Frequency of subgroups of the antigen “a” among volunteer donors

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Abstract

Introduction: The presence of weak variants of blood type A represents a challenge in the practice of immunohematology for discrepancies in the time of the classification. It is common in blood banks to perform a forward and reverse typing for the purpose of confirming the blood type, but not all the people with a subgroup A2 have developed anti-A1 antibodies. **Objective:** We present a descriptive, observational and transversal study that establishes the proportion of subgroups of A antigen with the analysis of manual tube technique and monoclonal antibodies like anti-A, anti-A1 (Dolichus biflorus lectins extract) and anti-H. **Methods:** The analysis involved a total of 818 samples of voluntary blood donor, selected by random sampling, which were initially classified as 737 of Type A, and 81 as Type AB, with a confidence level of 95% (alpha error of 5% and 3% of precision). **Results:** The present study evaluated the existence of the subgroups A1, A2, A1B, A2B, A intermediate and A intB. **Conclusions:** It is recommended the identification of subgroups in different types of blood in the laboratory and blood banks.

KEY WORDS: Antigen A. Subgroup of A. Blood donors.

Introduction

Determining the presence of A and AB blood type subgroups will avoid problems not only at the blood transfusion level in blood banks, but also in cases of ABO-incompatible transplantations that cause the so-called graft-versus-host disease¹. Several studies demonstrate the importance of identifying antigen A subgroups, due to the immunogenic capacity they exhibit, which stimulates the production of anti-A1 antibodies². In the present study, the existence of the A2 and A2B subgroup was identified; in addition, an A intermediate and A intermediate B was detected with a frequency of 4% and 0.5%, respectively, which confirms that there is variability in blood group A in the Ecuadorian population. Another important aspect was associating the presence of A subgroups with the geographic place of origin, with a distribution of all A variants existing in the Imbabura province. These data are

related to those reported in the literature, which mentions that the A subgroups are more common in the Black than in the Caucasian race³. An estimate of 3.6% of the black population, 2.0% of mulattos and, country-wise, 75% of mestizos are settled in the Imbabura province⁴. The results of the subgroup variants we found corroborate what García mentions, “miscegenation or population mixture”³. This demonstrates the importance for A antigens and subgroups detection to be instituted in blood donors who were initially typified as A in order to prevent alloimmunization and to avoid hemolytic anemia of the newborn in women⁵.

Method

This study is of the cross-sectional, descriptive type. A total of 818 blood donors were analyzed, out of which 737 were initially typified as A and 81 as AB. Sample procurement: with good laboratory practice

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Table 1. Antigen A subgroups frequency with regard to blood donor's gender

Gender	A1		A1B		A2		A2B		Aint		AintB	
	n	%	n	%	n	%	n	%	n	%	n	%
Female	261	43.36	17	27.87	47	44.34	10	62.5	10	34.5	1	25
Male	341	56.64	44	72.13	59	55.66	6	37.5	19	65.5	3	75
Total	602	100.00	61	100	106	100	16	100	29	100	4	100

being adhered to, a segment was obtained of the tube of the red blood cell packs obtained from blood donors without producing ruptures or contamination of the red blood cell concentrate, thus obtaining the blood sample. During the study, there was no direct contact with the blood donor. Employed reagents: DiaClon immunoglobulin (Ig) M-type anti-A monoclonal antibodies for plates and tubes (Id. 10291), DiaClon IgM-type anti-B monoclonal antibodies for plates and tubes cell lines G1/2 (Id. 10301) and Bio-Rad anti-H lectin for in-tube test. Commercial cells: Diacell A1-A2, B (diapro) for quality control. Processing: all blood samples were diluted at 3% in saline prior to cell washing. Quality Control: following the protocol described in the article published by Franco and the American Association of Blood Banks Technical Manual^{6,7}, the avidness, specificity and potency parameters of these reagents were assessed. For the A1 and B cells, characteristics such as appearance, color, turbidity and affinity were verified. Subgroup determination: the in-tube methodology was used; by means of agglutination reaction, the presence or absence of the different A antigens was identified; a positive and a negative control were included for each test; reading was carried out with a visualization lamp. Statistical analysis: descriptive statistics was used for data analysis, and to verify if there was association between two variables, Pearson's correlation test was employed. Ethical aspects: All donors signed an informed consent authorizing the performance of "all the required tests" for the use of the donated blood (FOS.10).

Results

The data analysis determined the existence of A subgroups in the blood donors, with A1 being prevalent (73%), followed by A2 (13%); the presence of A1B and A2B was detected in a lower percentage (8% and 2%, respectively); an important finding was the existence of A intermediate and A intermediate B subgroups, with a frequency of 4 and 0.5%, respectively.

