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Frequency of ABO blood group system polymorphisms in *Plasmodium falciparum* malaria patients and blood donors from the Brazilian Amazon region

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ABSTRACT. We investigated the ABO genotypes and heterogeneity of the *O* alleles in *Plasmodium falciparum*-infected and non-infected individuals from the Brazilian Amazon region. Sample collection took place from May 2003 to August 2005, from *P. falciparum* malaria patients from four endemic regions of the Brazilian Amazon. The control group consisted of donors from four blood banks in the same areas. DNA was extracted using the Easy-DNA™ extraction kit. ABO genotyping was performed using PCR/RFLP. There was a high frequency of *ABO*O01O01*. *ABO*AO01* was the second

most frequent genotype, and the third most frequent genotype was *ABO*BO01*. There were low frequencies of the *ABO*O01O02*, *ABO*AA*, *ABO*AB*, *ABO*BB*, and *ABO*O02O02* genotypes. We analyzed the alleles of the O phenotype; the $O^{1variant}$ allele was the most frequent, both in malaria and non-malaria groups; consequently, the homozygous genotype $O^{1v}O^{1v}$ was the most frequently observed. There was no evidence of the homozygous O^{2} allele. Significant differences were not detected in the frequency of individuals with the various alleles in the comparison of the malaria patients and the general population (blood donors).

Key words: Malaria; *Plasmodium falciparum*; ABO blood system; Brazilian Amazon region; Genetic polymorphism

INTRODUCTION

The ABO system consists of the A, B and H carbohydrate antigens synthesized by a series of enzymatic reactions catalyzed by glycosyltransferase and antibodies against these antigens. The *A*, *B* and *O* genes are at the same genetic locus on chromosome 9 at q34, and the *A* and *B* alleles are co-dominant against the recessive *O* allele (Yamamoto et al., 1995). Several point mutations on the *A* gene have been described. They cause a number of amino acid changes and alter the glycosyltransferase from A to B. Single guanine deletions at position 261 on the *ABO* gene result in a truncated, enzymatically inactive O protein (referred to as O^{1}) (Roubinet et al., 2004). Another *O* allele (O^{2}), which lacks this deletion, has been identified. A commonly occurring variant of the O^{1} gene, $O^{1variant}$, has also been reported (Olsson and Chester, 1996). The combination of these alleles offers several genotypes, which result in four phenotypes, but the alleles have now been shown to be highly polymorphic (Chester and Olsson, 2001; Yamamoto, 2004). The importance of ABO histo-blood groups is supported by the observation that their geographical distribution varies significantly, suggesting that positive selective factors may have influenced gene spread (Loscertales et al., 2007).

Plasmodium falciparum infections can be linked to the severest form of human malaria, and virulence is associated with parasite reproduction and erythrocyte invasion (Chotivanich et al., 2000). In Brazil, its distribution is localized: it is more common than *P. vivax* in some areas, but very rare or absent in others (Camargo et al., 1999). The ABO blood group system is the most important system for blood group transfusion medicine. Its association with malaria disease has been proposed (Carlson et al., 1990; Lell et al., 1999), and the hypothesis that *P. falciparum* malaria shaped the distribution of ABO blood group in humans is supported by the literature (Athreya and Coriell, 1967). Additionally, there is evidence that the ABO histo-blood group is not related to the incidence of *P. falciparum* malaria (Fischer and Boone, 1998), but it has been linked as a co-receptor in parasite and vascular cytoadherence, with higher rosette rates among non-group O compared to group O erythrocytes (Barragan et al., 2000; Cserti and Dzik, 2007).

We, therefore, investigated the ABO genotypes and the heterogeneity of the *O* alleles in *P. falciparum*-infected and non-infected individuals from the Brazilian Amazon region.

MATERIAL AND METHODS

Study population and sample collection

Sample collection took place from May 2003 to August 2005 in four endemic regions of the Brazilian Amazon: Macapá, Amapá State (00°02'20"S; 51°03'59"W); Novo Repartimento, Pará State (04°19'50"S; 49°47'47"W), Porto Velho, Rondônia State (-08°45'43"S; 63°54'14"W), and Plácido de Castro, Acre State (10°16'33"S; 67°09'00"W). The malaria group was composed of male and female patients with positive thick blood film and molecular diagnosis results for *P. falciparum* single infection (N = 98). These individuals were randomly recruited to participate in this study at the health clinics in each area studied. They were all over the age of 18 with age ranging up to 65 years old. All had an absence of cerebral malaria, severe malarial anemia, and multiple organ dysfunction syndromes. We excluded pregnant women, patients under the age of 18 years, and patients with concomitant illnesses from the study. The control group consisted of both male and female donors from four blood banks in the areas studied (N = 142), and, following the policies for Brazilian blood banks, the donors met the following criteria: over 18 years old (ranging from 19 up to 57 years old), no previous malaria attack, and no signs of malaria reported during the initial interview. The consent form was co-signed by a staff member of the health clinic and a staff member of the blood bank. The control subjects were matched to the patients with respect to age (± 5 years), gender and ethnicity. All control subjects were genetically independent. The protocol for this study was reviewed and approved by the Research Board of the Faculty of Medicine of São José do Rio Preto.

