

Original Article

ABO system combination with Rh, Kell and MN group in Georgian blood donors

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Abstract: There are numerous scientific data about the study of the prevalence of blood group antigens in the different donor population. Several studies showed that the profile of major blood group antigens is not similar in blood donors from different local areas. Research objective: Our scientific goal was to study of the prevalence blood group antigens in the Georgian blood donor population. In the current study, we analyzed the 48 phenotypically combinations based on four major (ABO, Rh, Kell, and MN) blood groups. Research methods: The blood of 1009 donors has been studied on RBC antigens. The sample were collected from the diagnostic laboratory of Medina Ltd Health Centre of Batumi. Blood typing of the sample has been carried out on the basis of the immunogenetics laboratory of Batumi Shota Rustaveli State University. The universal monoclonal antibodies was used for identify minor blood group antigens. We used as forward as reverse grouping methods. For identification erythrocytes, blood group antigens also were used ID cards, such as ABO/D + Reverse Grouping. Result: 12 phenotypic combinations have been identified in each O, A, B, AB group of ABO system. Out of 48 theoretically possible phenotypic combinations, we can actually find 1,9 times less phenotypes and the real amount is 25 phenotypes. The remaining 23 phenotypic combinations have not been observed in the studied donors. 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⁺ MM; 19. AB, Rh⁺K⁺ MN; 20. AB, Rh⁺K⁺ MM; 21. AB, Rh⁺K⁺ NN; 22. AB, Rh⁺K⁺ NN; 23. B, Rh⁻K⁻ NN. The value of χ^2 in the case is equal to 3221,16. The P-Value is < .00001. The result is significant at P < .05. Out of 1009 studied donors 349 are carriers of phenotypic group A (II), while 19 donors carry AB (IV) group specification. This means that 36.23% of the studied donors have A antigen on the surface of erythrocyte membrane. The majority of them A1 subgroup. Conclusion: As our research showed there is a quit high polymorphism of blood group phenotype combinations in Georgian blood donors in the example of one clinic. This kind of data is very important for the clinics' rational preparation of whole blood or blood components.

Keywords: Blood donor, antigens, prevalence, combination, phenotype

Introduction

A blood donation occurs when a person voluntarily has blood drawn and used for transfusions. Potential donors are evaluated for anything that might make their blood unsafe to use. Blood donor's biological materials are also typing on blood group antigens. Blood group antigens are expressed on the membrane surface of blood cells, such as erythrocytes, leukocytes, and thrombocytes and they determine the specific blood group. From a clinical point of view, erythrocyte group systems are of vital importance among the listed systems of blood cell groups, since they are responsible for the

immune compatibility between the donor and recipient during blood transfusion [1-3]. The antigens of the ABO system are also considered tissue antigens and are therefore of particular importance in organ transplantation and epidemiology [4].

A total of 39 blood group systems are recognized by the International Society of Blood Transfusion (ISBT). Many scientific data shows the possible correlation between blood group antigens and different type of diseases [5-14].

There are numerous scientific data about the study of the prevalence of blood group antigens

Blood in the donors

in the different donor population. Several studies showed that the profile of major blood group antigens is not similar in blood donors from different local areas [15-22]. Our scientific goal was to study of the prevalence blood group antigens in the Georgian blood donor population. In the current study, we analyzed the 48 phenotypically combinations based on four major (ABO, Rh, Kell, MN) blood group systems.

Research materials and methods

Studying materials

1009 blood donors has been studied on erythrocyte group antigens. A total of 1 ml of peripheral venous blood was collected. Blood Samples from the donors were collected in special tubes by the method of venipuncture.

One inclusion criteria in this study for the blood donors is age. According to the recommended norms and regulations of World Health Organization (WHO), only people from the certain age group can donate blood. The age range of the observed patients is 18-60. Majority our studied donors are between 20-55 years. Other inclusion criterion in this study is weight. The minimum weight of the patients is 50 kg. One of the most necessary factors is also the level of hemoglobin in the blood. Hemoglobin level for the male donors should be at least 130.0 mg all and for the female donors 120 mg. Most of the studied donors are the males.

