The features of antigen prevalence of Rhesus system in donor population

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Abstract: Research materials and methods. 852 voluntary Georgian blood donors have been typed on red blood cells group antigens. The research materials have taken from the diagnostic laboratory of Health Centre of Batumi (Georgia republic). The immunoserological methods with monoclonal anti –AB, -B, -A, A1, -A2 (H), -C, -c, - D, -E, - e (Bio-Rad, cypress diagnostics) antibodies was used for typing blood. The ID cards, such as ABO/D $^{+}$ Reverse Grouping (Bio-Rad) were also used for typing of erythrocyte antigens.

Result. Prevalence of Rh system antigens in the studied group is looks like so: e antigens – 94,6%, c antigens - 85%, C-68,03, E antigens - 38,07%. The majority (84%) of the studied donors are Rh-positive (n=719), 133 (16%) donors are Rh-negative. C antigen most common is present in the combination with D antigen. 65, 8 % case donors had CD+ combination (n=561). 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. We have studied the Rh phenotypes prevalence in blood donors. According to RHD, RHC, and

RHE gene loci, there are 18 theoretically possible phenotypical groups. Among them half (nine) are Rh-positive and the rest of them 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 phenotype CdE, which belongs to Rh negative group, was not present in studied donors. Other 17 phenotypes showed different frequencies. Some of them were only in a single case, for example, cdEe, cdE, CdEe phenotypes had only one donor. The majority of the phenotype in he 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.

Conclusion. Our studied donors are characterized by rather high polymorphism. The Georgian donor's population is heterogenic, especially high heterogeneity are shown in Rh positive phenotypes. The obtained data is vital importance for the preparation of whole blood or certain blood components for the purpose of their rational usage in blood transfusion.

Kay words: Rh system, Phenotype, blood donor, erythrocytes, antigen.

I. INTRODUCTION

Group antigens determine the trophic and regulatory functions of blood cells. It is well known that they are part of cellular receptors, through which hormones, vitamins, enzymes, and other proteins are transported in the circulatory system and are the main structural elements of cell membrane adhesion [1] [2] [3]. Blood group antigens determine the adaptation of a human to the environment. Erythrocyte group systems are significant for ethnic anthropology. The combination of antigens of group systems in the human population is likely a result of balanced polymorphism. According to the erythrocyte group antigens, the correlation between balanced polymorphism and various types of infectious and noninfectious diseases was well established [4] [5] [6] [7].

blood group antigens have ability to initiate creation of antierythrocyte antibodies, which can damage the red blood cells. The antierythrocyte antibodies cause different types of transfusion reactions, post-transfusion difficulties and hemolytic disease in newborns [8] [9].

Currently, about 39 systems of erythrocyte groups are distinguished, which include about 346 antigens that can induce the severe transfusion reactions. From a clinical point of view, ABO, Rh, Kell, MNSs, and other systems are the most and vitally important.

Blood erythrocyte group antigens are the genetically strictly deterministic feature. In spite of resistant specific characteristics, the relevant hereditary factors within the ranges of species at the level of populations and individuals are characterized with rather high polymorphism [10] [11] [12] [13].

The Rhesus system consists of 49 defined antigens. Five Rh system antigens (D, C, c, E, and e) are the cause of the second most important adverse effects, after those due to ABO, when group incompatibility occurs in blood transfusion. Their function in evolution, genetic disease, and forensic medicine is being intensively investigated. The rhesus system D, C, c, E, and e antigens are determined by two genes RHD and RHCE encoding for RhD and RhCE proteins, respectively [14] [15].

The Rhesus blood group systems show considerable genetic variation between populations across the world. Many blood transfusions and blood banks from different countries and regions have many investigations about study the prevalence of Rh antigens [10] [12] [16] [17] [18].

Our goal was to study the Rh antigens (C, c, D, E, e) in the Georgian donor population. The only rhesus system D antigen has typing in donor-recipient throughout the region (Adjara, Georgia republic). It is interesting to find the frequency of other Rh antigens and Rh phenotypes and estimate the theoretical risk of sensibilization caused by Rh antigen.

