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Assessment of secretor status in Peruvian university students

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ABSTRACT

Objective: Soluble antigens of ABO blood group system are found in the mucosa, this characteristic is called secretor status, has been associated with various pathological conditions and is determined by the allele of the FUT2 gene present in the individual. The aim of the present study was to generate the first report of the secretor status in a Peruvian population. ***Materials and Methods:*** 102 undergraduate students were selected, requesting their consent to verify their blood group through direct hemagglutination and a saliva sample donation. Secretor status was determined by hemagglutination inhibition assay with saliva, being found that 5% of individuals were non-secretors. ***Results:*** Regarding the ABO system, 76% presented group O (soluble antigen H); 19%, group A and 5%, group B. ***Conclusion:*** A high frequency of the secretor phenotype was found, suggesting the importance of conducting studies to establish the heterozygosity of FUT2 gene and susceptibility to infections depending on the type of secreted antigen.

Keywords: hemagglutination inhibition, ABO blood group, saliva, secretor status.

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INTRODUCTION

Classification of blood groups allows to develop basic clinical activities such as blood transfusions, hemotherapy, and in the identification of people too. The ABO system evaluates the presence of A and B antigens at the red blood cells surface; however, these carbohydrates are not restricted to their surface, but also being found in body fluids such as saliva, gastric juice, breast milk and semen according to the secretor status of the person and independently of the blood group (1).

The secretor phenotype is defined by the *FUT2* gene, which encodes the enzyme fucosyltransferase-2 (FUT2), which catalyzes the addition of terminal fucose residues to cell surface glucidic chains, and it is essential in the synthesis of soluble H antigen, over which the ABO transferase can add glucosyl groups depending on the blood type of the individual (2). There is a polymorphism of *FUT2* that inhibits the activity of the enzyme in human populations and is responsible for a phenotype known as non-secretor status (3). In Caucasian populations, a greater presence of secretor individuals has been found (75%). In this population, a significant association between the secretor status of blood groups and the intestinal bifidobacteria composition has also been found (4).

Initial studies found a significant presence of the secretor status in patients with ankylosing spondylitis (5). Likewise, it has been reported that non-secretor individuals have a greater predisposition to certain types of cancers that are more prevalent in particular types of blood groups (6). Other studies have shown that the secretor status can be an influencing factor in the development of certain potentially malignant oral diseases (7). In particular, a predisposition to cancerous oral lesions was found due to the presence of the c.G428A polymorphism in the *FUT2* gene (8); however, resistance to norovirus infection is also attributed to this genotype (9).

In addition, the relationship between the secretion of blood antigens and the presence of gastric lesions generated by *Helicobacter pylori* infection has been evaluated. In some European and West African populations, no apparent relationship was found (10), in contrast to the relationship found in Pakistani patients (11).

There are no reports of the secretor status in Peruvian populations, therefore, the purpose of this work was to determine the phenotypic frequencies of the ABO, Rh and secretor ABH status in a sample of college students.

MATERIALS AND METHODS

Subjects

We evaluated 102 individuals, students at National University of San Marcos (UNMSM). The group of individuals included 49 (48%) women and 53 (52%) men with an average age of 20 ± 2.2 years. All the individuals were informed and requested their consent for their participation in the study, they accepted voluntarily and consistently, providing information on sex, age and blood group.

Direct hemagglutination

A small sample of blood obtained by capillary puncture was used for confirmation of the ABO-Rh blood group by agglutination with ABO-Rh monoclonal antibodies (Immucor).

Isolation of the soluble antigens

Approximately 3 mL of saliva was collected per individual in a sterile container. A portion of the collected saliva was transferred to 2 mL centrifuge tubes. To denature the salivary enzymes, the tubes were incubated to a water bath at 95 °C for 15 minutes, then centrifuged at 750 g for 10 minutes. The supernatant was transferred into a new centrifuge tube for the hemagglutination-inhibition assay.

Hemagglutination-inhibition assay

For A and B blood groups, a drop of saliva from the individual to be evaluated and a drop of the corresponding antibody (anti-A or anti-B) were mixed in a tube. It was incubated at room temperature for 10 minutes, a drop of human red blood cells (GRH) at 2% was added and the tube was centrifuged at 100 g for 1 minute. For O blood group, two drops of saliva and two drops of anti-H lectin (Immucor) were mixed. It was incubated at room temperature for 10 minutes, a drop of 4% GRH was added and incubated for 5 minutes according to the manufacturer's instructions. Finally, we proceeded with a centrifugation at 1000 g for 15 minutes. After a gentle mixing for antibodies and lectin reactions, the result was observed, where the hemagglutination indicates that the saliva does not contain the corresponding antigen (non-secretor status), while the absence of this is explained by the presence of soluble antigens, blocking the binding sites of the antibodies and avoiding their combination with red blood cells (Figure 1).

