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Topic: ,,The immunogenetic features of "hard defined" blood groups''

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Introduction

Group antigens determine the trophic and regulatory functions of blood cells. They are part of cellular receptors, through which hormones, vitamins, enzymes and other biologically active proteins are transported in the circulatory system and are the main structural elements of cell membrane adhesion (Минеева 2004, 2010; Westhoff ...2004; Kormoczi ... 2009). Blood group antigens determine the adaptation of a human as a biological species to the environment. The frequency of distribution of blood groups is not the same for different races and ethnic groups and is considered a manifestation of the genetic-geographical adaptation of a particular ecosystem in the process of evolution. (Makroo ...2013; Musa...2012). The clinical significance of blood group antigens is determined by their immunogenicity - their ability to create antibodies; they can damage erythrocytes, leukocytes, and thrombocytes. These antibodies cause post-transfusion difficulties, various types of transfusion reactions, neutropenia, and hemolytic disease in newborns. (Cheng ... 2012). Blood groups are determined by the absence or presence of a specific antigen on the surface of red blood cells. Currently, about 39 systems of erythrocyte groups are distinguished, which include about 346 antigens that can provoke the most severe transfusion reactions. (Lane…2015). From a clinical point of view, ABO, Rh, Kell, MNSs and other systems are the most important. The clinical significance of blood group antigens is determined by their immunogenicity - their ability to create antibodies; they can damage erythrocytes, leukocytes, and thrombocytes. These antibodies cause post-transfusion difficulties, various types of transfusion reactions.

The main biomedical value of the antigens of the erythrocyte group is largely related to the living immune properties.These antigens are of particular importance in blood transfusion, in particular, the exact blood grouping is very important when it comes to blood transfusion. The antigens of the ABO system are also considered tissue antigens and are therefore of particular importance in organ transplantation and epidemiology. Specific antigens of the erythrocyte group can cause immune sensitization and hemolytic diseases of various complexity during pregnancy or blood transfusion in case of incompatibility (Manoj… 2014). From the point of view of genetics, the study of blood group antigens is important for determining population characteristics (https://www.britannica.com/science/blood-group/The-importance-of-antigens-and-antibodies). Humans are so individualized by group antigens that they also serve as identifiers.

Antigens of different systems of blood groups are unevenly distributed in the population. This means that populations differ in the composition of antigens. Some antigens are very rare in a particular population and therefore it is difficult to find a compatible donor.

Technical mistake made in determining group specifics is also important. Erythrocyte group antigen A, which occurs on the membrane surface of human red cells with blood groups A (II) and AB (IV), is commonly represented in two subgroups: A1 and A2. Among them, both quantitative and qualitative distinctive features are noted. In contrast to the A1 antigenic determinant, erythrocytes with a specificity of the A2 su-group are characterized by weak agglutinating ability with the monoclonal anti-A antibodies used in the study. Therefore, the method for determining group affiliation creates a high probability of the risk of agglutination of erythrocytes of the A2 subgroup by the plate method. Especially when agglutination is assessed with the naked eye. Based on the above, it is possible that blood group A (II) may be mistakenly assigned to group O (I) and AB (IV) -to B (III). In most transfusion centers in the world, the blood of donors at transfusion stations and blood banks is examined at the level of minor erythrocyte antigens. Often about 12-13 immunogenic antigens important for blood transfusion is studied and the probability of compatibility between donor and recipient are assessed accordingly. Currently, two antigens of ABO system (A,B) and five antigens of Rh system((D, C, c, E and e) are considered due to high immunogenicity in blood transfusion. In theory, there is a risk of high alloimmune sensitization in people who do not have these antigens. The compatibility between the donor and the recipient in the region is assessed only by the similarity or difference of the three antigenic compositions, however, no literature mentions that, in addition to these three vital antigens, the compatibility between the donor and the recipient should be assessed by other dangerous antigens in terms of transfusion.

newborns create an interesting study group; in contrast to adults, antigens A and B in some newborns are poorly expressed in comparison with erythrocytes, and the corresponding agglutinins may not be present in the blood serum, which causes certain difficulties in determining the blood group. From a scientific point of view, the biological material of donors and newborns is an interesting object of research. The composition of donor's blood and the distribution of group antigens are still of great interest to a wide range of scientists, but they are not yet fully explored due to the multiplicity of group antigens and the variety of their combinations. It is also relevant to study of group composition and phenotyping so called hard-to-define blood groups of newborns using various method.

Research goals and objectives

The aim of our research was to study the screening characteristics of blood group antigens using various research methods. Both newborns and donors were targeted. The aim of our work was to study the features of antigen-antibody expression of the ABO system in newborns as well as the characteristics of expression of the erythrocyte group antigens in donors in one of the regional polyclinics.

Based on the above goals, we set the following objectives:

- Selecting and modifying research methods;
- screening of group-specific antigens of ABO system in the blood of newborns and donors using variousl serological methods;
- screening of group-specific antigens of ABO system in the plasm of newborns and donors using variousl serological methods;
- screening of A1, A2(H) antigens in the blood of newborns and donors;
- the study of Rh antigens (D, C, c, E and e) in research material;
- screening of group-specific antigens of Kell and MNS system in the blood of newborns and donors;
- the study of the frequency of combinations of phenotypes in donors with all four group systems (ABO, RH, KELL, MNS)
- assessment of the quantitative and qualitative characteristics of natural and immune anti-A and anti-B antibodies of the ABO system;
- creation of an online donor database;
- assessment of the quantitative and qualitative characteristics of anti-Rhesus anti-D antibody;
- highlighting rare and interesting combinations of phenotypes and providing information about them to the clinic representatives.

Used research methods and material and technical base.

The subjects of our study are blood donors and newborns. The material of the research is vein blood and the blood of the newborn, which has been taken either from the vein of umbilical cord or from the

peripheral vein. The blood samples of 85 newborns and 1009 donors have been studied for the erythrocyte group antigens.

According to the recommended norms and regulations of WHO (World Health Organization), only people from the certain age group can donate blood. The age range of the observed patients is 18-60. The minimum weight of the patients is 50 kg. One of the most necessary factors is also the level of hemoglobin. Hemoglobin level for the male donors should be at least 130.0mg all and for the female donors 120 g. Most of the observed donors are the males.

Only of 233 donors out of 1009 are the female. This is caused by the fact that the hemoglobin level goes down after the donation and women themselves have naturally lower hemoglobin level compared to men, which is caused by menstrual cycle.

As for the nationalities, most of them are Georgians, but there are people from Armenia, Azerbaijan Ossetia, Russia and others.

The material for the research has been taken adhering to the ethic norms. Based on the conclusion of the Clinic Bioethics Committee, we were able to use the laboratory and this means that we did not have to do an additional invasion for collecting blood samples of the donors and newborns. The research material for the doctoral thesis has been taken in the dynamics of five years (2015-2020).

The blood of 85 newborns was tested for antigen-antibodies of the ABO system, and the blood of 1009 donors - for group antigens of red blood cells. The material of the diagnostic laboratory was provided to Batumi health Center Medina Ltd. Laboratory analysis was carried out on the basis of the laboratory of immunogenetics at Batumi Shota Rustaveli State University.

The study used an express method of immunoserology using monoclonal antibodies. Monoclonal antibodies of the following specificity were used for the experiment: anti -A, -B, AB, A2 (H), -A1, D, C, c, E, e, K, k, M, N, S, s. For the screening of group-specific antigens and natural antibodies of the ABO system, the so-called crossing or reverse method was used. In addition, the donor group in some cases was also determined by column agglutination methods. AHG (anti human globulin) plates –so-called ID cards were used. In particular, special ID cards ABO/Rh (A, B, DVI+/A, B, DVI+) for donors and A1, A2, B/I, II, III for reverse phenotyping of the ABO system were used.

Natural anti-A and anti-B antibodies of the ABO system were determined by crossing methods. Standard erythrocytes were used to detect them. To determine the anti-A and anti-B immune antibodies of this

system, it is first necessary to disrupt the activity of natural antibodies. Natural group-specific antibodies are suppressed by temperature shock; as you know, they are associated with "cold agglutinins" and are easily dissipate at high temperatures; Therefore, plasma processing of patients took 30-40 minutes in a water bath at 70C. It was only then that it became possible to investigate immune anti A and anti B antibodies using the Combs experiment.

Result

III. 1. The Combination of Rh, Kell and MN Antigens in Donors for the groups - O, A, B, AB

As we have already mentioned in the second chapter, four types of group system phenotypic combinations have been studied in 1009 blood donors. Based on four group system combinations, we have identified theoretically possible 48 phenotypic groups. (Table N 1)

Table № 1 - The combination of Rh, Kell and MN antigens in donors for O, A, B, AB groups.

N	Phenotypic combinations	Number of donors (1009)	Percentage and Error	Df	χ^2	CV	P
1	O,Rh+ k+ MN	125	12,3% ±1,03	**47**	**3221,16**	**62.83**	The P-Value is < .00001. The result is significant at p <
	O,Rh+ k+ MM	96	9,51% ±0,9				
	O,Rh+ K+ NN	31	3,07% ±0,5				
2	O,Rh+ K- MN	38	3,7% ±0,5				
	O,Rh+ K- NN	2	0,19% ±0,1				

	O,Rh+ K- MM	112	11,10% ±0,9				.05.
3	O,Rh- k+ MN	47	4,65% ±0,6				
	O,Rh- k+ MM	0	0				
	O,Rh- k+ NN	6	0,59% ±0,2				
4	O,Rh-K- MN	0	0				
	O,Rh-K- MM	47	4,65% ±0,6				
	O,Rh-K- NN	0	0				
5	A,Rh+ k+ MN	37	3,66% ±0,5				
	A,Rh+ k+ MM	111	11% ±0,9				
	A,Rh+ K+ NN	27	2,67% ±0,5				
6	A,Rh+ K- MN	56	5,55% ±0,7				
	A,Rh+ K- NN	7	0,69% ±0,2				
	A,Rh+ K- MM	95	9,41% ±0,9				
7	A,Rh- k+ MN	0	0				
	A,Rh- k+ MM	0	0				
	A,Rh- k+ NN	0	0				
8	A,Rh-K- MN	16	1,58% ±0,3				
	A,Rh-K- MM	0	0				

	A,Rh-K- NN	0	0				
9	B,Rh+ k+ MN	27	2,67% ±0,5				
	B,Rh+ k+ MM	26	2,57% ±0,5				
	B,Rh+ K+ NN	0	0				
10	B,Rh+ K- MN	33	3,27% ±0,5				
	B,Rh+ K- NN	7	0,69% ±0,2				
	B,Rh+ K- MM	16	1,58% ±0,3				
11	B,Rh- k+ MN	0	0				
	B,Rh- k+ MM	0	0				
	B,Rh- k+ NN	0	0				
12	B,Rh-K- MN	0	0				
	B,Rh-K- MM	0	0				
	B,Rh-K- NN	0	0				
13	AB,Rh+ k+ MN	0	0				
	AB,Rh+ k+ MM	27	2,67% ±0,5				
	AB,Rh+ K+ NN	0	0				
14	AB,Rh+ K- MN	18	1,78% ±0,4				
	AB,Rh+ K- NN	0	0				
	AB,Rh+ K- MM	0	0				

