Abstract

Introduction: The Diego blood group is an irregular blood system which has been involved in cases of hemolytic disease of the newborn and post transfusion reactions, in this system have been identified 22 erythrocyte antigens, among which the pair Di^a/ Di^b is the most important because those have the most immunogenic potential. Objective: This research aims to determine the frequency of antigen Di^a and their respective alloantibody in the Ecuadorian population. Methods: It was performed a simple random sampling in the donor population, being later tested tube agglutination by the presence or absence of the antigen and its alloantibody Di^a applying gel agglutination technique. Results: It was observed an antigen prevalence of 25% against a 6.09% of percentage alloimmunization due to Di^a antigen, without significant differences between men and women and these being independent of the age and origin of the donor, showing that there are some Diego positive cases in Ecuadorian population as probably cases of transfusional alloimmunization or due to fetal-maternal alloimmunization. Conclusions and Recommendations: The frequency distribution of antigens and alloantibodies from the Diego blood group is almost uniform in the population, due presumably to the high incidence of miscegenation in our country. Therefore it becomes vitally important the implementation of this blood system inside the protocols of irregular antibodies identification in Ecuadorian blood banks.


Introduction

Within the classification of blood groups, the Diego antigen or factor was the tenth to be discovered among the red blood cell antigens. After this finding by Miguel Layrisse and Tuli Arends, identification of this antigen gained importance, with several studies being conducted, especially in indigenous groups of the Venezuelan and Caribbean populations, and a prevalence of 35.54% being found. This alerted Latin American countries about the existence of a new red blood cell antigen able to elicit the production of antigens that cause hemolytic disease of the newborn (HDNB). One of the particular characteristics of this antibody is that it can be present as an immune response or naturally and cause an immediate hemolytic reaction.

Many times, alloimmunization by red blood cell antigens of the Diego system goes unnoticed, and it is until transfusional reactions or fatal hemolysis occur in neonates when its existence is determined in compromised patients, thus giving raise to doubt about the percentage of the population that is inadvertently exposed to these antigens.

In Ecuador, information about the presence of Diego system antigens and alloantibodies is not known, and even more when it comes to studies on the prevalence of this system, about which information is nearly inexistent. Similarly, the incidence of HDNB cases caused...
by irregular blood systems is unknown, which opens
the question on the prevalence of this blood system
in the country, taking into account that the population
is not significantly or anthropologically different from
the rest of the Latin population of the continent. Fur-
thermore, in the transfusional field, reactions pro-
duced by antigens of the ABO and Rh system have
been given more attention, wrongly leaving aside the
reactions produced by irregular antibodies, including
those of the Diego system6,7.

All investigations related to this antigen provide the
scientific bases for including its identification in regu-
lar blood typing and pre-transfusion tests, especially
in populations with significant prevalence and indige-
nous population. In Ecuador, the National Institute
of Statistics and Censuses (INEC – Instituto Nacional de
Estadística y Censos) establishes that there is an el-
evated incidence of indigenous population (approx-
imately one million inhabitants), which determines for
7% of the population to be considered ethnic popula-
tion3,9. Therefore, Diego antigen screening should be
considered, especially in women of childbearing age
and prior to blood transfusions.

Methods

A cross-sectional, descriptive observational study
with simple random sampling was carried out for the
determination of both Diα antigens and anti-Diα alloan-
tibodies. Sample collection: pilot tubes obtained during
the donation process were used and maintained in the
serum bank of the blood center triage station. All do-
nors signed an informed consent authorizing the per-
formance of “all the required tests” prior to the use of
the blood products (FOS.10). Reagents used: Bio-Rad
ID-Diα (Diego)-positive; 0.8% suspension, Id. 05980;
ID anti-Diα dried-monoclonal antibody; Bio-Rad albu-
min 30% batch 11840 and: Bio-Rad DiaClon Coombs
Serum Green, Id. 14060 and Coombs-Gel Grifols
cards Id. 3009280. Sample processing: the in-tube
 technique was used and, by means of agglutination
 reaction, the presence or absence of Diα antigen was
determined. For alloantibody detection, serum and the
gel technique were used for higher sensitivity. Statis-
tical analysis: an Excel-based registry was maintained,
where the following data were captured: code, age,
gender, place of origin, results, SABO blood group, Diα
antigen and anti-Diα antibodies, with coding being
maintained for analysis with descriptive statistics,
whereas for the between-variables relationship, the
chi-square statistical test was used2.

Results

A total of 383 donors originating from 22 provinces
of the Ecuadorian territory were analyzed, with a prev-
elence of Diα antigen of 25% being determined, dis-
tributed in 22 provinces, with the Pichincha, Loja,
Manabí and El Oro provinces showing the highest
prevalence of this antigen; other provinces of the cen-
tral mountain range that showed high prevalence were
Chimborazo (9%), Tungurahua (8%) and Cotopaxi
(7%). The Manabí province also stands out, with 10% (Fig.
1).

The relationship between Diα antigen and donor
gender was not significant (p > 0.05), with a preva-
elence of 35% being identified in females and 20% in
males.

A percentage of alloimmunization of 6.09% was
identified in the population of Ecuadorian donors (for
this study, 18 provinces were included in the random
sampling). Diα antigen-alloimmunized individuals were
found in 12 of the 18 participating provinces. Manabí
had the highest prevalence with 31.32% alloimmuni-
ization, followed by Guayas with 13.64% and Los Ríos
and Pichincha with 9.09% each (Fig. 2).

There is a higher alloimmunization rate in females
(6.30%) than in males (5.52%); this may be due to
predisposing factors such as previous pregnancies or
transfusions. With regard to age, anti-Diα was found
to be distributed in all age groups; however, this rela-
tionship of variables was not significant (p > 0.05).