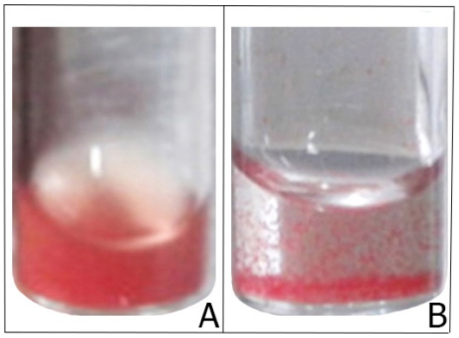

Figure 1. Controls for hemagglutination inhibition test. A) Positive control. Soluble antigens react with the commercial antibodies or the lectin, preventing erythrocytes agglutination. B) Negative control. The antibodies or the lectin react with erythrocytes without any interruption and agglutinate red blood cells.

RESULTS

National University of San Marcos student's population is constituted of 30 000 individuals (12), consequently, a sample of 102 individuals is representative with an error of 12.7 and a confidence interval of 99%. Blood groups in the sample were distributed as detailed below: 76% of individuals had O blood group, followed by A at 19% while B group was the least prevalent at 5%. As a consequence of multiallelic blood group inheritance, allele frequencies could not be calculated for this particular item. Regarding D antigen, 2% of individuals were Rh-negative, that means a relative allele frequency of 14% for recessive d allele. In relation to secretor status, 5 of the individuals were non-secretors (4 men and 1 woman). Non-secretor status establishes a recessive allele with a 22% relative allele frequency in the sample. These results are summarized in Table 1.

Phenotype	Phenotype frequency (%)
ABO blood group system (*ABO*)	
A	18.6
B	4.9
O	76.4
Rh blood group system (*RHD*)	
Rh-positive	98
Rh-negative	2
Secretor status (*FUT2*)	
Secretor	95
Non-secretor	5

Table 1. Blood group and secretor status frequencies.

DISCUSIÓN

The secretor status is a well-known biological condition, but, recently, its importance in clinical predisposition to certain pathologies have been analyzed. A vast number of this studies was done in European and Asiatic populations (1-7,11,13-15), where each region had a variable frequency of ABO blood group antigens in bodily fluids like saliva.

The non-secretor status was observed in 5% of samples processed, this condition is associated with a short number of variations, mainly in *FUT2* gene (3,4,8,10,13,14). Actually, for the Peruvian population, frequencies generated by the 1000 genomes project are available; however, our frequencies results are not identical to the ones found for most common mutations that cause the non-secretor status (FUT2: p.W134X, rs601338 and c.*1733A>G, rs632111) (14).

The 1000 genomes project accepted a maximum of two participants per family, with a total of 86 samples from Lima (16, 17). Our study considered 102 individuals, with major distribution and no family relationship among them. Thus, the information provided by 1000 genomes might not be accurate for this marker and its biological effect. According to their information for *FUT2* gene, Peruvian population had a MAF (Minor Allele Frequency) of 12.35% for the W134X mutation and 13.53% for the c.*1733A>G, where the non-secretor population might be equal to 3.35%, slightly different from the 5% reported by this study. Nevertheless, our study is limited by the phenotyping information and we need to establish what are the mutations involved in the absence of ABO blood group antigens in our sample´s saliva. In contrast, we found a Rh-negative relative allele frequency of 14%, value that is very similar to the 13.5% MAF found by the 1000 genomes project (17).

It is well known that the distribution of ABO blood group alleles varies between different populations (22). Our results match with the ones from a Mexican population (18), but are very different from a Turkish population, where the A group is predominant (19) or Indians, where the B group prevails.

When comparing our frequencies for non-secretor status with populations such as Greek (20) or Icelandic (21), we found great variations. In the Greek population the frequency of non-secretors was 23% (20), while in the Icelandic study control population, it was 42% (21). This last study also achieves to associate non-secretor status with the presence of caries, topic that have always been very controversial in scientific literature (20). Besides, secretor status and antigens from ABO and Rh systems had previously been associated with susceptibility to develop viral or microbial infections (9, 10) and oral lesions (6-8).

In Peru, no reports about ABO blood group antigen presence in fluids and its association with microbial infections had been presented. Thus, we present this report as a basis for future high-scale projects; we would suggest direct genotyping through PCR-based techniques and association studies with related pathologies as described before.

CONCLUSIONS

In the studied sample, the O blood group and the Rh-positive phenotypes prevail considering the ABO and the Rh systems, respectively. Most of individuals in the sample are secretor, that would be caused by a low frequency of the non-secretor mutant allele.

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