15	AB,Rh- k+ MN	0	0				
	AB,Rh- k+ MM	0	0				
	AB,Rh- K+ NN	0	0				
16	AB,Rh-K- MN	1	0,09% ±0,09				
	AB,Rh-K- MM	1	0,09% ±0,09				
	AB,Rh-K- NN	0	0				

According to the mentioned combinations, 12 phenotypic combinations have been identified in each O, A, B, AB group of ABO system. For example, O (I) phenotypic group of blood produces the following combinations in correlation with the other group systems:

1.O,Rh+ K+ MN; 2. O,Rh+ K+ MM; 3.O,Rh+ K+ NN; 4.O,Rh+ K- MN; 5.O,Rh+ K- NN; 6.O,Rh+ K- MM; 7.O,Rh- k+ MN; 8.O,Rh- k+ MM; 9.O,Rh- k+ NN; 10. O,Rh-K- MN; 11. O,Rh-K- MM; 12. O,Rh-K- NN . Similar combinations have been identified for A(II) blood phenotypic group. These combinations are the following: 1. A,Rh+ K+ MN; 2.A,Rh+ K+ MM; 3. A,Rh+ K+ NN; 4.A,Rh+ K- MN; 5.A,Rh+ K- NN; 6.A,Rh+ K- MM; 7. A,Rh- K+ MN; 8. A,Rh- K+ MM; 9. A,Rh- K+ NN; 10.A,Rh-K- MN; 11.A,Rh-K- MM; 12. A,Rh-K- NN. It is theoretically possible, that we can identify similar phenotypic groups for B(III) phenotypes: 1.B,Rh+ K+ MN; 2.B,Rh+ K+ MM; 3. B,Rh+ K+ NN; 4.B,Rh+ K- MN; 5.B,Rh+ K- NN; 6.B,Rh+ K- MM; 7.B,Rh- K+ MN; 8.B,Rh- K+ MM; 9.B,Rh- K+ NN; 10.B,Rh-K- MN; 11.B,Rh-K- MM; 12.B,Rh-K- NN. AB(IV) phenotypic group can produce 12 combinations in correlation with the other group systems. Among them one can find:

1.AB,Rh+ K+ MN; 2.AB,Rh+ K+ MM; 3.AB,Rh+ K+ NN; 4.AB,Rh+ K- MN; 5.AB,Rh+ K- NN; 6.AB,Rh+ K- MM; 7.AB,Rh- K+ MN; 8.AB,Rh- K+ MM; 9.AB,Rh- K+ NN; 10.AB,Rh-K- MN; 11.AB,Rh-K- MM; 12.AB,Rh-K- NN. As it is seen from Table N 6, out of 48 theoretically possible phenotypic combinations, we can actually find 1,9 times less phenotypes and the real amount is 25 phenotypes. As for the remaining 23 phenotypic combinations, they have not been observed in the donors we studied. These are: 1. O,Rh- K+ MM; 2. O,Rh-K- MN; 3. O,Rh-K- NN; 4. A,Rh- K+ MN; 5. A,Rh- K+ MM; 6. A,Rh- K+ NN; 7. A,Rh-K- MM; 8. A,Rh-K- NN; 9. B,Rh+ K+ NN; 10. B,Rh- K+ MN; 11. B,Rh- K+ MM; 12. B,Rh- K+ NN; 13. B,Rh-K-

MN; 14. B,Rh-K- MM; 15. B,Rh-K- NN; 16. AB,Rh+ K+ MN; 17. AB,Rh+ K+ NN; 18. AB,Rh+ K- NN; 19. AB,Rh+ K- MM; 20. AB,Rh- K+ MN; 21. AB,Rh- K+ MM; 22. AB,Rh- K+ NN; 23. B,Rh-K- NN.

As shown by a statistical study, the quantitative indicator X2 is quite high and is equal to 3221.16, while the critical value (CV) of the current degree of freedom (Df) for 48 categories is quite low and is equivalent to 62.83. this means that the frequency of distribution of phenotypic groups in the studied target group is uneven. The P-Value is < .00001. The result is significant at p < .05.

The data we have gathered has been analyzed in relation with ABO group system. O(I) phenotypic group combinations in relation with other system has actually produced only 9 phenotypic combinations out of 12 theoretical probabilities(fig. №1). Three phenotypes (1. O,Rh- K+ MM; 2. O,Rh-K- MN; 3. O,Rh-K- NN) have not been observed in the donors. As it seen from the list, the mentioned three combinations are connected to O Rh - phenotypes and in one case K+ is present in the combination with them.

All the six combinations from the O, Rh+ phenotypic group are found in the donors, but with a different prevalence. The distribution frequency of two of them (O, Rh+, K+, MN and O, Rh+, K-, MM) is very intense and nearly equal to each other (12,3% და 11, 1 %).The distribution frequency of O, Rh+, K-, NN phenotypic combination is the least intense among the O, Rh+ phenotypes and is equivalent to 0,19% (fig.1).

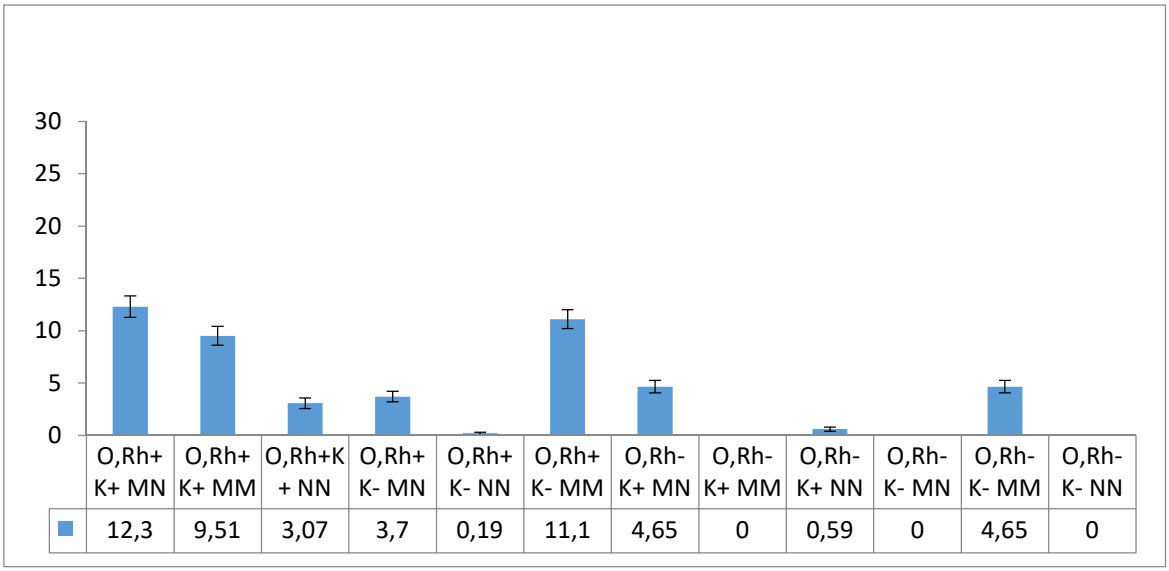

Figure № 1. The distribution of MN, KELL antigen combinations in O RH+ and 0RH- donors.

Different from the first group, the variations of combinations of A(II) phenotypic group is much less. Out of 12 theoretically possible cases, we have found nearly half of them. Actually, this group has only 7 combinations and 6 out of them is connected to A(II), Rh+. This means that the amount of theoretically possible phenotypic groups coincides with the actual amount (1.A,Rh+ K+ MM 11%; 2.A,Rh+ K- MM 9,41%; 3.A,Rh+ K- NN0,69%; 4. A,Rh+ K+ MN 3,66%; 5. A,Rh+ K+ NN 2,67% ; 6. A,Rh+ K- MN 5,55%).

As for the A(II), Rh- phenotypic group, only one phenotype(A,Rh-K-MN) has been found out of possible six. The distribution frequency is 1, 58%. (Fig. №2)

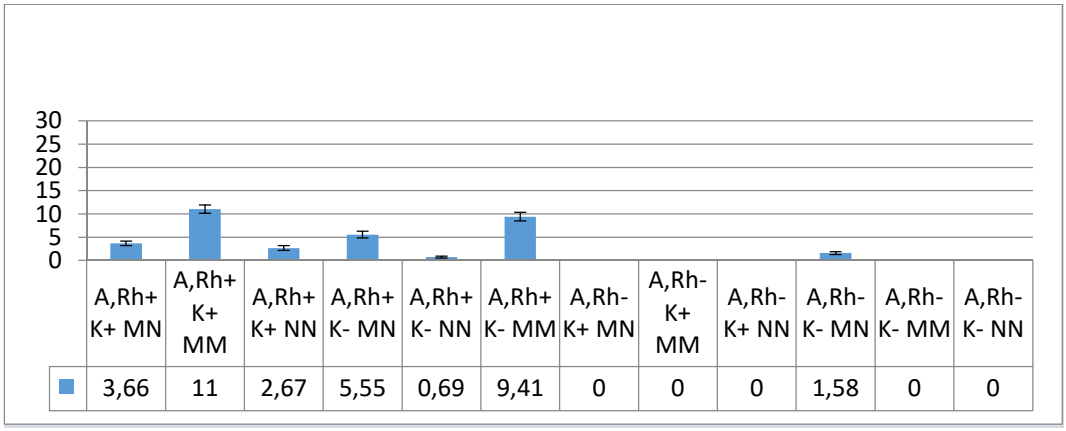

Figure №2. The distribution of MN, KELL antigen combinations in A,Rh-; A,Rh+, donors.

In case of B (III) phenotype group, the actual amount of the variations of phenotypic combinations has decreased even more. In case of phenotypic group B (III) only 5 phenotypic groups have been identified instead of 12 theoretically possible combinations. They are: (1. B,Rh+ K- MN 3,27%; 2. B,Rh+ K+ MM 2,57%;3. BRh+ K+ MN 2,67%;4. B,Rh+ K- MM 1,58%;5. B,Rh+ K- NN 0,69%). As seen from the picture №8, all the practically identified phenotypic combinations are of B,Rh+ phenotype and in case of B,Rh-, none of the 6 possible combinations has been detected.