DNA preparation and ABO histo-blood group system genotyping

DNA was extracted from frozen pellets of infected and non-infected erythrocytes using the Easy-DNA™ extraction kit (Invitrogen, Carlsbad, CA, USA). The ABO genotyping was assessed in 240 samples by amplifying fragments covering exons 6 and 7 using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). Moreover, a subset of peripheral blood samples from 30 *P. falciparum*-infected individuals and 27 blood donors were evaluated for the heterogeneity of the *O* alleles as previously described (Mattos et al., 2001). Briefly, four PCR were used. The *A*, *B*, O^1, and O^2 genes were identified in three amplifications using the fy46/fy47, fy43/fy31 and fy47/fy29 primer pairs, respectively. Additionally, the O^1 and O^{1v} genes were distinguished using the fy47/fy31 primer pair in the last reaction. PCR was performed in a 25-µL total reaction volume containing 200 ng genomic DNA, 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.25 µM of each primer and 0.5 U Taq DNA polymerase; 35 amplification cycles were carried out (94°C for 120 s, 56°C for 60 s and 72°C for 60 s). After PCR analysis, the amplified DNA was digested with the restriction enzymes *Kpn*I, *Nar*I, *Alu*I, and *Dde*I, respectively, for the four reactions, and the product was analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide and examined under UV light.

Statistical analyses

Analyses were performed using the R version 2.4.1 statistical software (The R Foun-

dation for Statistical Computing ISBN 3-900051-070 - http://www.r-project.org, Vienna, Austria). The Hardy-Weinberg equilibrium was tested by applying a hidden Markov chain with 10,000 steps (Guo and Thompson, 1992). To achieve independence among the proportions, the Fisher exact test was applied with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The genotype and allele frequencies of the ABO histo-blood group of the 142 blood donors and 98 patients infected with *P. falciparum* as determined by PCR/RFLP are summarized in Table 1. The data show a high frequency of the *ABO*O01O01* in 134 of the 240 individuals (55.83%) of the population studied. *ABO*AO01* was the second most frequent genotype in 46 of the individuals studied (19.16%). The third most frequent genotype was *ABO*BO01*, which was found in 26 individuals, or 10.84% of the population studied. Low frequencies were detected for the *ABO*O01O02*, *ABO*AA*, *ABO*AB*, *ABO*BB*, and *ABO*O02O02* genotypes.

Table 1. Comparison of genotyping results and allele frequency of the ABO histo-blood system among blood donors and patients infected with *Plasmodium falciparum* from the Brazilian Amazon region.

Genotype	Predicted phenotype of donors/patients	Total sample (N = 240)	Donors (N = 142)	*P. falciparum* patients (N = 98)	P
*ABO*AA*	A	5	4	1	0.6184
*ABO*AO01*	A	46	25	21	0.5668
*ABO*AB*	AB	8	5	3	0.8645
*ABO*BB*	B	5	4	1	0.6184
*ABO*BO01*	B	26	17	9	0.6370
*ABO*O01O01*	O	134	81	53	0.7476
*ABO*O01O02*	O	13	4	9	0.0641
*ABO*O02O02*	O	3	2	1	0.7451
Allele frequencies					
*ABO*A*		0.13	0.13	0.13	0.9709
*ABO*B*		0.09	0.11	0.07	0.2646
*ABO*O01*		0.74	0.73	0.74	0.9399
*ABO*O02*		0.04	0.03	0.06	0.1916

Among blood donors and patients, the frequency of individuals with the *ABO*A* allele was 13%, and the frequency of individuals with the *ABO*O01* allele was around 75%. Significant differences were not detected in terms of the frequency of individuals with all alleles studied between the two groups.