Our research also has clinical applications. Based on our research clinic has the possibility to have its donors' Rh phenotypes and combinations database. By using this database, it is quite easy to select fully compatible donors for recipients. This data is vitally important for patients with rare Rh phenotype combinations. A similar approach would certainly decrease the risks of post transfusion reactions caused by rare antigens.

The erythrocyte Rh blood group system shows high polymorphism and heterogeneity with different populations. Our study shows Rh blood group phenotype polymorphism in Georgian blood donor population.

II. Material and Methods

The subjects of our study are blood donors. The material of the research is vein blood samples. 852 donors have been typing on red blood cells' antigens. The majority of our studied donors were males (627 are male and 225 female). The most of our studied donors (92,5%) are Georgians, but there are some people from Armenia, Azerbaijan, Ossetia, Russia, Ukraine, India and others. The research was carried out 2016-2019 years.

According to the recommended norms and regulations of World Health Organization (WHO), only people from a certain age group can donate blood. The age range of the observed donors is 18-60 (The average age of the studied blood donors is 38 years). The minimum weight of the donors is 50 kg (The average weight of the studied blood donors is 65 kg). One of the most necessary factors for blood donation is also the level of hemoglobin in the blood. Hemoglobin level for the male donors should be at least 130.0mg all and for the female donors 120 g.

The material for the research has been taken adhering to the bioethics norms. Based on the conclusion of the Clinic Bioethics Committee, we were able to use the blood materials from the clinic's laboratory. We did not take an additional invasion for collecting blood samples from the donors and don't contact with donors. The studying materials were 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 (BSU).

The research methods were immuneserologically forward and reversed methods with monoclonal antibodies. We used following monoclonal antibodies: anti –AB, -B, -A, A1, -A2 (H), -C, -c, - D, -E, -e (Bio-Rad, cypress diagnostics). We used as a plate as tube methods during research.

In addition, the studied donor group in some cases was also determined by the column agglutination methods. Gel card system–so-called ID cards were used (Bio-Rad). 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 during research. Some Rh- blood donors additionally were typing for weak D (ID-Diaclon Anti-D for weak D, Bio-Rad).

All the research methods were based on specific antigen-antibody agglutination reaction. All negative reactions (without agglutination) were confirmed microscopically. We used an optic microscope with different magnification (10X4, 10X10, and 10X100). This method helps us to identify low-level agglutination reactions.

The Rh antigens, phenotypes and antigens combinations frequencies were expressed as percentages. This percentage is calculated by summing the number of donors, which contains the specific erythrocyte antigen, phenotype or antigens combination and dividing by the total number of studying materials. For calculated the Rh system allele frequency we used The Hardy-Weinberg equation. The frequency of Rh haplotype was calculated by using the formula shown in the table VI. We used the on-variable chi-square general formula for calculating chi-square ($\chi 2$). The level of statistical significance was set at 0.05.

III. RESULT

852 voluntary Georgian blood donors have been typed on red blood cells group antigens. As already was mentioned above The Rh group system consists of 49 defined blood group antigens [19] [20]. The five antigens D, C, c, E, and e are the vital important for transfusion. The study of Rh blood group antigens, phenotype, and Rh antibodies are very useful in routine and advanced clinical practice in blood transfusion centers. We study the prevalence of these five Rh antigens in Georgian blood donors (n=852) of both sex and different age (18-60 y.) (Table I).

The prevalence of Rh system antigens in the studied donors is looking like so: e antigen – 94,6%; c antigen - 85%; C-68,03%; and E antigen - 38,07%. The majority (84%) of our studied donors are Rh-positive (n=719), only 133 (16%) donors are Rh-negative. We used one-variable chi-square criterion. 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 the degree of freedom (d.f.=3), which is equal to 7,815 (Table I).

Table I . Prevalence and Chi-square analysis of Rhesus system antigens in the 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 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. Allele's distribution in the studying population is equal to 1 (p + q = 1). For calculated the allele frequency we used The Hardy-Weinberg equation. The distribution of two alleles of the RhE gene is as follows: E - 0,61 , e -0,40 (p + q = 1). RhD reveals a rather high frequency of distribution and it is 0,64 (Table II).

Table II. Frequency of distribution of alleles of the Rh system in donors.