The current retrospective study was carried out within the two years of 2018-2020. Our research was agreed with the hematology department and ethics committee of the clinics (ethics statement approval data is 11.10.2018). In some cases, in the clinic donors usually give blood when family members or friends need a transfusion (directed donation). We did not take the additional invasion from the blood donors and take that material directly from the laboratory of the clinic. The sample has been provided from the diagnostic laboratory of Medina Ltd Health Centre of Batumi. The clinic is one of the bigger in the Adjara region, which is the west part of the Georgia Republic. We didn't have any contact with blood donors and have only donors' special code and information about the age and gender.

Blood typing of the sample has been carried out on the basis of the immunogenetics laboratory of Batumi Shota Rustaveli State University (BSU).

Research methods

The following specific test-systems were used during the research: anti-AB, -B, -A, A1, -A2 (H), -C, -c, -D, -E, -e, -K, -k, -M, -N (Bio-Rad, cypress diagnostics), standard O (I), (II), (III) group erythrocytes and standard O (I), A (II), B (III), AB (IV) serums. For identification erythrocytes, blood group antigens also were used ID cards, such as ABO/D + Reverse Grouping (Bio-Rad). Both research methods are based of specific antigen-antibodies agglutination reaction.

Statistically methods

The obtained material has studied and processed statistically. Rates between groups were compared by Chi-square analysis of proportions. The level of statistical significance was set at 0.05. We used the social science statistics for analyses our obtained data (<https://www.socscistatistics.com/tests/chisquare2/default2.aspx>).

Result

ABO, Rh, Kell and MN blood group system antigens combination in the studied donors

Four types of group system phenotypic combinations have been studied. A total of 1009 donors were typed during the 2018-2020 studying period. Based on four major blood group system combinations, we have identified theoretically possible 48 phenotypic groups (**Table 1**).

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 χ^2 is quite effective for rejecting the null hypothesis ($E = 0$). The value of χ^2 in the case is equal to 3221,16. These numbers are much higher than the critical value (CV) of the criterion of the degree of freedom ($df = 47$), which is equal to 62.83. The P -Value is $< .00001$. The result is significant at $P < .05$ (**Table 1**).

Blood in the donors

Table 1. The combination of Rh, Kell, and MN blood group antigens for O, A, B, AB groups donors