The Brazilian population has a highly heterogeneous ethnic composition, which results from the hybridization of the numerous native indigenous populations and immigrants from Europe, Africa, and Asia. Waves of immigration occurred in unequal proportions in the different regions of the country (Zago et al., 1983). The homozygous genotype *ABO*O01O01* showed an expected higher frequency in the population studied, since phenotype studies of the ABO blood groups in Amerindian-descent populations have revealed that most individuals are exclusively of the O group (Franco et al., 1994; Olsson et al., 1998). The indigenous contributions in the ethnic formation of the North Brazil region are unquestioned, and the molecular basis of the O phenotype in Indians from the Brazilian Amazon was reported by Franco et al. (1994) as being the same G261 - single base deletion (O^1), and they found the absence of the O^2 allele in this population. We also observed a lower frequency of the O^2 allele in our sample,

which is in agreement with the studies in Amerindians (Franco et al., 1994; Olson et al., 1998).

According to data in Table 2, of 27 blood donor samples, 5 were homozygous for the O^1 allele, and 7 were $O^1O^{1variant}$. One sample was O^1O^2, and 3 were $O^{1variant}O^2$ heterozygotes. The 30 samples from the *P. falciparum*-infected patients included 12 $O^{1variant}$ homozygous individuals, 6 $O^1O^{1variant}$ individuals, 2 O^1O^2, and 4 $O^{1variant}O^2$ heterozygote. In the samples studied, there was no evidence of the homozygous O^2 allele. We have shown here that these individuals rarely have the O^2 allele, but do have a higher frequency of the $O^{1variant}$ allele. Thus, the gene frequency for the $O^{1variant}$ allele in these samples is 57% in the *P. falciparum*-infected patients and 60% in the control group. Comparing the genotype and *O* allele frequencies of the groups studied, no statistically significant difference between the blood donors and the individuals suffering from malaria was found. The loci were in Hardy-Weinberg equilibrium in the present sample.

Table 2. Comparison of genotyping results and allele frequency of the O histo-blood system among blood donors and patients infected with *Plasmodium falciparum* from the Brazilian Amazon region.

	P. falciparum patients (N = 30)	Donors (N = 27)	P (Fisher exact test)
O^1O^1	6 (20%)	5 (18.5%)	0.5781
O^1O^2	2 (6.66%)	1 (3.7%)	0.5402
$O^{1v}O^1$	6 (20%)	7 (25.9%)	0.4136
$O^{1v}O^2$	4 (13.33%)	3 (11.1%)	0.5607
$O^{1v}O^{1v}$	12 (40%)	11 (40.74%)	0.5840
Allele frequency			
O^1	0.33	0.33	1.000
O^2	0.10	0.07	0.9066
O^{1v}	0.57	0.60	0.9918

When we analyzed the alleles of the O phenotype, the $O^{1variant}$ allele was the most frequent both in malaria and non-malaria groups with a consequent highest frequency of the homozygous genotype $O^{1v}O^{1v}$. Indeed, previous studies have shown that several isolated Indian populations in South America comprise only blood group O individuals and that they rarely have the originally described *O* allele (O^1) or O^2 allele, but instead have almost exclusively the O^{1v} allele (Franco et al., 1995). In contrast, the frequencies found for this allele in Brazilian whites and blacks are around 40 and 30%, respectively (Olsson et al., 1998). Comparing the Brazilian population data, our results show that $O^{1variant}$ allele frequencies are lower than the frequencies reported for some Amerindians and higher than those for populations of Caucasian and African ancestry (Olsson et al., 1998), probably due to the influence of Portuguese colonization in the northern region of Brazil as well as the presence of Amerindians (Batista dos Santos et al., 1999). On the other hand, previous studies in Southeast Brazil (Mattos et al., 2001) have shown lower $O^{1variant}$ allele frequencies than those found in our sample, probably because the indigenous contribution in this region of country is the smallest. Along the same line, the frequency of the A phenotype reaches values around 35%, and the *A* allele frequency is more than 0.25 in these populations (Mattos et al., 2001), while in our sample of the North region the frequencies were 21.2 and 0.13%, respectively.

To our knowledge, this is the first evaluation of ABO genotyping of malaria patients in Brazil. In regions that are highly endemic for *Plasmodium falciparum* malaria, it is well recognized that a range of red blood cell polymorphisms associated with resistance to severe diseases have undergone positive selection (Min-Oo and Gros, 2005). Moreover, as mentioned

before, the blood group O proved to be a protective factor against severe malaria (Barragan et al., 2000; Chung et al., 2005). However, since in the Brazilian Amazon region malaria predominates in Mesoendemic conditions with wide variation in transmission, malaria endemicity could be viewed as a selective pressure for maintenance of the observed frequencies of genotypes of the ABO system, which could be very interesting as the focus of a new investigation, including analysis of the genotypes in severe and non-severe malaria patients, as well as in individuals living in non-endemic areas.

