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ORIGINAL ARTICLE

Rh Phenotype and Allele Frequencies Among 88,856 Patients in China

Yongqiang Shan [*], Yaohua Xu [*], Chunhong Du, Haitao Huang, Yuan Zhang

[*] *These authors contributed equally to this work*
Tianjin Medical University General Hospital, Tianjin, China

SUMMARY

Background: **This study was performed to provide information on the frequencies of Rh antigens, alleles, and phenotypes from our region in Tianjin, China.**
Methods: **This observational study was conducted on patients from January 2018 to March 2021 using a fully automated system for ABO and Rh typing of blood cells. The phenotypes of C, c, E, and e were detected by the slide method. The data were collected and calculations done to determine the antigen, phenotypes and allele frequencies.**
Results: **Four hundred thirty-three cases of Rh (D) negative phenotype were confirmed in 88,856 patients. Of the four Rh antigens (C, c, E, e) that were phenotyped by serological methods, the "e" antigen was found to have the highest frequency (99.74%). The most common Rh negative phenotype observed was ccdee, followed by Ccdee. The prevalence of Rh phenotypes ccdEe, CCdee, CcdEe, CCdEe, ccdEE were found to be rare in our population with percentages of 0.0473%, 0.018%, 0.018%, 0.0034%, and 0.0011%, respectively.**
Conclusions: **Knowledge of red cell antigen phenotype frequencies in a population is helpful in terms of their ethnic distribution. We have determined the prevalence of Rh antigens and Rh phenotypes in China. The Rh blood group distribution in this population was different from that in other populations.**
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Correspondence:
Chunhong Du
Tianjin Medical University General Hospital
Anshan Road 154
300052 Tianjin
China
Phone: +86 (022) 60362605
Email: 15822661978@163.com

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KEY WORDS

ABO and Rh D phenotypes, Rh (C, c, E, e) phenotypes, antigen and allele frequency

INTRODUCTION

The Rh blood group system is the most polymorphic of the human blood groups, consisting of more than 45 independent antigens and, next to ABO, is the most clinically significant in transfusion medicine. Most cases of alloimmunization following blood transfusions or pregnancy can be attributed to the five most common ones, namely D, C, E, c, and e [1]. Studies of Rh D phenotypes in Caucasian and African populations have provided considerable information about their frequency [2-9]. Relatively few studies of Rh D alleles have been conducted among Asians and, in particular, indigenous Chinese.
We conducted this study in order to provide information on the frequencies of Rh antigens and phenotypes in

China. In the present study, we compared our results with those from other places of the world, and there were significant differences between the different groups.

MATERIALS AND METHODS

This observational study was conducted at the Transfusion Department of Tianjin Medical University General Hospital between January 2018 and March 2021. A total of 88,856 patients were included in this study.

Samples

In order to test ABO grouping and Rh typing, 5 mL of ethylenediaminetetraacetic acid (EDTA) venous blood was collected according to standard blood banking practice. Then these blood samples were sent to the Blood Transfusion Department.

Major instruments and reagents

An ORTHO AutoVue Innova-OCD system (Johnson & Johnson company) to perform ABO and phenotype test. Monoclonal anti-A, anti-B, anti-D, anti-C, anti-c, anti-E and anti-e, and reverse typing reagents, as well as 5% standard red blood cells were purchased from Shanghai Blood Biological Pharmaceutical Co., LTD (Shanghai, China).

Methods

Routine ABO blood group and serum typing was performed using the ORTHO AutoVue Innova-OCD system. We did the test strictly based on the instruction book. The sampling was not done for the current experiment, it is rather a standard protocol. Samples from the blood bank were used for the study. We performed phenotype investigations by serological studies [14]. All blood units collected were phenotyped for ABO antigens and Rh D antigen. Units that tested positive for Rh D antigen were labelled as Rh positive.

All units except the newborns and the outpatients that tested negative for Rh D antigen were further tested for Rh C, c, E, and e antigen. Red blood cells were tested against specific antisera to observe antigen-antibody reactions (hemagglutination) by the slide method. For the slide test, we added two drops of whole blood from the sample (approximately a 40% - 50% suspension) to a slide and mixed it with one drop of anti-C, anti-c, anti-E, and anti-e over a 1.5 * 1.5-cm area with a clean wooden applicator. We tilted the slides gently and continuously and looked for agglutination within 2 minutes, according to the method described in the American Association of Blood Banks (AABB) Technical Manual. Direct agglutination of red cells with a particular reagent indicated the presence of the corresponding antigen. No agglutination indicated its absence.

We confirm that all methods were carried out in accordance with the guidelines and regulations required by the Tianjin Medical University General Hospital Subcommittee and AABB Standards for Blood Banks, all reagents used meet or exceed the requirements of the Food and Drug Administration (FDA).