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

As we see from Figure № 1 C antigen most common is present in the combination with D antigen. 65, 8 % case we had CD (CRH+) combination (n=561). A similar situation is with E antigen combination with D antigen. Erythrocyte E antigen in most donor cases is presented with a combination of D antigen (ED or ERH+). 36, 9% of the studied donors

(n=306) had ED+ combination. A miserable number of studied donors had CD – (CRH-) (2,23%; n=19) and ED – (ERH-) (1,17%; n=9) combinations. We can make the

conclusion that C and E antigens are more linked with D antigens based on our data.

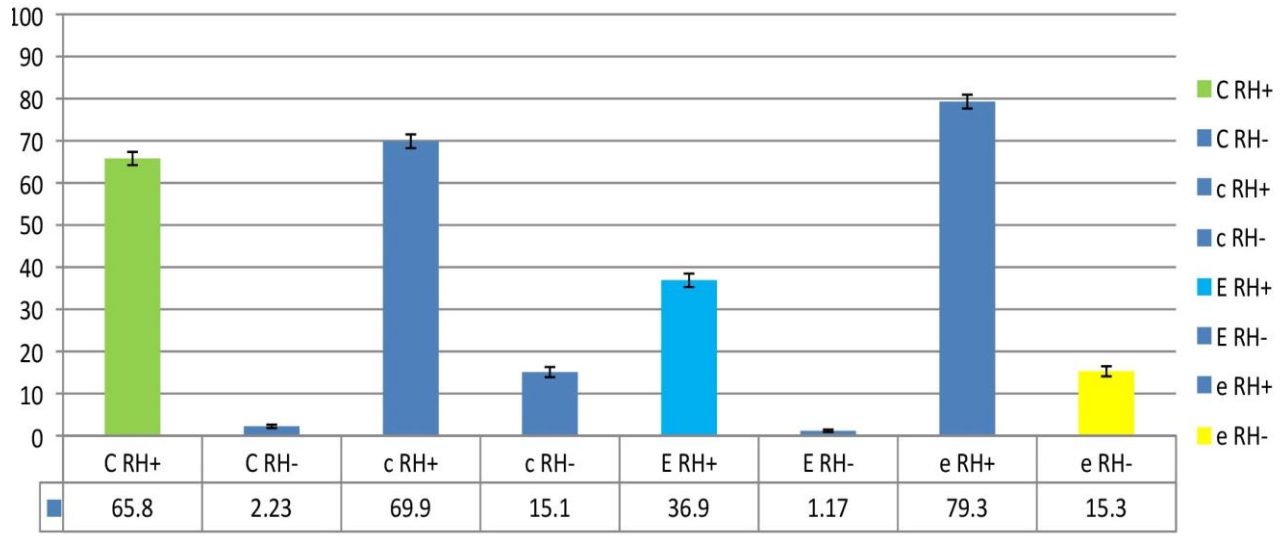

Figure № 1. C, c, E e antigens combination with D positive (Rh+) and D negative (Rh-) donors.

The Rh blood group system has two sets of nomenclatures . In our study, we used Fisher and Race CDE nomenclature [21].

We have studied the Rh phenotypes prevalence in blood donors. Phenotypic groups are divided according to serologically detected Rhesus system C, c, E, e, D antigens. For example phenotype D+C+E+c-e- means that donors blood have a positives reaction with anti-D, anti-C, and anti-E monoclonal antibodies, while anti-c and anti-e antibodies don't show any agglutination with donors' blood. D+C+E+c-e- phenotype also be written as CDE. D+C+E+c-e+ phenotype are written as CDEe and act.

According to RHD, RHC, and RHE gene loci there are 18 theoretically possible phenotypically groups. Among them, half (nine phenotypes) are Rh-positive and the rest of them 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 phenotype CdE, which belongs Rh negative group was not present in studied donors. Other 17 phenotypes showed different frequency (Table 3, Figure 2). Some of them were only in a 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 III, Figure 2).

Table III. 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 $D^-C^+E^+c^+e^-$	0	1	0	0	0	0	0	0	1
CcdEe $D^-C^+E^+c^+e^+$	0	4	0	2	0	0	0	0	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

There are some errors in determining the rhesus phenotype. Errors in determining the Rh factor is associated with a weak variation 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 weak D 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. No weak D antigen was observed in any of these 4 cases and accordingly, the primary detected phenotype of rhesus did not changed.