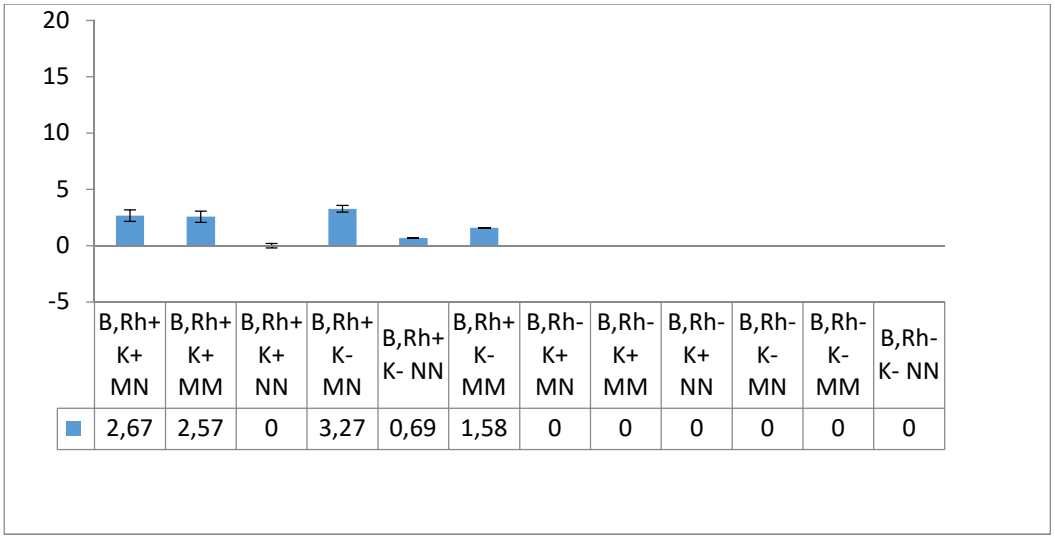

Figure №3. The distribution of MN, KELL antigen combination in B,Rh-; B,Rh+ donors.

The amount of variations of phenotypic group combinations has decreased even more in AB (IV) donors. In this case, out of 12 possible combinations, we have only identified 4, but with a very low prevalence. More specifically: 1.AB,Rh+K+MM - 2,67%; 2.ABRh+K-MN - 1,78%. As it is visible, both phenotypic combinations belong to AB,Rh+. While research, we have also found 2 phenotypic units of AB,Rh-, they are: AB,Rh-K-MN და AB,Rh-K-MM(fig. №4). The distribution frequency for each of them is only 0,09%.

While observing the phenotypic combinations due to antigens of four erythrocyte group systems, a comparatively high polymorphism has been found in case of O (I). It is followed by combinations of A (II) phenotypic group. B (III) phenotypic group holds the third place in a row with the multitude of variations, and AB (IV) is characterised by low polymorphism. Hereby, we can conclude that in the donors we have studied, the polymorphism characteristics have been distributed in the following sequence: O>A >B> AB.

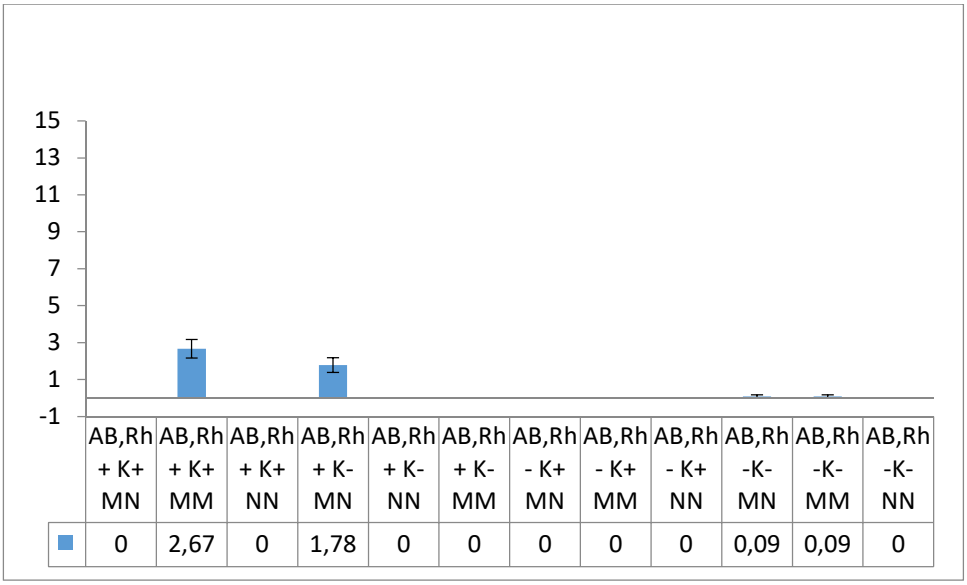

Figure №4. The distribution of MN, KELL antigen combination in AB,Rh-; AB,Rh+, donors.

We consider, that the existence of this data will really promote the increase of safety level of transfusion, will expand the database of the donors and will enable the clinics to easily detect the rarest combinations of blood groups in order to avoid post transfusion complications.

Gene distribution frequency of the ABO system in the studied donors was also analyzed. Their frequency was calculated using the formula used in the study of the three-allelic genetic system. In the study of the frequency of the r, p, q alleles, the r allele was detected with the highest frequency in the studied donors. Its distribution in the target group is 0.7, while the distribution of the q allele lags significantly behind and is 0.22, and the quantitative indicator of the frequency of the p allele is the lowest - 0.008 (Table 2).

table №2. Gene distribution frequency of the ABO system

Alleles of the ABO system	**Frequency**
$r = \sqrt{O}$	**0.7**
$p = 1 - \sqrt{A + O}$	0,22

$q = 1- \sqrt{B+O}$	0, 08

Where 0, A and B are the ratio of people carrying 0(I), A (II) and B (III) groups to the total number of research objects .

Alleles of the Kell system were also analyzed in the studied donors. Different frequencies of p (K) and q (k) were found in the target group. P (K) occurs with a high distribution frequency (0.77), and the frequency of the q (k) allele lags significantly behind and is 0.22. Table №.3

table №3. Allele frequency of the Kell system in the studied donors

	q	P
Kell	$\sqrt{\frac{n_{aa}}{N}}$ = 0.22	1-q 0.78

where n_{aa} is a recessive homozygote according to this locus and (kk), N is the total number of examined individuals.

Like the alleles of the Kell system, the frequency of distribution of the alleles of the MN system is also heterogeneous. As shown in the table below (Table N4), the frequency of distribution of the p allele is 0.72, and the frequency of distribution of the q allele is significantly lower and equal to 0.28.

Table №4.Allele frequency of the MN system in the studied donors.

	Q	P
MN	$\frac{n_A + \frac{1}{2} n_{AB}}{N}$ = 0,72	$\frac{n_B + \frac{1}{2} n_{AB}}{N}$ =0,28

where n_A means the number of carriers of the phenotype M, n_{AB} indicates MN phenotypes n_B means the number of carriers of the phenotype N .

We believe that the availability of data from our study will improve the safety of blood transfusion, expand the donor database and allow clinics to quickly find rare combinations of blood groups in order to reduce the risk of post-transfusion complications.

III.2. The features of antigen prevalence of Rhesus system in donor population

The blood of 852 donors (aged ≥18 years) has been investigated on erythrocyte blood group antigens. The Rh blood group system consists of 49 defined blood group antigens (14). among which the five antigens D, C, c, E, and e are the most important. Study of Rhesus (Rh) blood group antigens, phenotype, and Rh antibodies is very useful in routine and advanced clinical practice in blood transfusion centers. We study the prevalence of these five Rh antigens in blood donors (n=852) of both sex (male/female) and different age (18-55 y.) (Table №5).

Table №5. Prevalence and Chi-square analysis of C, c, E, e antigens in studied donors.

Rh antigens expressed on cell	Prevalence of antigens	Df	χ^2	CV	P
C	68,03%±1,5	3	211,46	7,815	The P-Value is < .00001. The result is significant at p < .05.
c	85%±1,22				
E	38,07 %±1,6				
E	94,6 %±0.77				

The prevalence of Rh system antigens is looks like so: e antigen – 94,6%, c antigen - 85%, C-68,03, E antigen - 38,07%. What about the D antigen majority (84%) of studied donors are Rh positive (n=719), 133 (16%) donors are Rh negative. In this case we used the one-variable chi-square criterion. Statistically revealed a high number of chi-square criteria. In this particular case the value $\chi 2$ is quite effective for rejecting the null hypothesis (E=0). The value of $\chi 2$ in the case is equal to 211,46. These numbers are much

higher than the critical value (CV) of the criterion of degree of freedom (d.f.=3), which is equal to 7,815. The P-Value is < .00001. The result is significant at p < .05 (Table 6).

Table N 6 Frequency of distribution of alleles of the Rh system in donors.

Rh system genes	Frequency
D	1 - $\sqrt{dd}$ = 0.64
C	*1 -* $\sqrt{cc}$ *= 0.48*
E	*1 -* $\sqrt{ee}$ *= 0.61*
c	$\sqrt{CC}$ *= 0.54*
e	*1 -* $\sqrt{EE}$ *= 0.40*

The frequency of distribution of Rh alleles in the studied donors was analyzed. Two alleles of the RhC gene occur with the following frequency: C – 0,48, c – 0,54. Their number is equal to 1 in the studied target group. The distribution of two alleles of the RhE gene is as follows: E - 0,61 , e -0,40. RhD reveals a rather high frequency of distribution and it is 0,64 (figure5).

As we see from Figure № 10 and Table №7 C antigen most common is present in the combination with D antigen. 65, 8 % case we had CD+ combination (n=561). A Similar situation is with E antigen combination with D antigen. E antigen in most cases is presented with a combination of D antigen. 36, 9% of the studied donors (n=306) had ED+ combination. A miserable number of studied donors had CD - (2,23%; n=19) and ED - (1,17%; n=9) combinations.

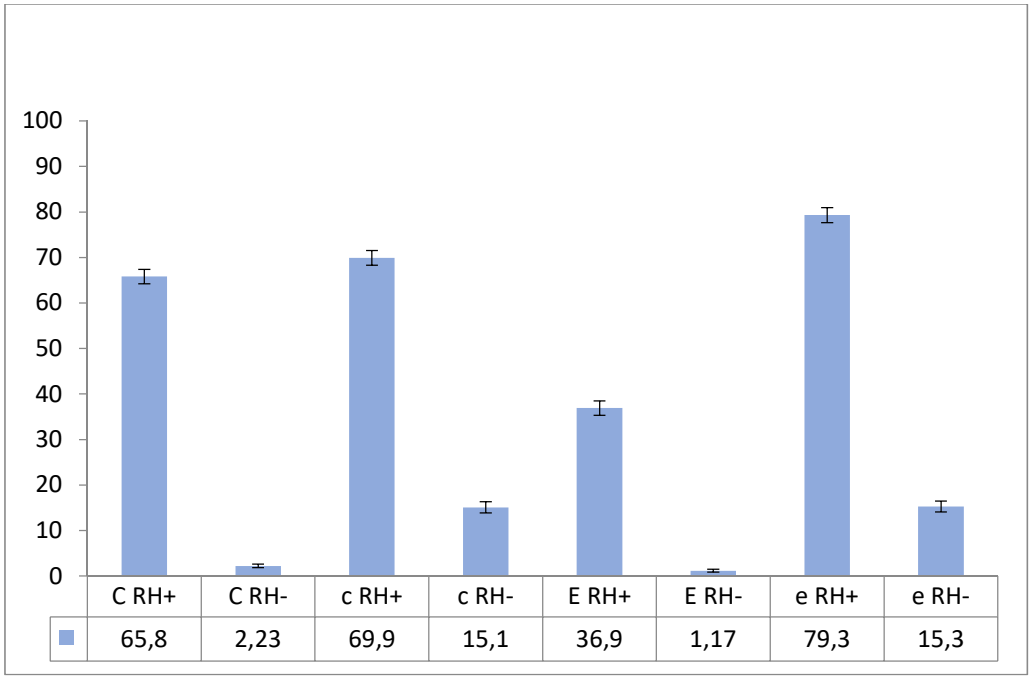

Figure № 5. C, c, E, e antigens combination in D positive and D negative donors

The Rh blood group system has two sets of nomenclatures: one developed by Ronald Fisher and R. R. Race, the other by Wiener. Both systems reflected alternative theories of inheritance. The Fisher–Race system, which is more commonly in use today, uses the CDE nomenclature. In our study we used Fisher and Race nomenclature.

