups. However, the absence of anti-*T. gondii* antibodies may be due to the lack of exposure to the parasite or even the action of other resistance factors not associated with the ABO blood groups and the secretor and non-secretor phenotypes. The differences not being statistically significant among the frequencies of ABO blood groups, secretor and non-secretor phenotypes when considered in isolation or in combination, in the presence of seropositive and seronegative tests for anti-*T. gondii* antibodies, suggests that the hypothesis that the ABH glycoconjugate profile in the gastrointestinal tract is associated with infection by *T. gondii* is not valid.

The results of this paper are in disagreement with those that stated that the B and AB blood group carriers are more susceptible to *T. gondii* infection and the B antigen may act as a receptor for this protozoan [7-10]. However, they are in agreement, at least in part, with other reports which also did not find any association with ABO blood groups and anti-*T. gondii* antibodies [11, 12]. Regrettably, all these reports evaluated only the ABO erythrocytic phenotypes, but the protozoan *T. gondii* does not infect red blood cells. Besides, these studies did not consider the influence of the *FUT2* gene on the control of the expression of ABH glycoconjugates in the gastrointestinal tract.

The proposition that B antigen may act as a potential candidate receptor for *T. gondii* [7, 8] is attractive due to the fact that some micro-organisms are able to bind carbohydrates such as those present in the ABO blood group structures [6, 23]. ABH glycoconjugate expression in the human gastrointestinal tract depends on the presence of at least one functional allele of the *FUT2* gene [4]. Homozygosity and heterozygosity for the *G* allele lead to expression of the FUTII enzyme, which is capable of incorporating a fucose molecule to the galactose terminal of type 1 oligosaccharide precursors (Galβ1→3GlcNAcβ1→R) to form the H type 1 antigen ([Fucα1→2]Galβ1→3GlcNAcβ1→R). This antigen, when glycosylated by α-3-D-N-acetylgalactosaminyltransferase or α-3-D-galactosyltransferase enzymes coded by *A* and *B* alleles of the *ABO* gene, results in A type 1 (NAcGalα1→ 3[Fucα1→2]Galβ1→3GlcNAcβ1→R) or B type 1 (Galα1→3[Fucα1→2]Galβ1→3GlcNAcβ1→R) antigens [5, 14]. These glycoconjugates are derived from a common precursor but present with variations in their spatial structures and chemical compositions [5, 6] which may not influence the selective binding of the tachyzoites from *T. gondii* to epithelial cells of the human gastrointestinal tract. Experimental trials have demonstrated that *T. gondii* is capable of binding different monosaccharides expressed on the surface of the vertebrate cells, including those present on the ABH glycoconjugates composition, but this ability seems not to be exclusive for galactose, which defines the specificity of the B antigen [3, 24].

Various factors may have contributed to the disagreement between the data of this study with those proposing that B and AB blood group carriers are more susceptible to *T. gondii* infection [7-10]. It is possible that the ABH glycoconjugate profile containing the B antigen constitutes a small risk for *T. gondii* infection. Besides, the Brazilian genetic background and the elevated prevalence of infection by this protozoan in the population living in the northwestern region of Sao Paulo State obscures its importance for susceptibility. Additionally, due to the variability of *T. gondii* strains that infect the Brazilian population [25], it is possible that only some may utilize specific ABH glycoconjugates as receptors in the gastrointestinal tract. Another aspect to be considered is that this study only analyzed a female cohort and so the influence of gender in the relationship between humans and anti-*T. gondii* antibodies was not considered [12].

As individuals of all ABO blood groups, both secretors and non-secretors, seem to be equally susceptible to *T. gondii* infection, in principle there is no reason to believe that ABH glycoconjugate profile may influence infection by this protozoan. However, the presence or absence of the FUTII enzyme, although necessary to create the differentiation of ABH glycoconjugate profiles in the gastrointestinal tract, is not sufficient to influence the susceptibility or resistance to *T. gondii* infection among females.

In conclusion, the ABH glycoconjugate profile in the human gastrointestinal tract controlled by the *FUT2* and *ABO* genes is not associated with the presence or absence of anti-*T. gondii* antibodies and thus it does not seem to be a crucial factor in increased or decreased risk of infection.

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