Rh positive donors, as we already mentioned above have nine phenotypes and their frequency was quite different. The studied population is heterogenic based this charachteristics. 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 these 2 phenotype characteristics on the erythrocyte. Another two phenotypes (CCDe – 17,38% and cDEe -12,1%) frequency equals 29,48%. The Rest of the five phenotypes (CDE, CDEe, CcDE, cDE, and cDe) prevalence was 14,47% (Figure 3).

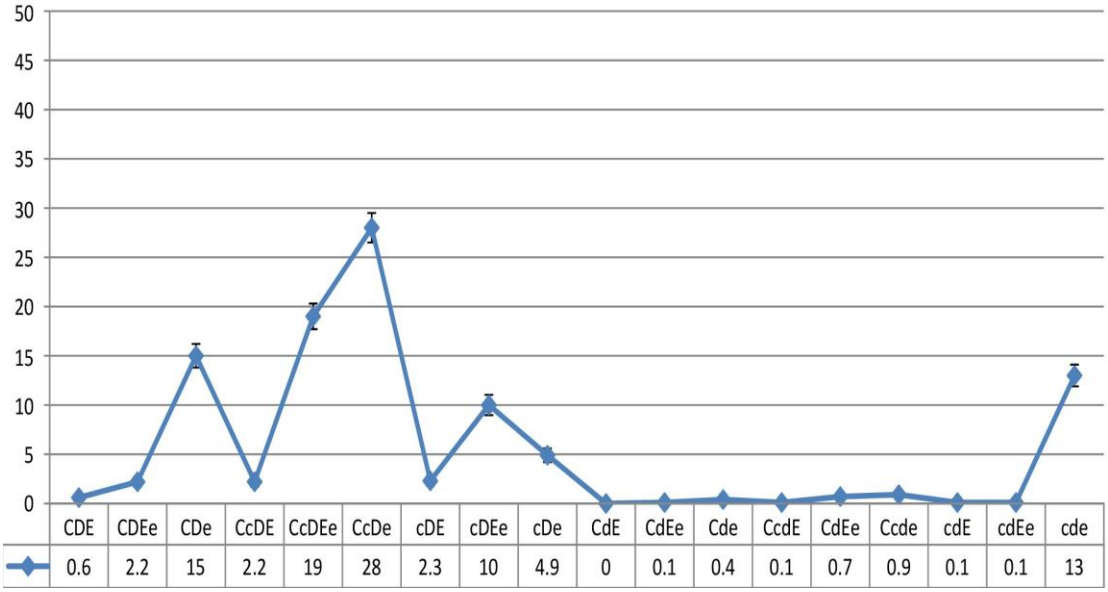

Figure №2. Frequency of Rh blood group phenotypes in studied donors (n=852).