We have studied the Rh phenotypes prevalence in blood donors. According to RHD, RHC and RHE gene locuses there are 18 theoretically possible phenotypical groups. Among them half (nine) are Rh positive and rest of them (nine) are Rh negative. The Rh positive phenotypes are: CDE; CDEe; CDe; CcDE; CcDEe; CcDe; ccDE; cDEe and cDe. Rh negative phenotypes are CdE; CdEe; Cde; CcdE; CcdEe; Ccde; cdE; cdEe; cde. We allocated 17 Rh phenotypes among studied donors. Only one phenotypes CdE, which belongs Rh negative group was not presents in studied donors. Other 17 phenotypes showed different frequency (Table 3, Figure 2). Some of them were only in single case, for example: cdEe, cdE, CdEe phenotypes had only one donor. Majority of the phenotype in the studied donors (27,8±1,53%) was CcDe (n=237). CcDEe - 19,3±1,35% (n=165); 125 donors have CDe phenotype (14,6±1,2); The frequency of cde was 13,1±1,5%, which means that 112 studied donors belonged to this phenotype group; 87 studied donors had cDEe

phenotype characteristics (10,2%); The frequency of cDe was 4,9% (n=42); 19 donors had CDEe phenotype. Other phenotypes (CDE, Cde, CcdEe, Ccde) frequency was very low (Table 7, Figure 6).

Table №7. The numbers of Rh phenotypes in the studied donors (n=852).

Rh phenotype	O(I), Rh+	O(I) Rh-	A(II)Rh+	A(II)Rh-	B(III)Rh+	B(III)Rh–	AB(IV)Rh+	AB(IV)Rh-	Total
CDE D+C+E+c-e-	3	0	2	0	0	0	0	0	5
CDEe D+C+E+c-e+	6	0	9	0	3	0	1	0	19
CDe D+C+E-c-e	64	0	48	0	12	0	1	0	125
CcDEe D+C+E+c+e+	9	0	9	0	1	0	0	0	19
CcD-ee D+C+E+c-e+	65	0	76	0	17	0	7	0	165
cDE D+C-E+c+e-	125	0	84	0	20	0	8	0	237
cDEe D+C-E+c+e+	11	0	7	0	1	0	1	0	20

ccD-ee **D+C-E-c+e+**	52	0	22	0	9	0	4	0	87
CdE **D-C+E+c-e-**	16	0	22	0	3	0	1	0	42
CCddEe **D-C+E+c-e**	0	0	0	0	0	0	0	0	0
Cde **D-C+E-c-e+**	0	1	0	2	0	0	0	0	3
CcddEE **D-C+E+c+e-** **CcdEe** **D-C+E+c+e+**	0 0	1 4	0 0	0 2	0 0	0 0	0 0	0 0	1 6
Ccde **D-C+E-c+e+**	0	4	0	3	0	1	0	0	8
cdE **D-C-E+c+e-**	0	1	0	0	0	0	0	0	1

cdEe **D-C-E+c+e+**	0	0	0	1	0	0	0	0	1
cde **D-C-E-c+e+**	0	62	0	34	0	14	0	2	112
Total	351	73	279	43	66	15	23	2	852

As mentioned in the literature section there are some errors in determining rhesus phenotype. Errors in determining the Rh factor are associated with a weak variation Du of the antigen D. According to the recommendations, additional studies should be carried out for all those cases where the Cde and cdE phenotypes are detected during the primary phenotyping of erythrocytes, since the Du antigen is most often found together with the C or E antigen. The studied donors had three cases of the Cde phenotype and one case of the cdE phenotype. The Du antigen has latent antigenic determinants that are expressed on the surface of erythrocytes, so we used an indirect Coombs test to detect them. No Du variation was observed in any of these 4 cases and accordingly, the primary phenotype of rhesus did not change.

From Rh positive donors as we already mentioned above have nine variated phenotypes and their frequency was quite different. Among Rh positive donors two (CcDEe – 22,9% and CcDe – 32,9) phenotypes were spread with high frequency. The Majority (55,8%) Rh positive donors had this 2 phenotype characteristic on the cell. Another two phenotypes (CCDe – 17,38% and cDEe -12,1%) frequency equals 29,48%. The Rest of the five phenotypes (CDE, CDEe, CcDE, cDE, cDe) prevalence was 14,47% (Figure 7)

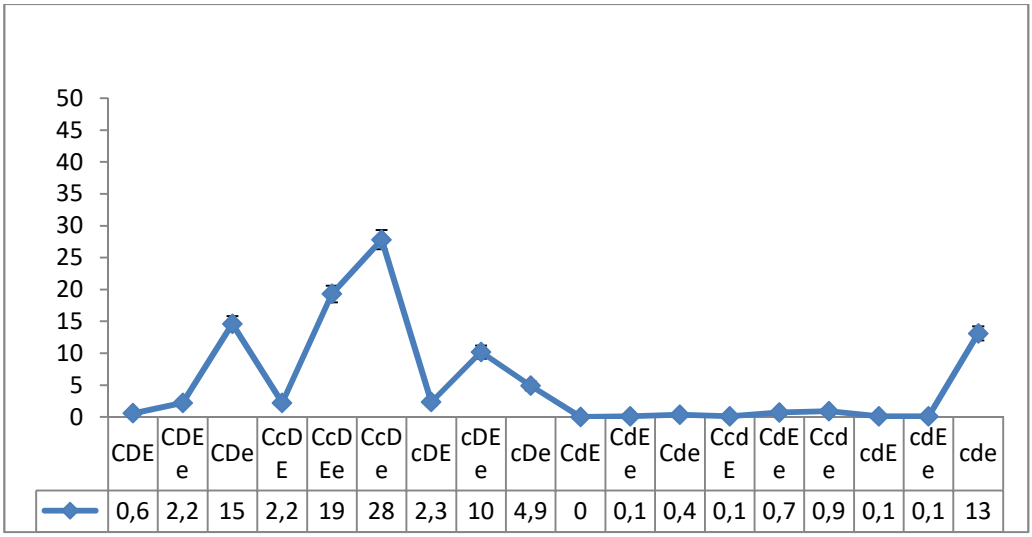

Figure №6. Frequency of Rh blood group phenotypes in studied donors (n=852).

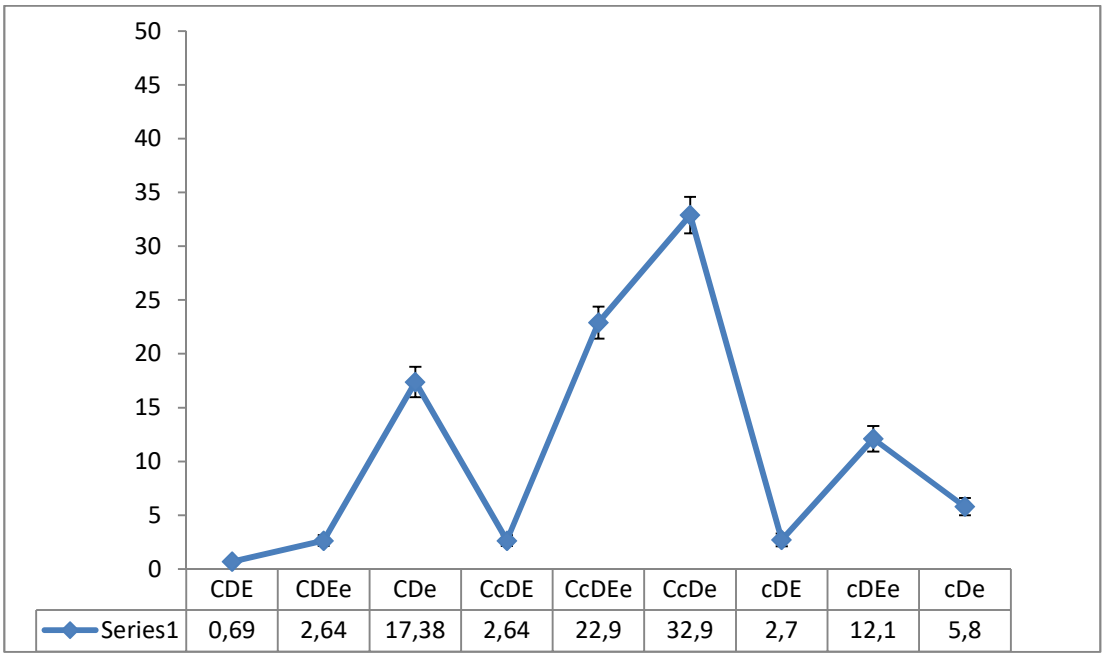

Figure 7. Phenotype variation in Rh positive donors.

In this case we used the one-variable chi-square criterion as it is mentioned above. Statistically revealed a high number of chi-square criteria, which indicates the unequal distribution of phenotypes. In this particular case the value $\chi 2$ is quite effective for rejecting the null hypothesis (E=0). The value of $\chi 2$ in the case is equal to 651. This is much higher than the critical value (CV) of the criterion of degree of

freedom (d.f.=8), which is equal to 15,51. The P-Value is < .00001. The result is significant at p < .05 (Table8).

Table 8. Rh positive phenotypes Chi-square analysis of proportions.

Rh positive phenotype	$(O-E)^2/E$	df	χ^2	CV	P
CDE	70,11	8	651	15,51	The P-Value is < .00001. The result is significant at p < .05.
CDEe	46,32				
CDe	25,6				
CcDE	46,32				
CcDEe	90,96				
CcDee	309				
cDE	44,21				
cDEe	0,65				
ccDee	17,9				

In contrast of Rh positive donors in the case of Rh negative dominant phenotypical characteristics was only one. This is cde phenotype. Totally in the studied donors we had 143 Rh negative donors, among them 112 donors had cde phenotypes. We can say that this phenotype is more common for Rh negative blood donors. The prevalence of this phenotype was 84,2 %. Three phenotypes (Ccde, CcdEe and Cde) prevalence was 7,8 times less then cde phenotype and totally was 12,77 % (Ccde- 6,01%; CcdEe - 4,51% and Cde – 2,25%). What about other four phenotypes (CddEe – 0,75%, CcdE-0,75, cdE – 0,75%, cdEe- 0,75%) total frequency in the studied donors was only 3% (Figure 8).