Discussion

The prevalence of antigens of the Diego system,
specifically of the Diα or Di1 racial antigen, is almost
inexistent in Afro-American and Caucasian popula-
tions, but it is relatively common in the Latin American
indigenous population3,9. In the present study, a prev-
elence of Diα antigen of 25% was found in Ecuadorian
blood donors, in contrast with a study carried out in
Guatemala, where total prevalence of Diego Di1
antigen was established at 7.5% in blood donors,
distributed between the Mestizo and indigenous pop-
ulations, with a prevalence of 12.99% and 3.90%,
respectively10. The present investigation does not al-
low determining ethnic differences because in the do-
nation form there is not a question specifying donor’sethnicity. However, according to INEC, in Ecuador
there is a high incidence of indigenous population,
approximately one million inhabitants, which deter-
mines for 7% of the population to be considered
as ethnic population and, therefore, the elevated prevalence of DiA antigen found in this study might be understood. In addition, said percentage is almost uniformly distributed throughout the national territory, with marked population foci observed in the andean provinces. Thus, in this study, 24% of donors with positive DiA antigen were identified to be found in the province of Pichincha, followed by Loja with 16%, Manabí with 10%, in addition to Chimborazo, Tungurahua and Cotopaxi with a prevalence of 9, 8 and 7%, respectively. This corroborates the distribution of this antigen in the andean provinces of the country, with the central mountain range being an important focus of DiA antigen distribution.

Identification and localization of blood donors who are carriers of the Diego antigen is highly useful for blood banks owing to the relationship between this antigen and the production of antibodies that cause post-transfusion hemolytic reactions. The study conducted in Guatemala determined that 3.5% of polytransfused patients had anti-DiA antibodies. In spite of not causing an immediate severe hemolytic response, it is one of the causes of early post-transfusion reactions, which are difficult to detect, so that a second incompatible transfusion might then cause a severe hemolytic reaction. In this study, the Pichincha province was established to have a higher prevalence of DiA antigen with regard to the other provinces, and identification of this antigen should be therefore performed, since this region is the major provider of blood to the country’s health centers.
Another aspect considered in this investigation was the prevalence of the Diego (Di) antigen with regard to gender, with a similar distribution being determined between males and females and their age groups. These data constitute relevant information due to the likelihood of alloimmunization in women of childbearing age; even when there is a high prevalence, there is also a percentage of women lacking the antigen, which might cause adverse effects in an incompatible blood transfusion, and even HDNB. Layrisse, Arends and Domínguez were the researchers who identified the first case of HDNB, determining that the child’s red blood cells were sensitized by anti-Di antibodies originating in the mother\(^1\)\(^{11}\).  

Subsequently, the Diego antigen, initially considered to be of low prevalence, was established to have become a “marker of Mongoloid populations with high genetic and anthropologic value”\(^3\). Several studies have established that the Diego antigen is part of a red blood cell membrane protein that accounts for 25% of total red blood protein, and for this reason, today it is one of the antigens that should be identified in populations with high prevalence\(^2\).

Screening for irregular antibodies or alloantibodies in blood donors is within the guidelines of blood bank standards\(^12\); however, commercial cells used for this purpose do not detect Diego system alloantibodies\(^13,14\), since these cells are prepared in countries where Diego antigens are not common, and finding cells with this phenotype is therefore not possible\(^2\). This is why introducing these reagents is necessary in countries with prevalence of these antigens. This way, in Singapore, the use of Di\(^a\) cells enabled determining the presence of alloimmunization by the Diego system in 19 out of 1,383 samples with inconclusive results in the panel of commercial cells\(^15\).

Different studies have determined the presence of anti-Di\(^a\) alloantibodies in blood donors within a range from 0.02% to 13.0%\(^16\). In this investigation, a prevalence of anti-Di\(^a\) of 6% was detected in blood donors, whereas in Texas, in the Corpus Christy area, a prevalence of anti-Di\(^a\) antibodies of 1.3% was reported in
individuals with previous transfusions\(^7\); in Brazil, 112 patients (3.6\%) with anti-D\(\text{a}\) were identified; in Singapore, a prevalence of 1.37\% was determined\(^2,15\), and in the study carried out in Guatemala, a prevalence of anti-D\(\text{a}\) of 3.5\% was found in multi-transfused patients\(^9\). Anti-D\(\text{a}\) antibodies are generally of the immunoglobulin (Ig) G1 and IgG3 types and are related to hemolytic disease of the newborn\(^18\). In a study carried out in Brazil, the presence of anti-D\(\text{a}\) in women sensitized by anti-D\(\text{a}\) during pregnancy; however, the newborns had no problems, owing to the follow-up throughout the natal period. This reinforces the need for screening to be implemented in countries with Diego antigens prevalence\(^9\).

The presence of anti-D\(\text{a}\) is directly related to transfusional hemolytic reactions. One case reported in 2012 referred the presence of a transfusional reaction in a woman after having received 800 mL of blood during surgery; pre-transfusion tests had been negative; however, she had symptoms related to hemolysis, with subsequent tests identifying the presence of anti-D\(\text{a}\) alloantibodies\(^14\).

Finally, inclusion of the Diego system in screening tests for irregular antibodies both in blood donors and multi-transfused patients constitutes an emergent aspect owing to the Ecuadorian population demographic characteristics and to the percentage of unidentified alloantibodies existing in the Ecuadorian Red Cross Blood Center and blood banks that perform these tests, in addition to the fact that commercial cells for screening lack these antigens. Therefore, one recommendation to the national Ecuadorian blood system is to see changes in the follow-up regulations for pregnant women within the Free Maternity Program, especially in those provinces where the existence of alloantibodies of the Diego system (D\(\text{a}\)) were reported.

**Conflict of interests**

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