N	Phenotypic combinations	Number number of donors (1009)	Percentage %	N	Phenotypic combinations	Number number of donors (1009)	Percentage %	χ^2 -chi-square criterion
1	O, Rh ⁺ K ⁺ MN	125	12,3±1,03	25	B, Rh ⁺ K ⁺ MN	27	2,67±0,5	3221,16
2	O, Rh ⁺ K ⁺ MM	96	9,51±0,9	26	B, Rh ⁺ K ⁺ MM	26	2,57±0,5	
3	O, Rh ⁺ K ⁺ NN	31	3,07±0,5	27	B, Rh ⁺ K ⁺ NN	0	0	
4	O, Rh ⁺ K ⁻ MN	38	3,7%±0,5	28	B, Rh ⁺ K ⁻ MN	33	3,27±0,5	
5	O, Rh ⁺ K ⁻ NN	2	0,19%±0,1	29	B, Rh ⁺ K ⁻ NN	7	0,69±0,2	
6	O, Rh ⁻ K ⁺ MM	112	11,10±0,9	30	B, Rh ⁻ K ⁺ MM	16	1,58±0,3	
7	O, Rh ⁻ K ⁺ MN	47	4,65±0,6	31	B, Rh ⁻ K ⁺ MN	0	0	
8	O, Rh ⁻ K ⁺ MM	0	0	32	B, Rh ⁻ K ⁺ MM	0	0	
9	O, Rh ⁻ K ⁺ NN	6	0,59±0,2	33	B, Rh ⁻ K ⁺ NN	0	0	
10	O, Rh ⁻ K ⁻ MN	0	0	34	B, Rh ⁻ K ⁻ MN	0	0	
11	O, Rh ⁻ K ⁻ MM	47	4,65±0,6	35	B, Rh ⁻ K ⁻ MM	0	0	
12	O, Rh ⁻ K ⁻ NN	0	0	36	B, Rh ⁻ K ⁻ NN	0	0	
13	A, Rh ⁺ K ⁺ MN	37	3,66±0,5	37	AB, Rh ⁺ k ⁺ MN	0	0	
14	A, Rh ⁺ K ⁺ MM	111	11±0,9	38	AB, Rh ⁺ k ⁺ MM	27	2,67±0,5	
15	A, Rh ⁺ K ⁺ NN	27	2,67±0,5	39	AB, Rh ⁺ k ⁺ NN	0	0	
16	A, Rh ⁺ K ⁻ MN	56	5,55±0,7	40	AB, Rh ⁺ k ⁻ MN	18	1,78±0,4	
17	A, Rh ⁺ K ⁻ NN	7	0,69±0,2	41	AB, Rh ⁺ k ⁻ NN	0	0	
18	A, Rh ⁻ K ⁺ MM	95	9,41±0,9	42	AB, Rh ⁻ k ⁺ MM	0	0	
19	A, Rh ⁻ K ⁺ MN	0	0	43	AB, Rh ⁻ k ⁺ MN	0	0	
20	A, Rh ⁻ K ⁺ MM	0	0	44	AB, Rh ⁻ k ⁺ MM	0	0	
21	A, Rh ⁻ K ⁺ NN	0	0	45	AB, Rh ⁻ k ⁺ NN	0	0	
22	A, Rh ⁻ K ⁻ MN	16	1,58±0,3	46	AB, Rh ⁻ k ⁻ MN	1	0,09±0,09	
23	A, Rh ⁻ K ⁻ MM	0	0	47	AB, Rh ⁻ k ⁻ MM	1	0,09±0,09	
24	A, Rh ⁻ K ⁻ NN	0	0	48	AB, Rh ⁻ k ⁻ NN	0	0	

CV-critical value -62.83; Df-degree of freedom-47; P value -the probability of obtaining results. The P-Value is < .00001. The result is significant at P < .05.

According to the above mentioned combinations, 12 phenotypic combinations have been identified in each O, A, B, AB group. For example, O (I) phenotypic group has following combinations with 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 to identify similar phenotypic groups for B (III) phenotype: 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 has 12 combinations in correlation with the other group systems. Among them are: 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 1**, out of 48 theoretically possible phenotypic combinations, we can actually find 1,9 times less phenotypes and the real amount phenotypically combination is 25. The remaining 23 phenotypic combinations have not been observed in the studied donors. 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.

Blood in the donors

Table 2. Prevalence of A and AB sub-groups among the studied donors (n = 368)

ABO phenotype	subgroup	N	%
A	A1	324	32,11
	A2	25	2,47
AB	A1B	12	1,18
	A2B	7	0,69
Total		368	36,45

N-number of the studied blood donors.

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.

A antigens subgroups in the studied donors

The antigen A is expressed on the erythrocyte membrane of people with A (II) and AB (IV) blood group. A antigen is generally present in several subgroups that show different prevalence. These are: subgroup of A1, A2 and weak A antigens. In the next step all A (II) and AB (IV) phenotypes were tested using A1 lectin. The sample is considered a subgroup A1 when both anti-A and A1 lectin shows well expressed agglutination reaction. 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.

Out of the 1009 studied donors, 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 majority of them carry A1 subgroup (**Table 2**).

As shown in the **Table 2**, the frequency of subgroups A1 and A2 is not equal. In our Studied donors 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 donor population is characterized by a rather low prevalence. The rate of distribution of A2 subgroup is only 4.05%.