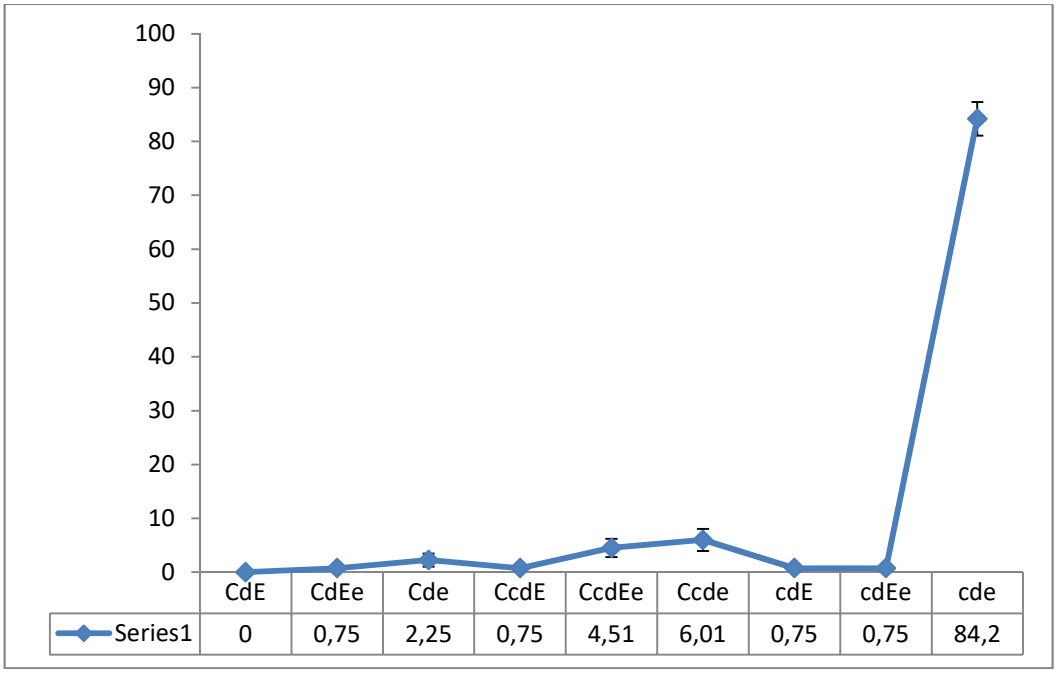

Figure № 8. Phenotype variation in Rh negative donors.

Table № 9. Rh negative phenotypes Chi-square analysis of proportions.

Rh negative phenotype	**$(O-E)^2/E$**	**df**	**χ^2**	**CV**	**P**
CdE	14,7	8	727	15,51	The P-Value is < .00001. The result is significant at p < .05.
CdEe	12,76				
Cde	9,31				
CcdE	12,76				
CcdEe	5,1				
Ccde	3,05				
cdE	12, 76				

cdEe	12,76				
cde	644				

In this case statistically revealed a high number of chi-square criteria and it is equal to 727. This is much higher than the critical value (CV) of the criterion of degree of freedom (d.f.=8), which is equal to 15,51. The P-Value is < .00001. The result is significant at p < .05 (Table 10).

The haplotypes of the Rh system were calculated by us in the studied donors. Seven haplotypes were isolated in the target group. They are:*cde*, *Cde*, *cdE*, *cDe*, *cDE*, *CDe*, *CDE.* Among them, the cde haplotype is most often present in donors and is equal to 0.33. the lowest frequency of distribution shows the CDE haplotype (table10)

Table № 10 Haplotypes of the Rhesus system in the studied donors.

Frequency of phenotypes
$cde = 0{,}33 \quad \sqrt{ccddee}$
$Cde = 0.1 \quad \frac{Ccddee}{2cde}$
$cdE = 0.1 \quad \frac{ccddEe}{2cde}$
$cDe = 0.13 \quad \frac{ccDee}{2cde}$
$cDE = 0{,}23 \quad \sqrt{ccDEE + cdE^2} \; - \; cdE;$
$CDe = \; 0.1\sqrt{CCDee + Cde^2} \; - \; Cde$
$CDE = 0.02 \quad \frac{CCDEe}{2(CDe + cde)}$

In our work we analyzed the combination of ABO blood groups and Rh phenotypes. We allocated eight phenotypical groups with combination ABO blood group and D positive and D negative groups. The above mentioned phenotypical groups were: O (I), Rh+; O (I), Rh-; A (II), Rh+; A (II), Rh-; B (III), Rh+; R (III), Rh-; AB (IV), Rh +; AB (IV), Rh-. As it is showed from the figure № 5 majority (41,19%) of the studied donors were O (I), Rh+ (n=351). 32,7 % donors belonged to A (II), Rh+ phenotypical group. The frequency of B (III), Rh+ phenotypes was 7,74%. The less frequently (2,69%) from Rh positive phenotypes is spread Rh-; AB (IV), Rh + (Figure 9).

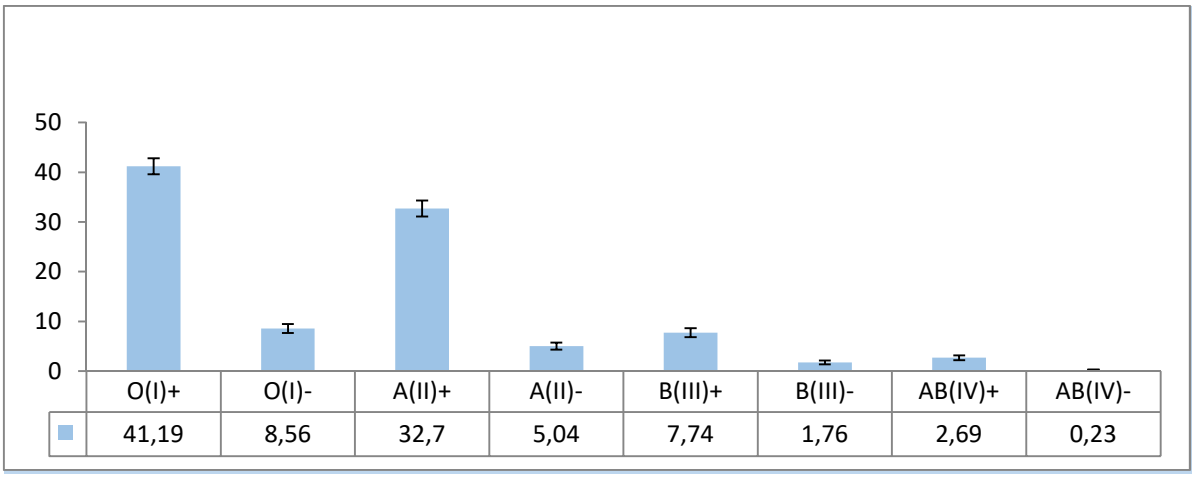

Figure № 9. Frequency of ABO and Rh blood group phenotypes in studied donors (n=852).

Today in our clinics two antigens (A, B) of ABO system and D antigens for Rh system are taken into account during a blood transfusion. For the individuals where those antigens do not occur the theoretical risk of

alloimmunosensibilization is high. In the viewpoint of transfusion c antigen, among resus system antigens, is also significant. c antigen clinically is the most important Rh antigen after the D antigen. Numerous data about alloimmunosensibilization caused by this antigen are presented in the scientific literature.

The Distribution frequency of c antigens within world population is 80-82%. 18-20 % of humans don't have this antigen and are revealed in CC state. Individuals with just this genotype belong to high-risk group of alloimmunosensibilization.

We took our attention to cc and Cc genotypic donors, because in both cases their erythrocyte membrane contains c antigens. As we see in our study frequency of cc genotype in the studied donors is 30,72% (ccD+ 17,42% and ccD- 13,3%), Cc genotype frequency was more high and equals 50,82 (ccD+ - 49,06% and CcD- - 1,76%). As we discussed above the recipient who has CC genotype the sensibilization by anti-c antibodies is higher. The frequency of CC genotype in studied donors was 17,88% (Table №11).

Table №11 . CC, Cc,cc, EE, Ee, ee phenotypes combination with Rh+ and Rh- case.

	Rh+	Rh-
CC	17,42%±1,29	0,46%±0,2
Cc	49,06%±1,7	1,76%±0,4
Cc	17,42%±1,29	13,3%±1,1
EE	5,1%±0,7	0,23%±0,1
Ee	31,8%±1,5	0,9%±0,3
Ee	47,44%±1,7	14,43%±1,2

In our previous work we studied the distribution of these genotypes in Adjara population (M. Nagervadze, 2011). As they are potential recipients. The distribution frequency of CC genotype in Adjara population was equaled to 8%. Implying that carriers of this genotype don't consist in c antigen and during transfusion only 17,88 % of the cases they received the blood from CC donors. In the majority of cases they are at high risk of immunosensibilization by anti-c antibodies, because theoretically 82,12% of cases they received the blood from Cc and cc donors. The immunization risk by anti-c antibodies is 82,2% cases. Only 17,88% of the cases of transfusion with CC genotypic donors are safe.

We have found differences in the distribution of Rh phenotypes between blood donors and Adjara population, for instance, there are more phenotypic variation among blood donors than Adjara population. Six Rh-phenotypic groups with various frequency distributions were fixed for Adjara region population (18). In the same region example of one clinic blood donors we allocated 2,8 times more Rh phenotypic characteristics (Figure 2). We think that this differences reason is that on the study of Rh antigens in Adjara population level we took our attention on the nationality. All participants were Georgens. In case of blood donors as they are officially donors nationality is different and donors belong different ethnic groups.

III.3. Antigens A1 and A2 in blood donors

Group-specific antigen A is expressed on the erythrocyte membrane of people with two phenotypic groups. These are phenotypes A(II) and AB(IV). As discussed in the literature section, the A antigen is generally present in several subgroups that show varying prevalence (Saboor M…2010; Mohieldin E…..2015).

These are: variations of A1, A2 and weak A antigens. In the next step All with phenotypes A (II) and AB (IV) were tested using A1 lectin. The sample is considered a subgroup A1 in the case of both anti-A and A1 lectin, when the degree of agglutination is well expressed. The sample was considered a subgroup A2, when the degree of agglutination with anti-A-antibody was assessed as 4^{+}, and the response to anti-A-lectin was negative. The sample was considered as a weak sub-group of antigen A in the case of weak agglutination (1^{+} or 2^{+}) with anti-A antibody and negative response to anti-A lectin. We were interested in how these variations were distributed among the studied donors.

Of the 1009 donors studied, 349 are carriers of phenotypic group A (II) while 19 donors carry AB (IV) group specification. This means that 36.23% of the donors studied have antigen A on the erythrocyte membrane. The vast majority of them carry this antigen in the form of A1 variation (tabela12).

Table № 12. prevalence of A and AB sub-groups of phenotypes among donors (n=368).

ABO phenotypes	Subgroup	n	%
A	A1	324	32,11
	A2	25	2,47

AB	A1B A2B	12 7	1,18 O,69
Total		368	36,45

As shown in the table, the distribution frequency of subgroups A1 and A2 is uneven. A2 and A2B are considered relatively rare phenotypes. These two phenotypes differ from A1 and A1B phenotypes by a negative response to Anti-A1 lectin. Subgroup A1 occurs in 324 cases among donors with A (II) phenotypic group. A small proportion of this group of donors (n = 25) belong to subgroup A2. As for the AB (IV) phenotypic group, two subgroups A1B and A2B were identified in the studied donors. 63% (n = 12) of nineteen donors with phenotypic group AB (IV) is characterized by phenotypic specification A1B, and 37% have A2B.

