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Research Article

The expression of ABH and Lewis antigens in Brazilian semi-isolated Black communities

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Abstract

The expression of the ABH and Lewis blood groups was determined in blood and saliva samples from two semi-isolated Black communities of Northern Brazil: Cametá and Alcântara. The distributions of ABO blood group phenotypes and the ABH secretor status frequencies showed no significant differences between these populations. In contrast, there was a difference regarding the frequency of the red blood cell Le(a-b-) phenotypes, associated with erythrocyte/saliva discordance, as confirmed by the observation that individuals with Le(a-b-) red cells have the Lewis antigen in their saliva, resulting in a nongenuine Le(a-b-) phenotype, whose frequency was higher in Alcântara.

Key words: semi-isolated black communities, Lewis blood group, ABH secretor status, dot-blot-ELISA.

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Introduction

The ABH and Lewis blood group systems were initially determined in erythrocytes, followed by other human tissues and secretions. In their systemic relationships, both competition and cooperation exist between the glycolsyltransferases produced by the Secretor, H, Lewis, and ABO loci (as reviewed by Clausen and Hakomori, 1989; Henry *et al.*, 1995; Oriol *et al.*, 1986; Watkins, 1980).

Four phenotype groups are usually observed: Lewis-positive or Lewis-negative, and secretor or nonsecretor of ABH soluble substances. In Lewis-positive and ABH secretor individuals, the main antigen is called Le^b. If allele Se is absent, the type 1 precursor chain is converted by the Le enzyme only into the Le^a determinant. In Lewis-negative individuals, the secretor phenotype has no influence.

The frequency of Le(a-b-) in American Blacks is almost 30%, and some individuals secrete Lewis substances in their saliva (Miller *et al.*, 1954). In African populations, the same phenotype can reach 40% (Mourant *et al.*, 1976). The contribution proportions of the Black component has been evaluated in some interracial populations. In Brazil, the frequency of Le(a-b-) is 25% in the Caucasoid population, and 27% in the Black population (Palatnik *et al.*, 1987).

A number of studies (Hammer *et al.*, 1981; Hirano *et al.*, 1987; Langkilde *et al.*, 1991; Makni *et al.*, 1987; Ørntoft *et al.*, 1991; Stingendal *et al.*, 1984; Yazawa *et al.*, 1988) have shown differences in the phenotypic expression of the Lewis antigens. Individuals with a Le(a-b-) phenotype, presenting no Lewis antigen expression in their erythrocytes, showed Lewis activity in their secretions.

Because of these variations, several studies (Elmgren *et al.*, 1993; Koda *et al.*, 1993; Liu *et al.*, 1996; Mollicone *et al.*, 1994; Nishiara *et al.*, 1993; Pang *et al.*, 1998) were carried out, to determine the most common frequencies of the Lewis phenotypes in different ethnic groups. Thus, the study of semi-isolated Black communities represents a contribution to the investigation of their variability.

The Brazilian Blacks descend from slaves brought from different regions of the African Continent, and are genetically very different from North-American and Caribbean Blacks. Predominantly genes of Bantu origin, but also of Benin, and some very rare ones of Senegambia origin have been observed. (Bortoline *et al.*, 1992; Pante 1995; Vergolino and Figueiredo, 1990).

In the present study, we report on the results of ABH and Lewis blood group system analyses of blood and saliva samples from two Brazilian Black semi-isolated communities.

Material and Methods

Populations sampled

Blood and saliva samples were collected from Black members of semi-isolated communities in the Brazilian cities of Cametá (State of Pará) and Alcântara (State of

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Maranhão). These communities consist of descendants of former slaves, who fled to isolated areas and formed villages called "quilombos", which still conserve their roots. If we consider all Blacks as one group, the degree of racial mixture may be estimated at about 20% with Caucasoid, and 30% with American Indian genes, respectively.

Voluntary participants were identified, and subsequently specimens were collected. Fifty-eight individuals were tested in Cametá, and 69 in Alcântara.

Samples: blood and saliva

Five-milliliter whole blood samples were drawn with heparin by venipuncture. Samples were stored at 4 °C and tested within 48 h. The remaining red cells were treated with glycerol and frozen at -20 °C. Simultaneously, saliva samples were collected and, within 1 h of collection, boiled during 15 min, centrifuged (at 3500 rpm for 10 min), and supernatants separated and stored at -20 °C for further testing.

Salivary ABH and Lewis Phenotypes

The presence or absence of ABH and Lewis antigens in saliva was determined by Dot-blot-ELISA with monoclonal antibodies (Ortho Diagnostic Systems), including anti-Le^a, anti-Le^b, anti-A, anti-B, and anti-H (Fresenius Diagnostics). The saliva samples were diluted at 1:2; 1:10; 1:50; and 1:100, in TRIS buffer, pH 7.4, and 5 µL of appropriate dilution plotted onto nitrocellulose membranes, as described by Pflug *et al.* (1989).