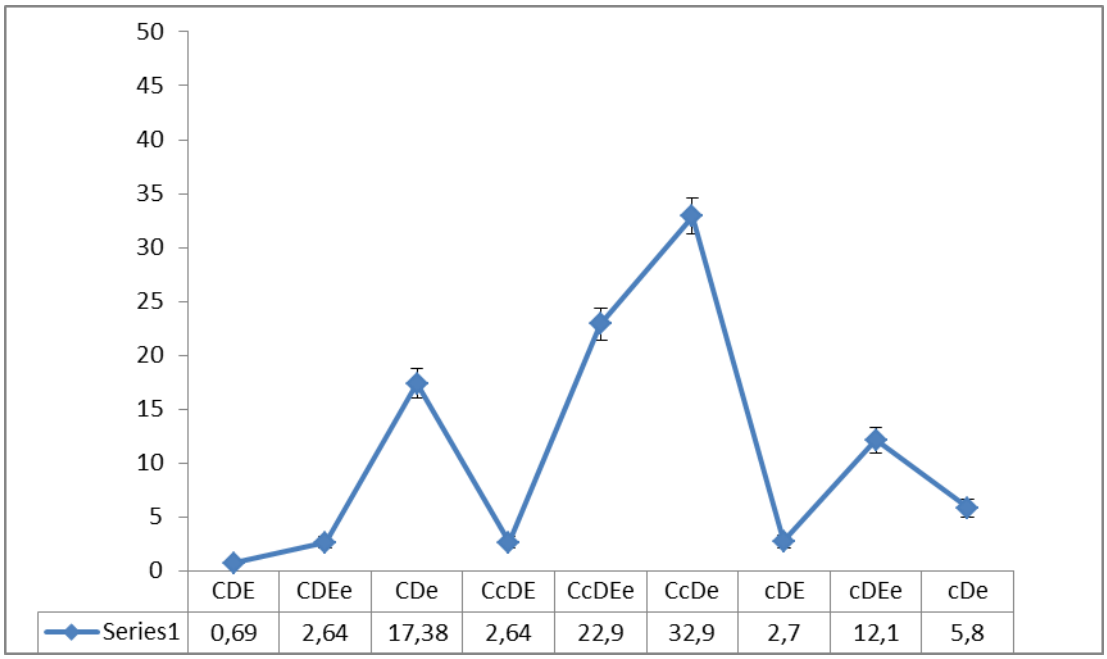

Figure 3. Phenotype variation in Rh positive donors.

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 this case, is equal to 651. This is much higher than the critical value (CV) of the criterion of the 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 IV).

Table IV. 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				

All donors which blood doesn't show agglutination reaction with anti-D antibodies belong to Rh negative group. Rh negative group is less polymorphic in our donors. In contrast to Rh-positive donors in the case of Rh-negative dominant phenotypically characteristics were 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 than cde phenotype and totally was 12,77 % (Ccde- 6,01%; CcdEe - 4,51% and Cde – 2,25%). What about the 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 4).

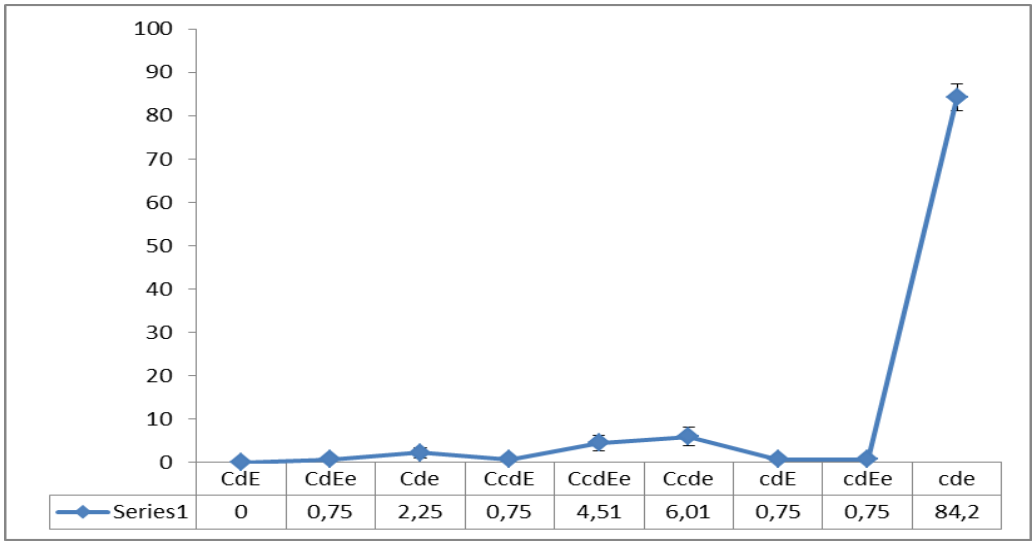

Figure № 4. Phenotype variation in Rh negative donors.

Table № V. 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				

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 the 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 5).

The haplotypes of the Rh system were calculated by us in the studied donors. We used the specially formulas for calculation of Rh haplotypes. It was described above. Theoretically, there are seven haplotypes. All of them 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 (Table 6).