From the above, it can be noted that the A2 subgroup in the studied population is characterized by a rather low prevalence. The rate of distribution is only 4.05%. It should be noted here that among the studied A (II) and AB (IV) phenotypic groups, weak subgroups were not identified.

III.4. Natural and immune antibodies in donors

As it is known, the presence of natural group-specific antibodies is characteristic only for the ABO system. Natural antibodies (anti-A and anti-B) were detected in donors by the reverse method of determining the blood group, for which the donor plasma and standard erythrocyte masses A (II) and B (III) were used. Natural antibodies of 237 donors (anti-A and anti-B) were studied.

The distribution frequency of natural anti-A antibody in the studied donors is 57%, and for anti-B antibody - 87% (Figure 10).

10% of the donors studied by us carried only anti-A antibody, which is characteristic of the phenotypic group B (III). 39.6% of the plasma of the examined donors carried only anti-B antibodies, which are usually characteristic of donors with the A (II) phenotype. 47.6% of donor plasma contains both anti-A and anti-B antibodies, and, accordingly, these donors belong to the blood group O (I). We identified a case with a low

percentage, when the plasma did not contain any of the above antibodies due to the fact that the frequency of distribution of the phenotypic group AB (IV) is low in the studied target group (figure 11).

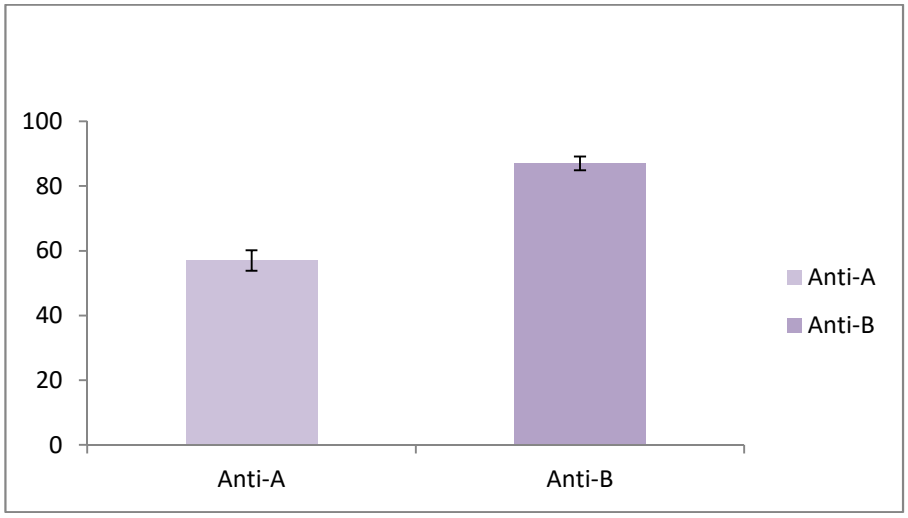

Figure №10. Frequency of distribution of natural antibodies (anti-A and anti-B) in the studied donors (n=237).

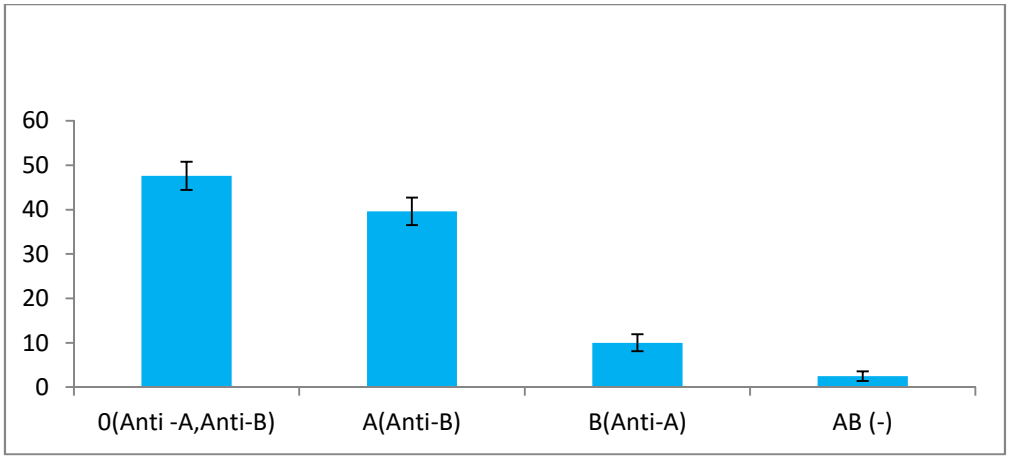

Figure №11. Frequency of distribution of natural antibodies (anti-A and anti-B), taking into account the blood group of the studied donors.

We were also interested in studying the quantitative characteristics of antibodies. (titr). To do this, we randomly selected 20 donors. Natural antibodies of 20 donors were studied and their titer was determined (5 samples were taken for each phenotypic group). The expected results were observed in all of them: both anti-A and anti-B antibodies were found in donors of blood group O (I), in the case of group A (II) only

anti-B antibodies were detected while none of the group-specific antibodies was found in individuals with blood group AB (IV) (table 6). We also studied the titer of these antibodies. In all cases, antibodies were present with a high titer (minimum 1: 512), but it should be noted that the titer of anti-A-antibody is much higher in all cases studied in comparison with anti-B-antibody (table 13,14,15,16).

Table 13. group-specific antigen-antibodies

Blood groups	Erythrocyte antigen	Natural antibody (corresponding titre)
O (I)	-	Anti–A, Anti–B
A (II)	B	Anti–B
B (III)	A	Anti – A
AB (IV)	A, B	--

Table № 14. Titer of group-specific antibodies in samples of group O (I)

Anti–A	Anti–B
1:1024	1:1024
1:1024	1:1024
1:2048	1:512
1:1024	1:1024
1:2048	1:1024

table № 15. titer of group-specific antibodies in samples of group A (II)

	1	2	3	4	5
Anti–A	1:1024	1:1024	1:512	1:1024	1:1024

table № 16. titer of group-specific antibodies in samples of group B (III)

	1	2	3	4	5
Anti–B	1:1024	1:1024	1:512	1:512	1:1024

In none of the cases studied, immune anti-A and anti-B antibodies were not detected. Research in this area requires a larger target group. In this regard, it is necessary to carry out more tests, since immune antibodies are produced in isolated cases associated with pregnancy with an immune conflict or incompatible blood transfusion.

III. 5. Features of the determination of antigens and antibodies of the erythrocyte group in newborns.

Determining the blood group of a newborn is one of the first laboratory tests carried out for a newborn after birth. Biological material can be taken from both the umbilical cord and the peripheral blood of the newborn. The collection of blood from the umbilical cord requires some care so as not to contaminate biological material, which may affect the serological expression of antigens and cause false agglutination and / or non-specific reactions which leads to misinterpretation of results.

We examined biological material of 85 newborns. Phenotypic groups of the ABO system in newborns are unevenly distributed. Of the 85 examined newborns, 34 had group I (O), 38 had group A (II), 11 had phenotypic group B (III), and 2 newborns had blood group AB (IV) (figure 12).

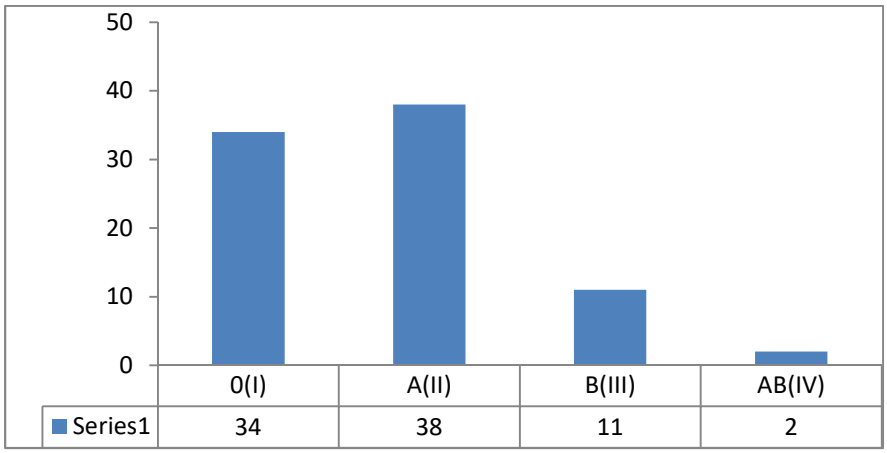

Figure №12. frequency of distribution of ABO phenotype in the studied newborns

Table №17 frequency of distribution of genes of ABO system in newborns

$r = \sqrt{O}$	**0.6**
$p = 1 - \sqrt{A + O}$	0,1
$q = 1 - \sqrt{B + O}$	0, 3

Where 0, A and B are the ratio of people carrying 0(I), A (II) and B (III) groups to the total number of research objects .

Gene distribution frequency of the ABO system in the studied newborns was also analyzed. Their frequency was calculated using the formula used in the study of the three-allelic genetic system. r, p, q alleles was detected with the highest frequency in the studied donors and is equal to 0,6, while the prevalence of the q allele lags significantly behind and is 0.3, and the frequency of the p allele is the lowest - 0.1(table 17).

In parallel with the screening of group antigens of newborns, we were interested in the features of detecting group-specific antibodies in this target group. As known from the literature, carriers of the O (I) group have both anti-A and anti-B antibodies in their blood plasma. The expression of group-specific antigens in

newborns with group O (I) differs from that in adults. 2.3% of the examined newborns with the O (I) group carried both anti-A and anti-B antibodies, while none of the antibodies was detected in 33.3% of cases, and 23.3% of newborns carried only anti-A antibody while 20% carry anti-B antibodies only (figure 13).

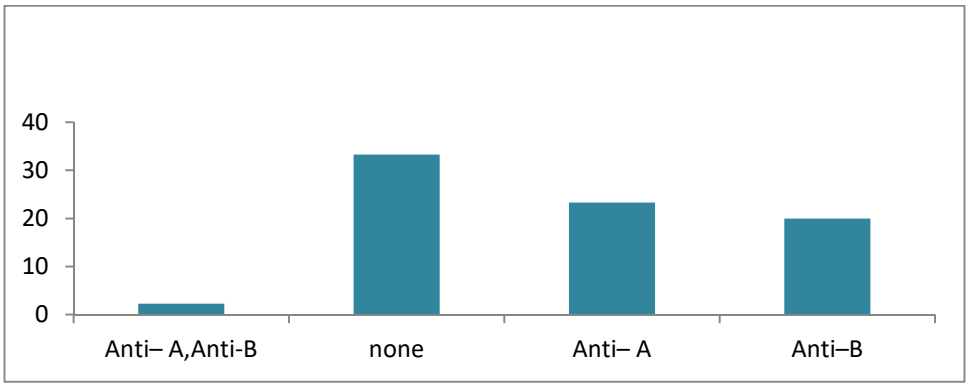

Figure № 13. Presence of natural antibodies in newborns with O (I) group

As known, only anti-B antibodies are expressed in the blood plasma of an adult with group A (II). of the 38 newborns examined, the expected result was found in 23 cases, just as it should be in adults, and none of the natural antibodies was detected in 14 newborns. There was one interesting and unexpected result when both anti-A and anti-B were found. (figure 14). It is possible that the anti-A antibody is of the immune type.