Erythrocyte phenotypes

ABO, including the A_1 subgroup, and Lewis were determined in fresh blood samples by standard agglutination methods. Red blood cells were washed and suspended in a 2-3% (v/v) saline solution, 50 µL of this suspension were mixed in tubes with 50 µL of specific antisera, then incubated for 45 min at room temperature, and the results were read by naked eye after centrifugation (at 1000 rpm for 1 min). All assays included appropriate known controls. The antisera used were the following: 1) human polyclonal anti-A and anti-B Ortho Diagnostic Systems; 2) anti-Le^a and Anti-Le^b of goat origin Ortho Diagnostic Systems; 3) anti-H (*Ulex europeaus*) and anti-A_1 (*Dolichus biflorus*) lectins were locally prepared, according to methods described by Boyd and Shapleigh (1954).

All red cell samples were also tested by dot-blot-ELISA, with the same monoclonal antibody batches used in the specific assay reported above for the evaluation of salivary antigens. The red cell suspensions were prepared at a concentration of 1:4 (v/v) in 0.09% saline solution, and 5 µL of appropriate dilution were plotted onto the nitrocellulose membrane.

Statistical analysis

The statistical analyses were carried out using the nonparametric Chi-square (χ^2) and G tests, with a significance level of 5%. In the case of polyallelism, the comparison between the gene frequencies of the ABO system blood groups was made using the graphic method of equivalent circles (Stevens, 1950); by this method, when representative circle images partially overlap, it shows that the population samples are homogeneous: in our case, with no significant difference between the gene frequencies of the ABO system.

Results

The distribution of ABO blood groups in two semi-isolated Black communities of North Brazil (Table I) showed no significant differences between either their phenotype frequencies or their gene frequencies (Figure 1). Both population samples fit the genetic equilibrium estimated for the ABO locus.

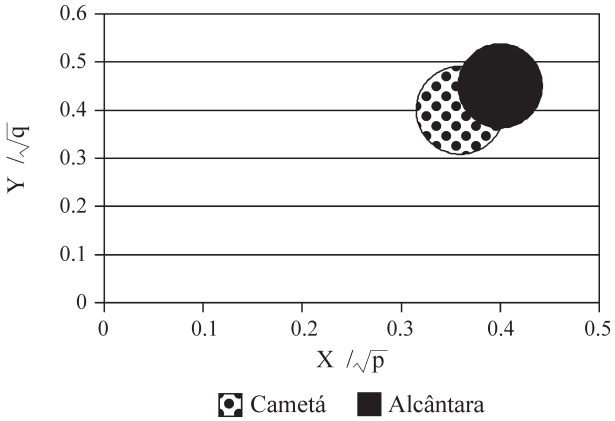

Figure 1 - Comparison of ABO gene frequencies by equivalent circles with 95% probability, in two Brazilian semi-isolated Black communities located in Cametá (PA) and Alcântara (MA).

Table I - Distribution of ABO blood group phenotypes and gene frequencies in Brazilian Black populations.

Population sample	N	ABO phenotypes (%)				Gene frequencies		
		A	B	AB	O	I^A	I^B	I^O
Cametá	91	18(20)	22(24)	05(05)	46(51)	0.14	0.16	0.70
Alcântara	104	26(25)	33(32)	04(04)	41(39)	0.16	0.20	0.64

G (Williams) test = 3.1261; (p) = 0.3726; df = 3.

Analyses of Lewis antigen expression, both in erythrocytes and in saliva, were used to determine the Lewis blood group phenotypes (Table II). Three groups were identified:

1) Lewis-positive ABH nonsecretor individuals, with red cell phenotype Le(a+b-), also expressing Le^a phenotype in saliva;

2) Lewis-positive, salivary ABH secretor individuals, with red cell phenotype Le(a-b+), producing Le^b antigen in saliva. The majority also produces Le^a antigen, detectable in saliva, but usually not in erythrocytes;

3) Lewis-negative, ABH secretor and nonsecretor individuals, with red cell phenotype Le(a-b-). However, these communities show a high degree of variation in the frequency of red cell Lewis phenotypes. Thus, they can be subdivided into two groups named nongenuine Lewis-negative individuals, with normal Le^a and/or Le^b antigen expression in saliva, and genuine Lewis-negative individuals, not expressing Lewis antigens, neither in erythrocytes nor in saliva.

In this survey, the red cell Lewis phenotype distribution in Cametá differed significantly from that found in the Alcântara population ($\chi^2 = 12.17$; df = 2; p = 0.002). A different range of variation also exists in the red cell Le(a-b-) blood group frequency. Indeed, the Lewis-negative frequency approaches 14% in the Cametá population, whereas, in the Alcântara population, the frequency of the Lewis-negative phenotype exceeds 40%. But, considering all the Lewis-negative salivary phenotypes (Table II), the prevalence of genuine Lewis-negative individuals is about 3.5% in Cametá, and 1.5% in Alcântara. However, the prevalence of nongenuine Lewis-negative individuals is 10.5% in Cametá and 40.5% in Alcântara. In marked contrast to this, secretor and nonsecretor frequencies (Table II) do not significantly differ in the two populations examined ($\chi^2 = 0.47$; df = 1; p = 0.49).