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REFERENCES

Athreya BH and Coriell LL (1967). Relation of blood groups to infection. I. A survey and review of data suggesting possible relationship between malaria and blood groups. *Am. J. Epidemiol.* 86: 292-304.

Barragan A, Kremsner PG, Wahlgren M and Carlson J (2000). Blood group A antigen is a coreceptor in *Plasmodium falciparum* rosetting. *Infect. Immun.* 68: 2971-2975.

Batista dos Santos SE, Rodrigues JD, Ribeiro-dos-Santos AK and Zago MA (1999). Differential contribution of indigenous men and women to the formation of an urban population in the Amazon region as revealed by mtDNA and Y-DNA. *Am. J. Phys. Anthropol.* 109: 175-180.

Camargo EP, Alves F and Pereira da Silva LH (1999). Symptomless *Plasmodium vivax* infections in native Amazonians. *Lancet* 353: 1415-1416.

Carlson J, Helmby H, Hill AV, Brewster D, et al. (1990). Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* 336: 1457-1460.

Chester MA and Olsson ML (2001). The ABO blood group gene: a locus of considerable genetic diversity. *Transfus. Med. Rev.* 15: 177-200.

Chotivanich K, Udomsangpetch R, Simpson JA, Newton P, et al. (2000). Parasite multiplication potential and the severity of *Falciparum malaria*. *J. Infect. Dis.* 181: 1206-1209.

Chung WY, Gardiner DL, Hyland C, Gatton M, et al. (2005). Enhanced invasion of blood group A1 erythrocytes by *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 144: 128-130.

Cserti CM and Dzik WH (2007). The ABO blood group system and *Plasmodium falciparum malaria*. *Blood* 110: 2250-2258.

Fischer PR and Boone P (1998). Short report: severe malaria associated with blood group. *Am. J. Trop. Med. Hyg.* 58: 122-123.

Franco RF, Simoes BP, Guerreiro JF, Santos SE, et al. (1994). Molecular bases of the ABO blood groups of Indians from the Brazilian Amazon region. *Vox Sang.* 67: 299-301.

Franco RF, Simoes BP and Zago MA (1995). Relative frequencies of the two O alleles of the histo-blood ABH system in different racial groups. *Vox Sang.* 69: 50-52.

Guo SW and Thompson EA (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361-372.

Lell B, May J, Schmidt-Ott RJ, Lehman LG, et al. (1999). The role of red blood cell polymorphisms in resistance and susceptibility to malaria. *Clin. Infect. Dis.* 28: 794-799.

Loscertales MP, Owens S, O'Donnell J, Bunn J, et al. (2007). ABO blood group phenotypes and *Plasmodium falciparum malaria*: unlocking a pivotal mechanism. *Adv. Parasitol.* 65: 1-50.

Mattos LC, Sanchez FE, Cintra JR, Salles CFAB, et al. (2001). Genotipagem do locus ABO (9q34.1) em doadores de sangue da região noroeste do Estado de São Paulo. *Rev. Bras. Hematol. Hemoter.* 23: 15-22.

Min-Oo G and Gros P (2005). Erythrocyte variants and the nature of their malaria protective effect. *Cell Microbiol.* 7: 753-763.

Olsson ML and Chester MA (1996). Frequent occurrence of a variant O1 gene at the blood group ABO locus. *Vox Sang.* 70: 26-30.

Olsson ML, Santos SE, Guerreiro JF, Zago MA, et al. (1998). Heterogeneity of the O alleles at the blood group ABO locus in Amerindians. *Vox Sang.* 74: 46-50.

Roubinet F, Despiau S, Calafell F, Jin F, et al. (2004). Evolution of the O alleles of the human ABO blood group gene. *Transfusion* 44: 707-715.

Yamamoto F (2004). Review: ABO blood group system - ABH oligosaccharide antigens, anti-A and anti-B, A and B glycosyltransferases, and ABO genes. *Immunohematology* 20: 3-22.

Yamamoto F, McNeill PD and Hakomori S (1995). Genomic organization of human histo-blood group ABO genes. *Glycobiology* 5: 51-58.

Zago MA, Costa FF, Tone LG and Bottura C (1983). Hereditary hemoglobin disorders in a Brazilian population. *Hum. Hered.* 33: 125-129.