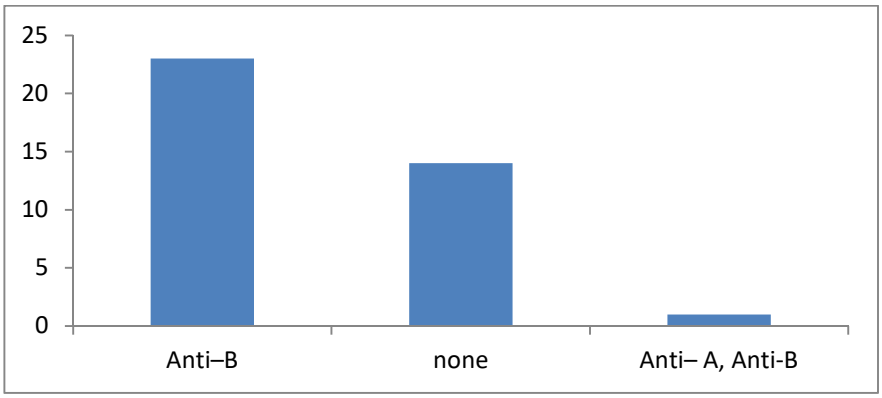

Figure № 14. the frequency of detection of antibodies in the plasma of newborns with group A (II)

Anti-A antibodies alone were to be detected in the plasma of 11 newborns with group B (III). Only 8 of them showed an antibody anti-A, and in the remaining three cases no antigen was detected (Figure 15).

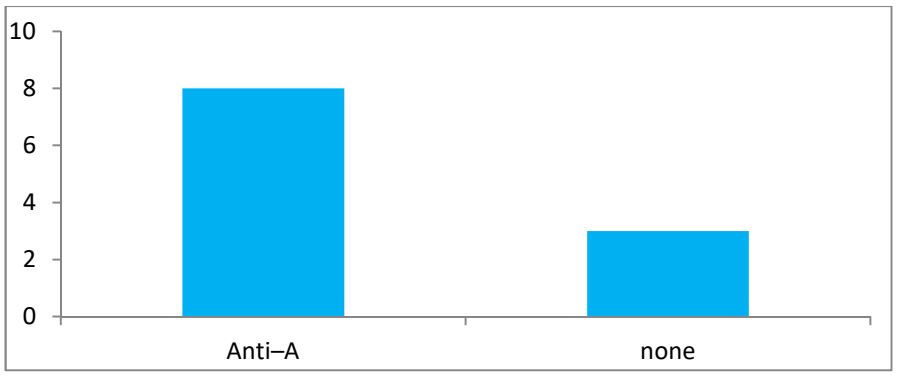

Figure № 15. the frequency of detection of antibodies in the plasma of newborns with group B (III

Of the 85 examined newborns, only two belonged to the AB (IV) group. As it is known, the plasma of adults of the fourth group does not contain any antibodies. A similar picture was observed in newborns.

III.6. Subgroups of antigen A in newborns with the group A(II) and B(IV)

To determine the blood group of newborns, we used a serological plate and test methods. An additional study using anti-A1 and anti-H lectins was performed in each newborn with phenotypes A (II) and AB (IV). Based on serological tests, we had the opportunity to study subgroups A1/A1B, A2/A2B and weak A. The sample is considered a subgroup A1 in the case of both anti-A and A1 lectin, when the degree of agglutination is well expressed. The sample was considered a subgroup A2, when the degree of agglutination with anti-A-antibody was assessed as 4^{+}, but the response to anti-A-lectin was negative. In the case of weak agglutination (1^{+} or 2^{+}) with anti-A antibody and negative response to anti-A lectin the sample was considered as a weak sub-group of antigen A. We were interested in how these variations were distributed among the studied newborns. when studying subgroups, a completely different picture developed in newborns compared to donors. 46% of the studied donors carry antigen A, 44 of them belong to the phenotypic group A (II), and the remaining 2 are carriers of the AB (IV) phenotype.

Our research has shown that the response of erythrocytes to anti-A1 lectin in newborns is very low. In most cases, there is no reaction. In rare cases, very low agglutination is expressed. The table below shows the four variants identified in the studied samples of newborns (table 18).

Table № 18 Serologic tests of samples from newborns.

samples	Anti-A	Anti_B	Anti-AB	Anti-A lectin	Anti-H lectin	Interpretation of results
1	+	-	+	-	+	A2
2	+	-	+	+ (very weak agglutination)	+	A1
3	+	+	+	-	+	A2B
4	+	+	+	+ (very weak agglutination)	+	A1B

As shown in the table below, the frequency of distribution of subgroups A1 and A2 is uneven in newborns. If A2 and A2B are considered rare phenotypes in donors, their number prevails over A1 and A1B phenotypes in newborns. 7% of the studied newborns carry subgroup A1. Subgroup A2 occurs in 37% of cases. As for the two newborns with the AB (IV) phenotype group, A2B was serologically detected in them.

Table № 19. Subgroups of antigen A in newborns.

ABO phenotypes	Subgroup	N	%
A	A1 A2	6 32	7 37
AB	A1B A2B	0 2	0 2
total		2	46

It should be noted that the prevalence of the A2 and A2B phenotypes identified serologically in newborns is associated with an incomplete synthesis of blood group antigens. As we have already mentioned in the

literature section, for the full expression of antigens of the ABO system, the postnatal period of development is necessary, therefore, erythrocytes of group A1 in newborns do not show a serological reaction to anti-A lectin due to incomplete synthesis. Thus, serologically the A1 subgroup reveals signs of mimicry in the A2 subgroup, which is a variable feature at the next stage of ontogenesis.

III.7. H antigen and features of its screening in newborns and donors.

The H group system is considered one of the most important systems among the erythrocyte group antigens. Unlike other antigens of the erythrocyte group, this system contains only one minor -the H antigen. As it was mentioned in the literature section, the H antigenic system is a genetic system independent of the ABO system. The loci of the coding genes of antigens of the H and ABO systems are located on different non-homologous chromosomes. However, it is generally known that the H antigen plays an important and crucial role in the serological formation of antigens of the ABO system. In the synthesis of antigens, A and B, they play the role of so-called precursor substance. We were interested in studying the serological properties of the H antigen in both donors and newborns.

Serological properties of the H-antigen were analyzed in 40 donors and 17 newborns studied by us. Each phenotypic group (0 (I), A (II), B (III) and AB (IV) of the ABO system) is taken from 40 donors in equal amounts (10-10). Also, 5 samples for each group were analyzed in newborns with group 0 (I), A (II), B (III), and in the case of group AB (IV), only two samples (due to the fact that out of 85 examined newborns, only two of them were carriers of the AB specificity).

In all donors of the O (I) phenotypic group taken for analysis, the H antigen showed strong agglutination, which means that the H antigen is present in large quantities in the erythrocytes of the O (I) group carriers (figure 22;table 16).

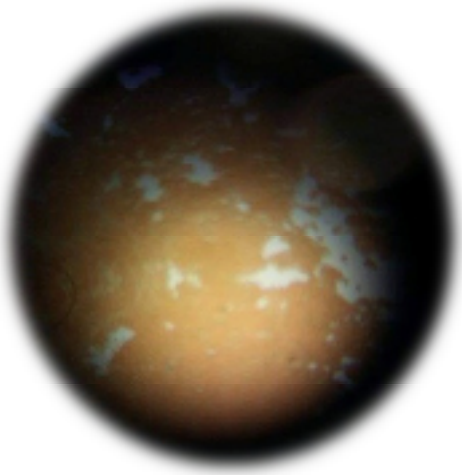

Figure № 16. Strong ability of agglutination H antigen in donors with phenotypic group O (I)

Table № 20. serological properties of donors with group 0 (I)

samples	Anti-A	Anti-B	Anti-AB	Anti-H	A erythr.	B erythr.
1	-	-	-	+ (strong agglutination)	+	+

Serological expression of the H antigen in other cases of groups A (II), B (III) and AB (IV) differs from the phenotypic group O (I). In this case, moderate or weak H-antigen agglutination was detected (figure 17). All this indicates its minimal amount in carriers of groups A (II), B (III) and AB (IV). The H antigen showed particularly weak agglutination in the case of the AB (IV) phenotypic group.

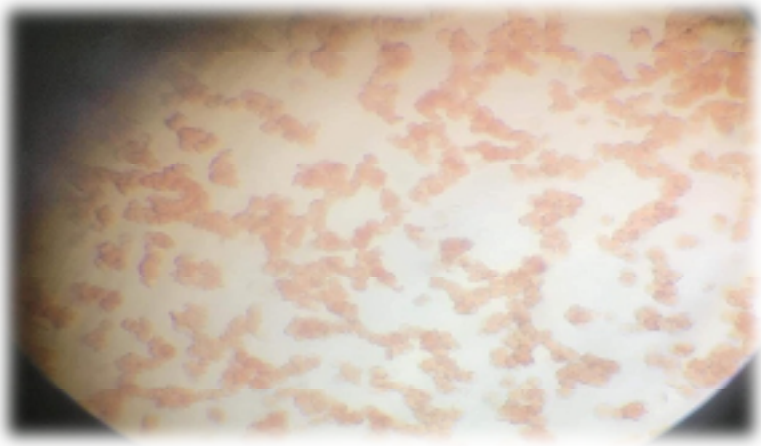

Figure № 17. Moderate agglutination of H antigen in donors with A(II), B(III) and AB (IV) phenotypic groups.

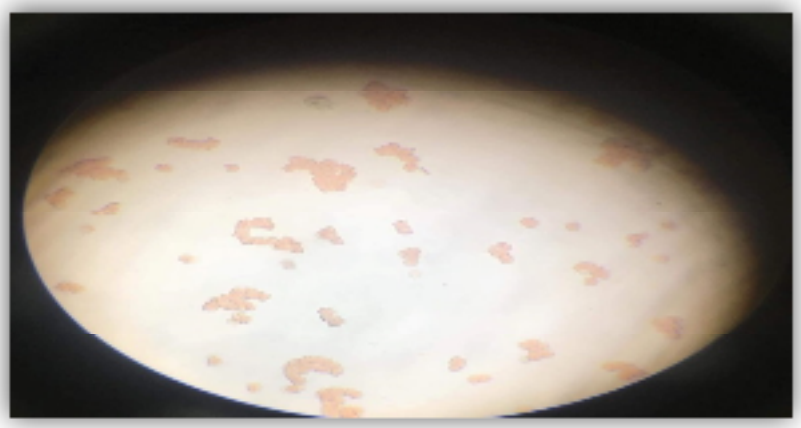

Figure № 18. Weak agglutination of H antigen in donors with A(II), B(III) and AB (IV) phenotypic groups.

As shown in the table below (table 21), H antigen in donors with group A (II) is detected serologically. In most cases (6/10) occurs its ability to moderate agglutination. 4 out of 10 donors were found to have very weak serological properties of the antigen H. Taking this factor into account, we can talk about homo- or heterozygous versions. In particular, there is a homozygous situation where a very weak agglutination of the H antigen is found which is the next stage of research.