Table II - Distribution of Lewis blood groups and salivary secretor phenotypes in Brazilian Black populations.

Serum red cells	Salivary antigens		Cametá		Alcântara		
Lewis phenotype	Le^a	Le^b	ABH secretor status*				Total
			S	NS	S	NS	
Positive							
Le (a+b-)	+	-	0	9	0	7	16
Le (a-b+)	+	+	31	0	29	0	60
	-	+	10	0	4	0	14
Negative §							
G Le (a-b-)	-	-	0	2	1	0	3
NG Le (a-b-)	+	-	0	1	0	3	4
	-	+	5	0	21	0	26
	+	+	0	0	4	0	4
Total			46	12	59	10	127

*S = Secretor; NS = Nonsecretor.
§G = Genuine; NG = Nongenuine.

Discussion

The Lewis system is a histo-blood group producing antigens in secretions and plasma, which are subsequently adsorbed onto red cells (Marcus and Cass, 1969; Sneath and Sneath, 1965). However, these antigens can be more accurately detected in saliva secretions, due to secondary acquisition of Lewis antigens by red cells (Makni *et al.*, 1987). The Lewis phenotype is not easily distinguished by the conventional hemoagglutination test, which appears to be partly caused by the labile condition of the Lewis antigen expression and by the small number of Lewis antigens on the erythrocytes, resulting in sometimes very weak hemoagglutination reactions (Wang *et al.*, 1994).

The results presented show that the red cell Lewis antigen reactivity does not appear to be associated with the Lewis phenotype in the saliva, a conclusion supported by the observation that some individuals with Le(a-b-) red cells show Lewis reactivity in their secretions. Such individuals have been classified as nongenuine Lewis-negative, since they present α(1-4) fucosyltransferase activity and Lewis antigens in saliva (Langkilde *et al.*, 1991; Ørntoft *et al.*, 1991).

Analysis of the results shown in Table II raises the question of whether the observed prevalence variability of nongenuine Lewis-negative individuals in the isolated groups studied is biologically true, or rather a result of methodology.

This discrepancy, however, does not seem to conflict with the genetics of the ABH secretor status and Lewis antigen biosynthesis, which is a result of the enzyme products of alleles Se and Le. In saliva, Le^a has been found in Le(a-b-) nonsecretors, while Le^b can be identified in Le(a-b-) secretors.

Other investigators (Makni *et al.*, 1987; Miller *et al.*, 1954; Mollicone *et al.*, 1994; Ørntoft *et al.*, 1991; Palatnik *et al.*, 1987) have reported the existence of paradoxical erythrocyte/saliva discordance, that may have been explained previously by the presence of the weak Lewis allele (Le^w), which originates a fucosyltransferase that is less efficient than that codified by the dominant allele, thus determining a quantitative variation.

Recently published data (Elmgren *et al.*, 1993; Koda *et al.*, 1993; Liu *et al.*, 1996; Mollicone *et al.*, 1994; Nishiara *et al.*, 1993; Pang *et al.*, 1998), however, show the presence of a single-point mutation (T59G) in the transmembrane domain of the Lewis enzyme, which seems to be more globally distributed. This 59 mutation allele, when present in double dose, leads to a reduced enzyme activity, resulting in the nongenuine Le(a-b-) phenotype. This 59

mutation polymorphism was not investigated here, ruling out a comparison with the population samples studied.

Comparing our results with those previously reported for Black populations, we observed a difference in the red cell Lewis-negative phenotype frequency between the Cametá and Alcântara populations. A high frequency of the red cell Le(a-b-) type, already described in Black populations, was observed only in Alcântara (Table II). On the other hand, the results obtained for the ABO system show the expected rise in frequency of the I^B gene in both populations, with no significant differences between the two samples, showing them to be homogeneous with regard to the gene frequencies of ABO blood groups, therefore being considered as originating from the same African parental ethnic stock.

However, in accordance with previous publications (Hammer *et al.*, 1981; Hirano *et al.*, 1987; Langkilde *et al.*, 1991; Makni *et al.*, 1987; Ørntoft *et al.*, 1991; Stingendal *et al.*, 1984; Yazawa *et al.*, 1988), we believe that the high frequency of the Lewis (a-b-) red cell phenotype in the Alcântara population is probably a consequence of the change from Lewis-positive to Lewis-negative, predisposed to the nongenuine Lewis-negative phenotype, due to decreased concentration of circulating Lewis antigens occurring during diseases caused by parasites, infections, or other pathologic conditions.

This change may also have been produced by intrapopulational microdifferentiation of the population structure, caused by stochastic factors, particularly genetic drift, which is frequently observed in small and relatively isolated populations. Further studies focusing on codifier alleles of these fucosyltransferases could determine if this genetic variability adjusts to the differentiated expression of the Lewis antigen.

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