

A, A1, H (A2) and B red blood cell antigens in blood donor

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ABSTRACT

154 blood samples were tested for the antigens of red blood cell groups. The samples were provided from the Hematology laboratory of “Iris Borchashvili Health Center Medina Ltd” (on of the biggest local hospital). The laboratory analysis was conducted in the immunogenetic laboratory of Batumi Shota Rustaveli State University. The immunoserological express method was used for screening the erythrocyte blood group antigens. We used the following monoclonal antibodies: anti- A,-A1,- H (A2),-B,-AB. A reverse method was also used for the study. The distribution of the ABO group system in the studied donor population is as follows: O > A > B > AB. Among the studied blood donors, antigen A occurs in 61 donors, antigen B in 23 cases, and H (A2) antigen was found in 82 donors. Accordingly, H (A2) antigen is found at a frequency of 53.24%, the distribution of antigen A is 39.1%, and antigen B occurs with the frequency of 14.93%. The vast majority of donors of the phenotypic group A (II) contain A1. A2(H) antigens were detected only in single cases. As for the phenotypic group AB (IV), only subgroup A1 antigen was found.

Key words: Blood-antigens-erythrocyte-distribution-donors.

1 INTRODUCTION:

Red blood cell (RBC) blood group antigens are polymorphic, inherited, carbohydrate or protein structures located on the extracellular surface of the RBC membrane. They contribute to the architecture of the RBC membrane, and their individual function(s) are being slowly revealed. The biological qualities assigned to these RBC membrane structures are based on observed physiological alteration in RBCs that lack the component, by documenting similarities in its protein sequence (predicted from the nucleotide sequence of the gene) to proteins of known function and by extrapolation to identified functional homologues in other cells. The varied roles of RBC antigens include membrane structural integrity, the transport of molecules through the membrane, as receptors for extracellular ligands, adhesion molecules, enzymes, complement components and regulators, and in glycocalyx formation [1]. At present, the International Society of Blood Transfusion (ISBT) approves as

29 human blood group systems [2].

The H blood group system, ISBT symbol H (018), consists of a single antigen (H) defined by a terminal fucose residue found on red blood cells and in secretions formed by the action of α -1,2-fucosyltransferases 1 (α 2FucT1) and 2 (α 2FucT2), respectively. Mutant alleles of the corresponding FUT1 and FUT2 genes result in either a H- phenotype (Bombay phenotype, Oh) or a weak H phenotype (para-Bombay, H+w). In addition, the FUT2 gene is the molecular basis of the secretor (Se) status, and homozygosity or compound heterozygosity for null alleles is associated with the nonsecretor (se) status. H individuals have natural anti-H (mostly IgM), which can cause severe hemolytic transfusion reactions with intravascular hemolysis [3]. The ABO blood group system consists of four antigens (A, B, O and AB). These antigens are known as oligosaccharide antigens, and widely expressed on the membranes of red cell and tissue cells as well as, in the saliva and body fluid. The ABO blood group antigens are one of the most important issues in transfusion medicine to evaluate the adaptability of donor blood cells with bone marrow transplantations, and lifespan

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of the hemocytes [2].

At present, Increasing numbers of blood donors are recruited to participate in biomedical research. As blood services depend on voluntary donors, successful recruitment calls for a better understanding of donors' expectations and attitudes toward the use of samples in research [4].

Donor and recipient factors play a role in RBC alloimmunization and range from characteristics of particular blood group antigens to the recipient's ability to present the antigens to their immune system. In addition to genetic factors, emerging data emphasize the importance of environmental factors in the formation of RBC alloantibodies [5].

Our aim is to study the distribution of erythrocyte A, A1, H (A2) and B antigens in donors. There are several data where is study the distribution of erythrocyte blood group antigens in different groups, where is shown the high polymorphisms of this characteristics [6–9].

2 MATERIALS AND METHODS:

154 blood samples were tested for the antigens of red blood cell groups. The samples were provided from the Hematology laboratory of "Iris Borchashvili Health Center Medina Ltd" (on of the biggest local hospital). The laboratory analysis was conducted in the immunogenetic laboratory of Batumi Shota Rustaveli State University. Written approval of the study was obtained from the Ethics Committee of the Clinic's.

The gender balance of donors is disrupted, in particular, the majority of donors are men ($n = 127$). Only a small proportion of the studied donors are women ($n=27$). In accordance with the permitted and recommended norms, a certain age group of people is involved in donation. The age of donors varies from 18 to 60 years with a personal weight above 50 kg, and who met the hemoglobin cut off criteria. All donors were required to have a hemoglobin level of at least 12.0 g/dL for females and 13.0 g/dL for males as per WHO standards. Donor samples were taken within 2018-2019.