When the presence of antigen A subgroups was correlated with gender and age, subgroups A1 and A1B

were established to be more common in the male gender, as well as A-intermediate and A-intermediate B. The A2B subgroup is more common in females; however, this relationship was not statistically significant ($p > 0.05$). Table 1 shows the existence of antigen A variants that give rise to the existence of 6 blood subgroups in the Ecuadorian population. The A subgroups age distribution analysis established that, between 17 and 49 years of age, there are A variants in 28.9% and 36.8%, respectively. With these results, the search for anti-A1 antibodies was carried out, with only one donor being found to be alloimmunized. The analysis of red blood cell antigen A subgroups distribution and the Ecuadorian blood donors place of origin was significant ($p < 0.05$) in subgroups A1 and A2; subgroups A1 and A2B are distributed in the Esmeraldas, Imbabura, Santo Domingo, Pichincha and Tulcán provinces, whereas A2, A2B and A-intermediate were localized in 3 Ecuadorian provinces and A-intermediate B only in the Pichincha province. Table 2 shows A subgroups distribution in Ecuadorian provinces with important population migration.

Discussion

The methods for blood selection and classification such as direct and inverse typing, antibody screening and irregular antibody identification are the processes that are commonly carried out in the country's blood banks¹²; however, A and AB blood subgroups typing is not systematically performed, since anti-A1 antibodies were considered to be alloantibodies; i.e., that they are produced after previous contact in 1% of people considered to be A2⁷. However, several studies establish the importance of antigen A subgroups identification in post-transfusion reactions, hemolytic anemia of the newborn, and in renal and stem cell transplantations owing to the immunogenicity presented by these antigens by stimulating the production of anti-A1 antibodies². One case of alloimmunization by incompatible blood transfusion was reported after the patient received a second AB blood transfusion; initially, he didn't suffer any hemolytic reaction; however, when he received incompatible blood again, he

Table 2. Frequency of antigen A subgroups per province

	Esmeraldas		Imbabura		Pichincha		Sto. Domingo		Tulcán	
	n	%	n	%	n	%	n	%	n	%
A1	3	75.00	26	68.42	541	75.24	31	55.36	1	100
A1B	1	25.00	5	13.16	46	6.40	9	16.07		
A2			3	7.89	95	13.21	8	14.29		
A2B			3	7.89	11	1.53	2	3.57		
Aint			1	2.63	22	3.06	6	10.71		
AintB					4	0.56				
Total	4	100	38	100	719	100	56	100.00	1	100

experienced hemolytic anemia owing to the presence of anti-A1 IgG antibodies that were developed after the first transfusion⁸.

In the present study, the existence of subgroup A2 was determined in 13% of donors, A1B in 8%, A2B in 2% and A-intermediate and A intB at a frequency of 4 and 0.5%, respectively, which confirms that there is antigen A variability in the Ecuadorian population. Red blood cells that share type A1 and A2 characteristics are defined as A-intermediate, and are more common in the Black than in the Caucasian race. In this study, a prevalence of 4% was detected, just as in the study carried out in Cuba. This group A variant is relevant in population immunogenetics studies, and has contributed to the knowledge about population miscegenation with the Black race⁹. The presence of A blood group weak variants should also be considered as representing a challenge in the practice of transfusional medicine due to the discrepancies at the moment of typing⁹. The anti-A1 antibody is generally a cold agglutinin that atypically appears in the serum of A2 or A2B individuals lacking this antigen; however, in alloimmunization cases, clinically significant IgG-type antibodies are produced, which is highly common after blood transfusion or during pregnancy; at that moment, it turns into a hemolytic reaction-producing antibody, and it is therefore necessary for the donor and/or patient to know his/her true subgroup in order to prevent unnecessary alloimmunization⁷.

Anti-A1 antibodies are found in between 1% and 2% in A2 people, and in 25% of A2B subjects¹⁰. In this study, a percentage of 1% was determined; the remaining 99% are not alloimmunized yet, and prevention is therefore important, with this being achieved by identifying the presence of A subgroups. In this study, a distribution of A subgroups in groups considered to be of

reproductive age was determined. This is an important finding, since this is the young age group that comprises blood donors that can become repetitive volunteers; furthermore, it includes women of child-bearing age. All these variables turn into risk factors for alloimmunization, either by blood transfusion or pregnancy. The immune response that is produced against antigens of the ABO system ends up in complement activation, which elicits a hemolytic reaction that can be mild or serious^{8,11}. ABO system incompatibility during pregnancy is a common hematologic problem that is going to affect the newborn; it is usually benign, but if the mother has been previously alloimmunized, it can cause hemolytic anemia of the newborn^{3,11}.

The presence of A subgroups was also correlated with the place of origin, with a percentage of A1 of 75% being found both in donors who were native to Esmeralda and Pichincha, with all A variants being distributed in the Imbabura province. These data are consistent with those reported in the literature, which refer that the A subgroups are more common in the Black than in the Caucasian race³. About 3.6% of the black population, 2.0% of mulattos and, country-wise, 75% of mestizos⁴ are estimated to be settled in the Imbabura province. The results of the identified subgroups variants corroborate what García refers, "miscegenation or population mixture"³. All these analyses demonstrate the importance for A antigen and subgroups detection to be instituted not only in blood donors, but also in clinical laboratories, hospitals and transfusional medicine centers users in order to prevent alloimmunization⁷.

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