Discussion

Most of the studied donors are the males (79.9%). Only 233 out of 1009 studied donors were females. In this case our data is similar as world donor population. 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. The iron deficiency anemia has occurred more frequently in female blood donors than male. Iron is necessary for maintaining the hemoglobin level in the blood [23-25].

From a transfusion point of view ABO erythrocyte group system is vital importance, since they are responsible for the immune compatibility between the donor and recipient during blood transfusion. Secondary important is Rh system. In most transfusion centers in the world, the blood of donors at transfusion stations and blood banks are 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 (A, B, D) antigenic compositions, however, 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 [14, 26].

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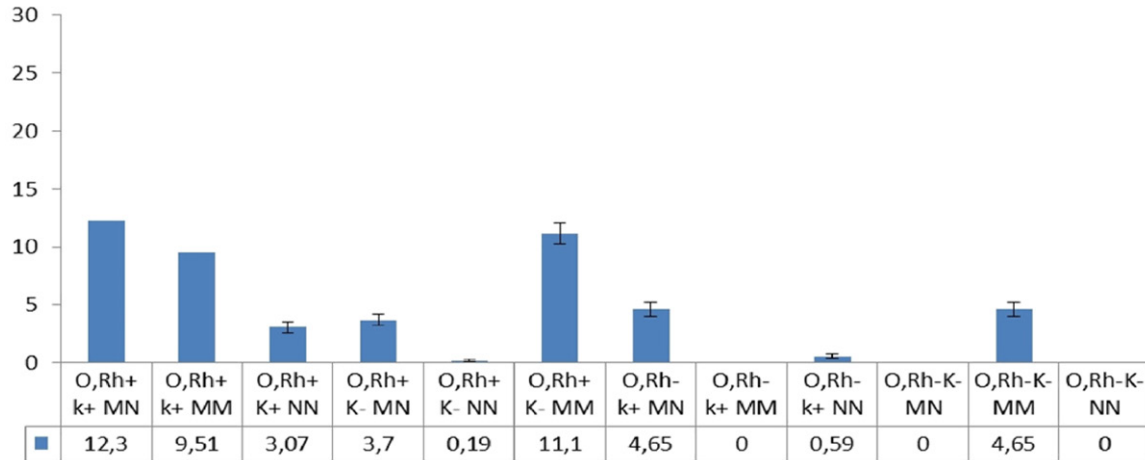


Figure 1. The distribution of MN, KELL blooda group antigen combinations for O RH⁺, and ORH-donors.

The data we have gathered has been analyzed in relation to ABO group system. O (I) phenotypic group combinations in relation to other systems have actually produced only 9 phenotypic combinations out of 12 theoretical probabilities (**Figure 1**). Three phenotypes (1. O, Rh⁺K⁺ MM; 2. O, Rh⁺K⁻ MN; 3. O, Rh⁺K⁻ NN) have not been observed in the O (I) blood group donors. As is seen from the list, the mentioned three phenotype combinations belong to O, Rh⁻ phenotypes, and in one case K⁺ (O, Rh⁺K⁺ MM) is present in the combination with them.

All the six combinations from the O, Rh⁺ phenotypic group are found in the studied donors, but with different prevalence. The 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% and 11.1%). The frequency of O, Rh⁺, K⁻, NN phenotypic combination is the least intense among the O, Rh⁺ phenotypes and is equivalent to 0.19%.

Different from the O (I) blood group, the variations of combinations of A (II) phenotypic group is much less. Out of 12 theoretically possible combinations we have found nearly half of them. Actually, this group has only seven combinations and six out of them belong to A (II), Rh⁺ group. 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⁻ NN-0.69%; 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 theoretically possible six combinations. The frequency of A, Rh⁻K⁻ MN phenotype is 1.58% (**Figure 2**).