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

Rh haplotypes	Calculated formula	**Frequency of haplotypes**
Cde	$cde = \sqrt{ccddee}$	0,33
Cde	$Cde = \frac{Ccddee}{2cde}$	0.1
cdE	$cdE = \frac{ccddEe}{2cde}$	0.1
cDe	$cDe = \frac{ccDee}{2cde}$	0.13
cDE	$cDE = \sqrt{ccDEE + cdE^2}$	0,23
CDe	$CDe = \sqrt{CCDee + Cde^2}$	0.1
CDE	$CDE = \frac{CCDEe}{2(CDe + cde)}$	*0.02*

IV. DISCUSSION

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

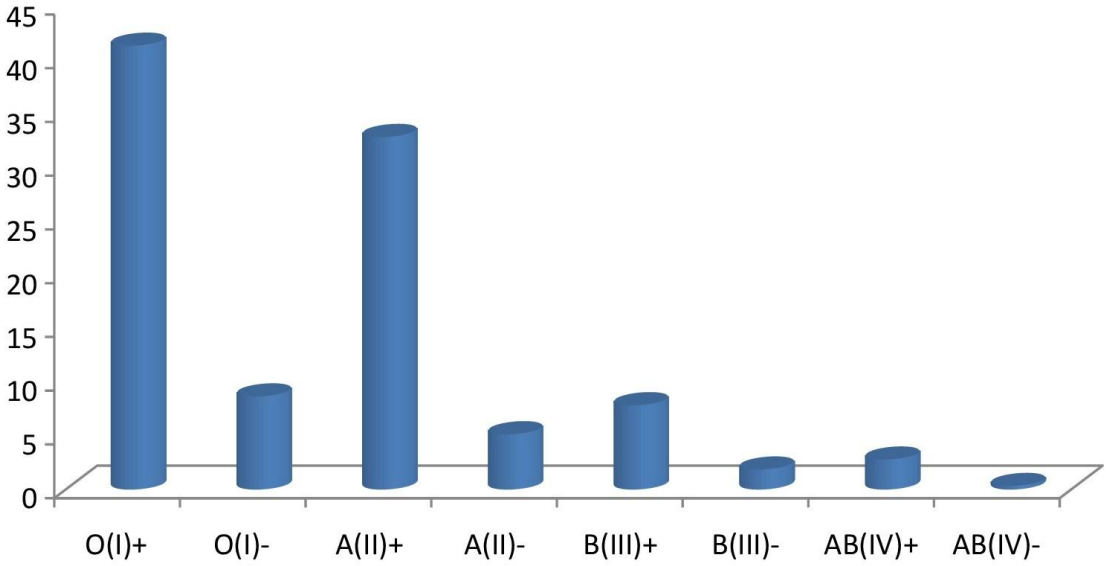

Figure № 5. Frequency of ABO and Rh blood group phenotypes in studied donors (n=852).

Today in our clinics two antigens (A, B) of ABO system and D antigens for the 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 is also significant. Numerous data about alloimmunosensibilization caused by this antigen are presented in the scientific literature [8] [22] [23].

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

We took our attention to cc and Cc genotypic donors because in both cases their erythrocyte membrane contains rhesus system 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 immunosensibilization by anti-c antibodies is higher. The frequency of CC genotype in studied donors was 17,88% (Table №VII).

Table №VII . 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 the Adjara population [24]. They are potential recipients. The distribution frequency of CC genotype in the 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 a more phenotypic variation among blood donors than the Adjara population (19). Rh haplotype in the current study is 7, while in the Adjara region population we fixed only 3 haplotypes (Table VIII).

Table VIII. Rh haplotype in blood donors and Adjara population.

Rh haplotype	Blood donors	Adjara population
cde	0,33	0,42
Cde	0.1	0
cdE	0.1	0
cDe	0.13	0,11
cDE	0,23	0
CDe	0.1	0,14
CDE	*0.02*	0

In the same region example of one clinic blood donor, 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 Georgians. In case of blood donors as they are officially donor's nationality is different and donors belong to different ethnic groups.

V. Conclusion

According to the Rh blood group antigens and phenotypes our studied donors are characterized by rather high polymorphism. The Georgian donor's population is heterogenic, especially high heterogeneity are shown in Rh-positive phenotypes. Each geographic area has its specific characteristics. Alloimmunization rates caused by Rh blood group antigens are not similar in different geographic area and dependent on the Rh phenotype of that population. The study was helpful for us to establish a database of clinic donors according to Rh group antigens. In our work, we used serological methods, while a molecular study is much informative. In the future, we are planning to include molecular investigations and also study the donors immunosensibilization with anti-Rh antibodies.

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