Table № 21 serological properties of donors with A(II) group.

samples	Anti-A	Anti-B	Anti-AB	Anti-H	A erythr.	B erythr.
1	+	-	+	+ (Moderate level of agglutination)	-	+
2	+	-	+	+ (Moderate level of agglutination)	-	+
3	+	-		+ (Moderate level of agglutination)	-	+
4	+	-	+	+ (Moderate level of agglutination)	-	+
5	+	-	+	+ (Moderate level of agglutination)	-	+
6	+	-	+	+ (Moderate level of agglutination)	-	+

7	+	-	+	+(very weak agglutination)	-	+
8	+	-	+	+(very weak agglutination)	-	+
9	+	-	+	+(very weak agglutination)	-	+
10	+	-	+	+(very weak agglutination)	-	+

The antigen H of newborns with the group B (III) is also determined serologically (table22). The antigen H of newborns with the group B (III) is characterized by moderate agglutination (table). Like above mentioned, the H antigen with its serological properties in newborns of group B(III) is almost equal to the agglutination ability of the H antigen in donors with B (III) group.

Table № 22. serological properties of newborns with B (III) group.

Samples	Anti-A	Anti-B	Anti-AB	Anti-H	A erythr.	B erythr.
1	-	+	+	+ (Moderate level of agglutination)	+	-
2	-	+	+	+ (Moderate level of agglutination)	+	-

3	-	+	+	+ (Moderate level of agglutination)	+	-
4	-	+	+	+ (Moderate level of agglutination)	+	-
5	-	+	+	+(very weak agglutination)	+	-
6	-	+	+	+(very weak agglutination)	+	-
7	-	+	+	+(very weak agglutination)	+	-
8	-	+	+	+(very weak agglutination)	+	-
9	-	+	+	+(very weak agglutination)	+	-
10	-	+	+	+(very weak agglutination)	+	-

In the case of the two studied newborns of AB (IV) groups, the agglutination ability of the H antigen is relatively weak, as in donors (table23).

Table № 23. serological properties of donors with AB (IV) group.

samples	Anti-A	Anti-B	Anti-AB	Anti-H	A erythr.	B erythr.
1	+	+	+	+(very weak agglutination)	-	-
2	+	+	+	+(very weak agglutination)	-	-

III.8. Quantitative characteristics of natural antibodies

It was interesting for us to study the titer of natural antibodies in newborns.Here too, we randomly selected 10 cases (table 24). Of course, those samples were taken for analysis in which natural antibodies were detected. As mentioned above, they are mostly not synthesized in newborns.

Table № 24. The titer of natural antibodies in newborns.

N	Anti–A	Anti–B
1.	1:64	1:64
2.	1:64	1:64
3.	1:128	1:128
4.	1:128	1:128
5	1:256	1:128
6	1:128	1:32
7	1:64	1:32

8	1:64	1:64
9	1:64	1:128
10	1:32	1:128

A serious difference will arise if we compare the quantitative characteristics of antibodies anti- A andante- B in newborns and donors. Unlike adults, natural antibodies were not detected at all or were present in a rather low amount in newborns. It should also be noted that the anti-A antibody titer is much higher compared to the anti-B antibody titer. A similar picture was observed in the case of donors (adults).

III . 9. Newborns with severe hemolytic anemia

Here we would like to highlight newborns with severe hemolytic anemia, for whom phenotypic determination of the blood group becomes even more difficult and there is a need for genotyping. The difficulty is as follows: different group affiliation is detected using various immunoserological methods.

Of the 85 newborns examined, 25 were considered difficult due to a hemolytic reaction in them. using the Coombs test, a positive reaction was detected in a single case. Also, no incompatibility of the ABO system was found, since immune anti-A and anti-B antibodies were not detected in the maternal serum. However, an interesting nuance was revealed in a single case, in particular, a newborn (n = 4) has a blood group K +, and a mother has a K- phenotype (figure 19). It is possible that the hemolytic reaction is caused by this particular antigen. Unfortunately, we have not studied anti-Kell antigens.

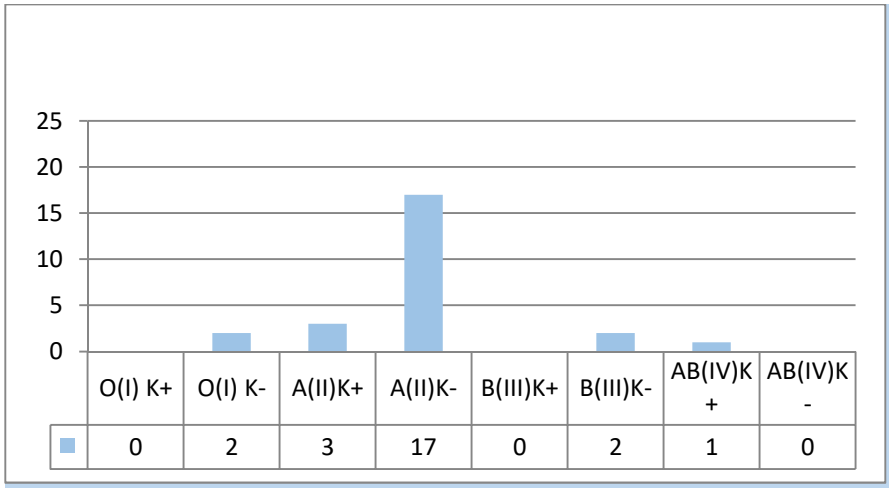

Figure № 19. K^- and K^- phenotypes in newborns with sevre hemolytic anemia.

Attention has also been drawn to the c antigen of the rhesus system as one of the immunogenic antigens that can provoke immune reactions during pregnancy, as well as hemolytic reactions in newborns. One of 25 newborns with hemolytic jaundice we studied had the c (cc) antigen in a recessive homozygous state, while the mother had a CC version instead. It is possible that hemolytic jaundice was caused by anti-c antibodies. Anti-c antibodies have not been studied by us in this particular case either.

III . 10. Characteristics of Rh antigens in newborns with severe hemolysis

None of the examined newborns had any difficulty in identifying the group with the Rh factor, which, apparently, is caused by a strong ability of agglutination during prenatal development. Most of the 25 difficult newborns are carriers of antigen D. This antigen was not detected in only two cases. (figure 20). Here I would like to note that the agglutination caused by antigen D, in all studied variants, was detected by the plate method within 2-3 minutes and was visible even with the naked eye. and agglutination corresponding to 4+ and 3+ was detected by the column agglutination method.

In contrast to antigens of the ABO system, in the case of antigen A, when using the column method of agglutination, rather low variability was observed, in particular, agglutination corresponding to 3+, 2 + was mainly detected. Agglutination of degree 1+ was also detected in a single case.

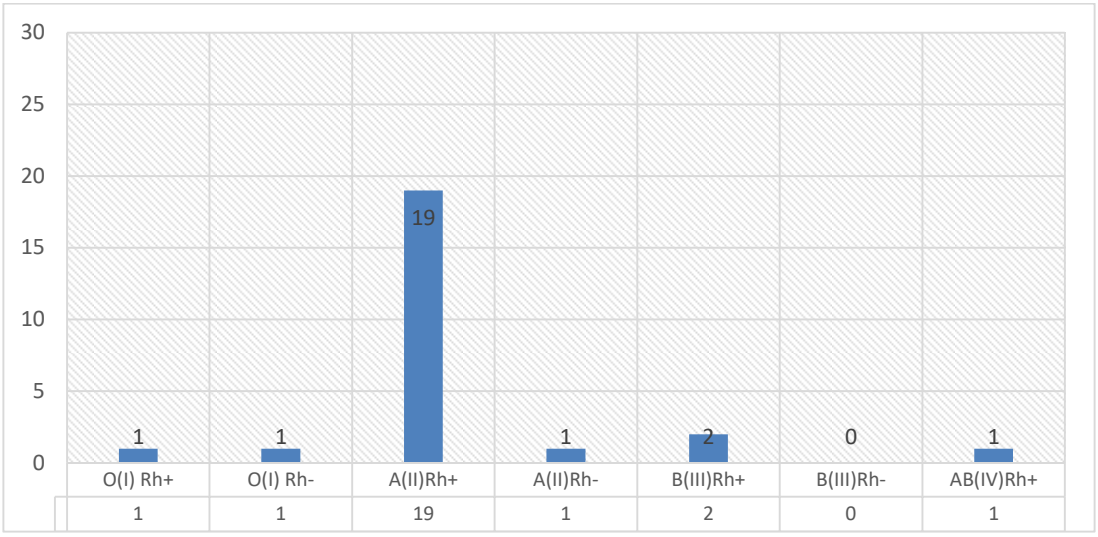

Figure № 20. Belonging to the Rhesus group in combination with antigens of the ABO

Conclusions:

1. Group-specific antigens in newborns are characterized by a lower ability of agglutination in comparison with erythrocyte antigens in adults. Especially noteworthy is the antigen A;

2. the frequency of detection of natural group-specific antibodies in newborns is lower than in adults, which increases the risk of incorrect blood group determination in newborns;

3. Difficulties in determining the blood group and rhesus arise in complex newborns (in the case of hemolytic anemia and other modern problems) and often become the cause of misinterpretation of the blood group.

4. For phenotyping of the ABO system, it is better to use blood from a peripheral vein than blood from the umbilical cord, especially when it comes to newborns with a complex anamnesis.

5. Monoclonal antibodies should be used to identify subgroups of both antigen A and antigen B, for complete blood typing and elimination of errors.

6. Sufficiently high polymorphism was observed in group O (I) when studying phenotypic combinations of antigens of four erythrocyte group systems in donors. It is followed by combinations of the phenotypic group A (II). Group B (III) ranks third in the number of phenotypic variations, and group AB (IV) is characterized by low polymorphism. That is, we can conclude that the polymorphism in the donors studied by us was distributed in the following order: O> A> B> AB.

7. The investigated donors showed signs of high polymorphism of the Rh system, namely: (27.8 ± 1.53%) most of them belonged to the CcDe phenotype (n = 237). The CcDEe phenotype is presented with a prevalence of 19.3 ± 1.35% (n = 165); 125 donors studied were carriers of the CDe phenotype (14.6 ± 1.2); the prevalence of the cde phenotype is 13.1 ± 1.5%, which means that 122 donors studied belonged to this phenotypic group; 87 donors revealed the phenotypic characteristics of the cDEe (10.2%); the prevalence of the cDe phenotype was 4.9% (n = 42); 19 donors have the CDEe phenotype. The prevalence of other phenotypes (CDE, Cde, CcdEe, Ccde) was significantly low.

8. We found differences in the prevalence of rhesus phenotypes between blood donors and the population of Adjara; For example, donors have much more phenotypic variations than the Adjara population. In the region of Adjara, six phenotypic groups of rhesus were identified with different frequency of distribution. In the same region, using the example of blood donors from one clinic, 2.8 times more phenotypic signs of rhesus were identified. We believe that the reason for these differences lies in the fact that we pay attention to nationality when studying Rh antigens at the level of the population of Adjara. Each participant in the previous study was ethnic Georgian. In the case of blood donors, since they are officially donors, their nationality is different and they belong to different ethnic groups.

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