In the case of B (III) phenotype group, the actual amount of the variations of phenotypic combinations has decreased even more. In the case of phenotypic group B (III), only five 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. B, Rh⁺K⁺ MN-2.67%; 4. B, Rh⁺K⁻ MM-1.58%; 5. B, Rh⁺K⁻ NN-0.69%). As seen from **Figure 3**, all the practically identified phenotypic combinations are of B, Rh⁺ phenotype and in the case of B, Rh⁻, none of the six possible combinations has been detected.

The amount of variations of phenotypic group combinations has decreased even more in AB (IV) blood donors. In this case, out of 12 possible combinations, we have only identified four, but with a very low prevalence. More specifically are two phenotypes. They are: 1. AB, Rh⁺K⁺ MM-2.67%; 2. AB, Rh⁺K⁻ MN-1.78%. As it is visible, both phenotypic combinations belong to AB, Rh⁺. While research, we have also found two phenotypic combinations of AB, Rh⁻, they are AB, Rh⁻K⁻ MN, and AB, Rh⁻K⁻ MM (**Figure 4**). The distribution frequency for each of them is only 0.09%.

While observing the phenotypic combinations due to antigens of four erythrocyte group

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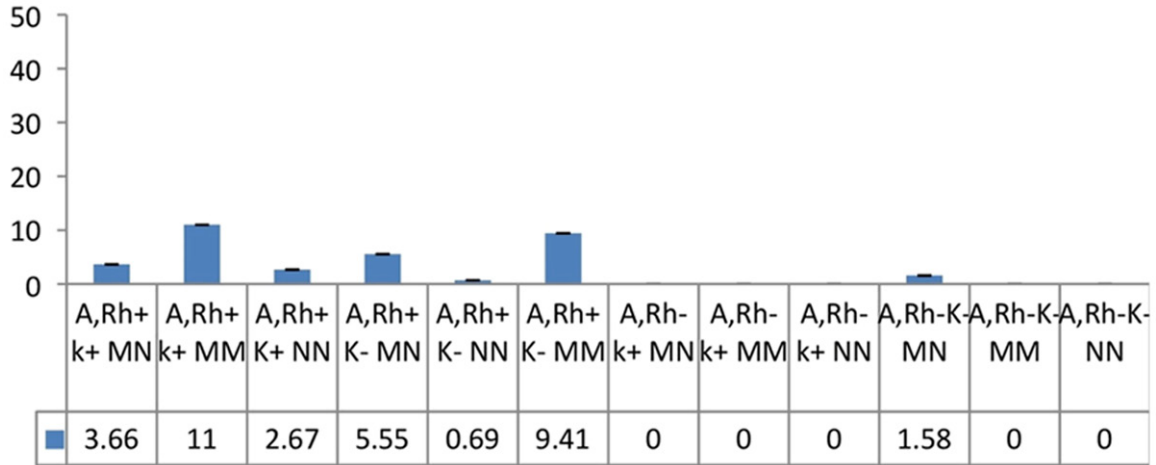


Figure 2. The distribution of MN, KELL antigen combinations in A, Rh; A, Rh⁺, donors.