The samples were collected in special tubes (2 ml) with an anticoagulant (EDTA). We used blood samples from the blood bank department of the clinic's and, did not performed additional invasion and had no contact with donors. The material provided was anonymous. Both plasma and red blood cell were used to detect specific antigens and antibodies of red blood cells.

The immunoserological express method was used for screening the erythrocyte blood group antigens. We used the following monoclonal antibodies: anti- A,-A1,- H (A2),- B,-AB. This method allows to detect of specific antigens on the surface of red blood cell membranes on the basis of agglutination ability. We screened A, A1, H (A2) and B antigens in the RBC of the donors.

A reverse method was also used for the study, which is widely used to determine the blood group, which considers the detection of both A and B antigens on the erythrocyte membrane (direct method) and Anti-A and Anti-B in the plasma (reverse method) simultaneously.

3 RESULTS:

The distribution of the ABO group system in the studied donor population is as follows: $O > A > B > AB$. As can be seen in the following diagram, the O (I) phenotypic group is represented with a high distribution frequency (50.65%) ($n = 78$), the distribution frequency of group A (II) is 34.42% ($n = 53$), B (III) phenotype occurs among donors with a percentage 9,74% ($n=9,74$). AB blood group is represented with less frequency (5,19 %) ($n=8$)Figure 1.

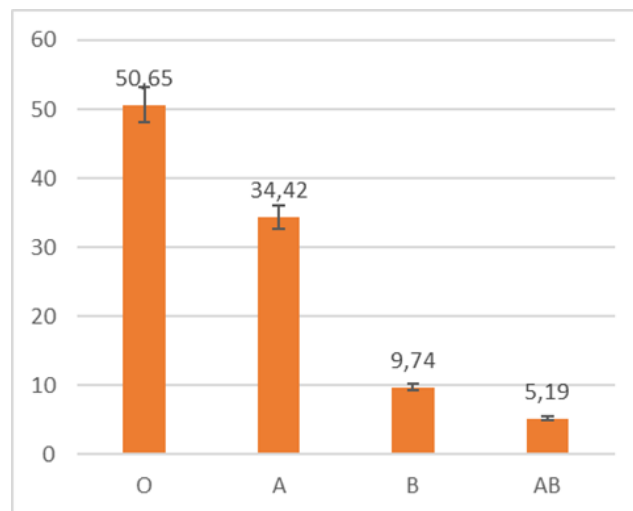


Figure 1. The distribution of of ABO group system phenotypes in blood donors.

Among the studied blood donors, antigen A occurs in 61 donors, antigen B in 23 cases, and H (A2) antigen was found in 82 donors. Accordingly, H (A2) antigen is found at a frequency of 53.24%, the distribution of antigen A is 39.1%, and antigen B occurs with the frequency of 14.93% Figure 2.

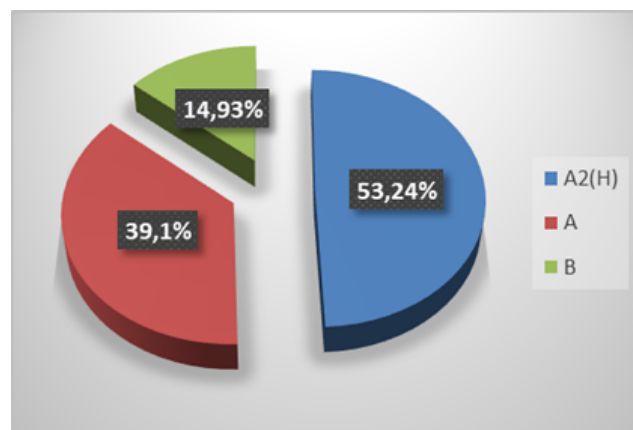


Figure 2. A, B and H (A2) antigens in the studied blood donors.

As is known, antigen A occurs in the case of the phenotypic group A (II) and AB (IV). We were interested in looking at subgroups of antigen A-A1 and A2 in the donor population. The vast majority of donors of the phenotypic

group A (II) contain A1. A2(H) antigens were detected only in single cases. As for the phenotypic group AB (IV), only subgroup A1 antigen was found.

4 DISCUSSION:

As mentioned in the literature, the H group system contains only one H antigen and is genetically independent of the ABO system. Antigen H is an intermediate, precursor substance in the biosynthesis of determinants A and B antigens. Due to this, this antigen plays the largest role in determining the blood group. To detect antigen H, we used the monoclonal antibody Anti-H (A2). We evaluated the agglutination ability of antigen H with both the naked eye and a microscope with various magnifications, in particular, we used an optical microscope with a 4X10, 10X10, and 40X10 magnification lens Figure 3 .