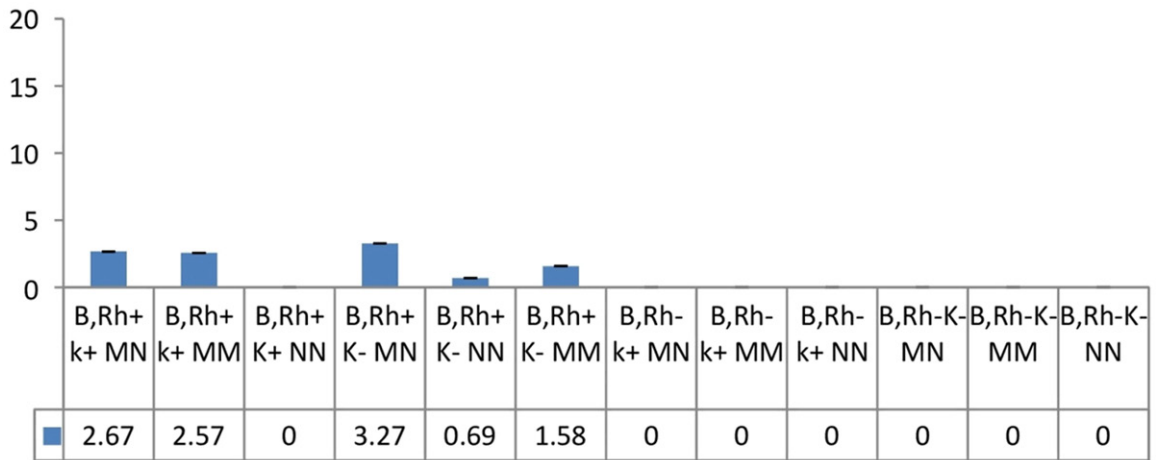


Figure 3. The distribution of MN, KELL antigen combination in B, Rh; B, Rh⁺ donors.

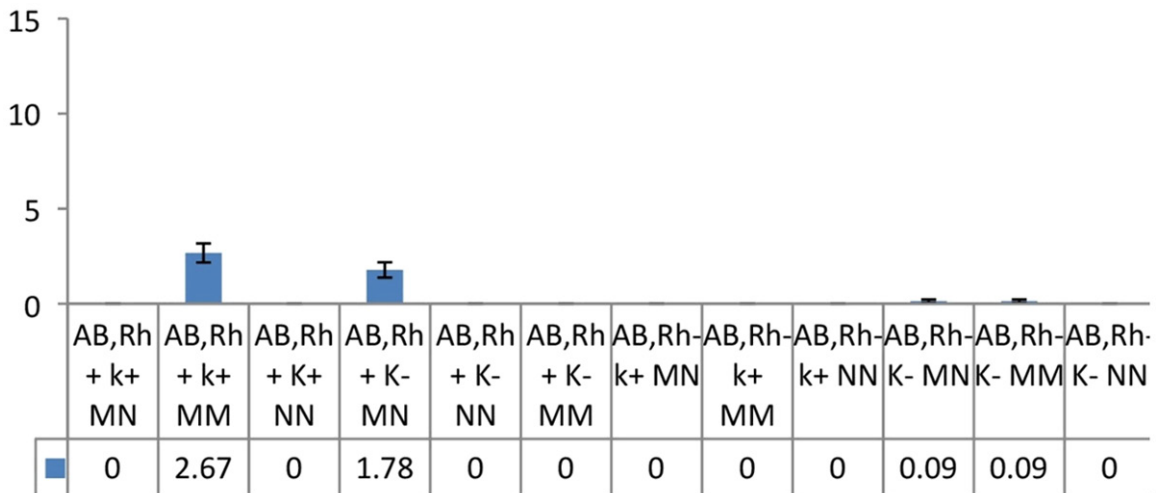


Figure 4. The distribution of MN, KELL blood group antigen combination for AB, Rh; AB, Rh⁺, donors.

systems, a comparatively high polymorphism has been found in the case of O (I) blood group. It is followed by combinations of A (II) phenotypic groups. B (III) phenotypic group holds third place in a row with a multitude of variations, and AB (IV) is characterized 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.

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 [27]. In contrast to the A1 antigenic determinant, erythrocytes with a specificity of the A2 subgroup are characterized by weak agglutinating ability with the monoclonal anti-A antibodies used in the majority clinics of Georgia. 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).

Conclusion

As our research showed there is a quit high polymorphism of blood group phenotype combinations profile in Georgian blood donors in the example of one clinic. This kind of data is very important for the rational preparation of whole blood or blood components for the clinics. We consider that the existence of this data will really promote the increase of safety level of transfusion.

Disclosure of conflict of interest

None.

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