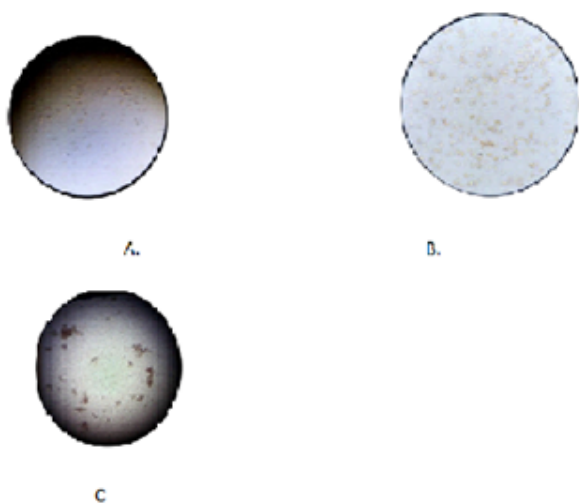


Figure 3. Different agglutination ability of H antigen. A. lack of agglutination, B. weak agglutination; C. strong agglutination (optical microscope with a 10X10 lens).

Agglutination of antigen H was detected in almost every donor of the phenotypic group O (I). In some cases, clear agglutination can be seen even with the naked eye, but in most cases an optical microscope with a lens of various magnifications were used. An interesting picture can be seen among donors of the group A(II). As mentioned above, 53 studied donors belong to the phenotypic group A (II), and most of them are characterized by the ability to agglutinate with monoclonal antibodies anti-A, anti-A1, anti-AB; They do not show agglutination with monoclonal anti-H (A2) at all. In this case, a variation of the A1 group without agglutination of the H antigen was detected in the donor. This donor is homozygote the genotype A1/A1. In three cases, in donors having a phenotypic group A (II), agglutination was detected with all monoclonal antibodies Anti-A, Anti-A1,

Anti-H (A2) and anti-AB. Most likely, these donors belong to the heterozygous A1 / O genotype Figure 4 .

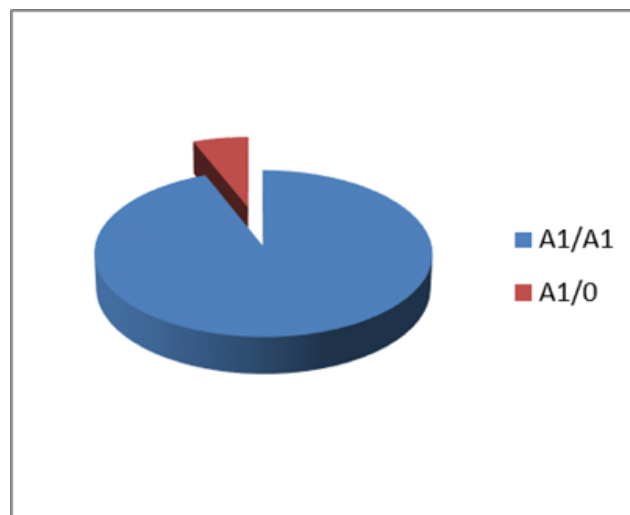


Figure 4. Genotypes A1/A1 and A1/ O in donors having phenotypic group A(II).

A similar pattern was observed in the case of phenotypic groups B (III). 15 cases were identified among donors of the phenotypic group B (III). Among them, erythrocytes of 14 donors show agglutination only with Anti-B and anti-AB antibody, and the absence of agglutination occurs with monoclonal Anti-H. In the first case, the donors of group B (III) are homozygotes with the B / B genotype, and in the second case, most likely, we have the case with the heterozygous B / O genotype, where agglutination were expressed with monoclonal anti-B, anti-AB and anti-H antibodies Figure 5 .

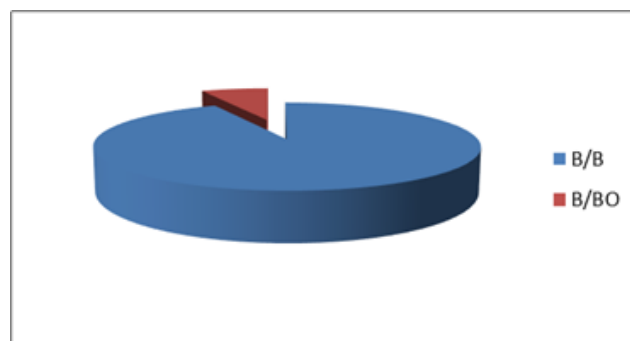


Figure 5. B/B and B/ O genotypes in donors belonging to phenotypic group B (III).

The donors of group AB (IV), which we studied, contain only subgroup A1 of antigen A and correspond to genotype A1B. In no case was the A2B genotype detected.

5 CONCLUSION:

We believe that these data are important for donor centers in terms of rational preparation of blood components; In

addition, the role of H antigen should be considered for the proper identification of blood groups.

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