We confirm that all experimental protocols were approved by the Tianjin Medical University General Hospital Subcommittee, according to the AABB Standards.

Statistical analysis

SPSS 17.0 software was used for statistical analysis. The chi squared test and Fisher's exact test were used for assessment of statistical significant differences between antigen frequencies when comparing two groups. First, we should check if the population we studied obeyed the Hardy-Weinberg equilibrium. Now consider a diploid individual who has two alleles, C and c, on one gene locus.

The following formulae were used for calculation:

N: The population of our study,
n1: The observed number of individuals carrying CC genotypes,
n2: The observed number of individuals carrying Cc genotypes,
n3: The observed number of individuals carrying cc genotypes,
p: frequency of C allele,
q: frequency of c allele,
E_{n1}: expected values of CC genotype,
E_{n2}: expected values of Cc genotype,
E_{n3}: expected values of cc genotype.

(1) $p = n1/N + n2/(2N)$;
(2) $q = n2/N + n2/(2N)$;
(3) $E_{n1} = p^2 \times (n1 + n2 + n3)$;
(4) $E_{n2} = 2pq \times (n1 + n2 + n3)$;
(5) $E_{n3} = q^2 \times (n1 + n2 + n3)$;

Observed and expected values of genotype frequencies were compared using the chi-squared test. If $p > 0.05$, the genotype frequencies obeyed the Hardy-Weinberg equilibrium. The test showed that the allele frequencies were stable. Otherwise, it was considered to indicate a significant difference.

In the present study, $p > 0.05$, the allele frequencies were calculated under the standard assumption of a Hardy-Weinberg equilibrium, using the counting method of Mourant AE et al. [15] According to the Hardy-Weinberg principle, for a sufficiently large population for sexual reproduction, the allele frequencies will remain constant across generations if the individuals are allowed random breeding without gene mutation, introduction of new genes, or natural selection. Therefore, the sum of allele frequencies will be 1, and the sum of all genotype frequencies will be 1 or 100%.

Calculations of antigen and phenotype frequencies were expressed as percentages and for allele frequencies under the standard assumption of Hardy-Weinberg equilibrium.

Calculation of red cell antigen and phenotype frequencies of the blood group system was calculated by totaling the number of patients positive for a particular anti-

Table 1. Prevalence of the various ABO phenotypes in the study population.

Phenotype	The no. of Rh D negative n = 433	The no. of Rh D positive n = 88,423	Total no. n = 88,856	Prevalence of phenotype (%)	
				Rh D negative	Rh D positive
A	116	23,776	23,892	0.13	26.76
B	145	29,825	29,970	0.16	33.57
O	126	25,935	26,061	0.14	29.19
AB	46	8,887	8,933	0.05	10.00

Table 2. Percentage prevalence of the various Rh phenotypes in our study population.

Phenotype	Total no. of patients	The no. of male patients	The no. of female patients	A	B	O	AB	Prevalence of phenotype (%)	
								Our study n = 88,809	95% CI **
Ccdee	89	22	67	27	28	27	7	0.1002	0.079 - 0.121
CCdee	16	2	14	6	6	3	1	0.018	0.009 - 0.027
ccdEe	42	12	30	13	11	16	2	0.0473	0.033 - 0.062
ccdEE	1	1	0	0	1	0	0	0.0011	-0.001 - 0.003
ccdee	219	62	157	52	76	58	33	0.2466	0.214 - 0.279
CcdEe	16	4	12	2	9	4	1	0.018	0.009 - 0.027
CCdEe	3	1	2	1	2	0	0	0.0034	0.000 - 0.007

**** - 95% confidence intervals.**

Table 3. Percentage prevalence of the various Rh phenotypes in the study population and people from other regions of the world.

Phenotype	Chinese (our study) n = 88,809	Indian n = 51,857	Caucasian [2] n = 624,163	Black [10]
Ccdee	0.1002	2.32	0.8	NA
CCdee	0.018	0.05	0.01	NA
ccdEe	0.0473	0.05	0.43	NA
ccdEE	0.0011	0.004	0.002	NA
ccdee	0.2466	4.76	15.1	6.8
CcdEe	0.018	0.075	0.05	NA
CCdEe	0.0034	NA	NA	NA
CcdEE	0	0.002	NA	NA
p-value *		Chinese/Indian $p < 0.05$	Chinese/Caucasian $p < 0.05$	NA

**** - p-value calculated by Fisher's Exact Test.**
NA - indicates not available.

gen phenotype divided by the total number of patients tested. Results were expressed as a percentage.

Table 4. Allele frequency and distribution of Rh antigens in D negative population.

Traditional nomenclature	ISBT nomenclature	Allele frequency in study population	Total no. of patients	Percentage prevalence of antigens		
				Chinese (Our study) n = 386	95% CI **	Indian n = 82 [11]
C	RH2	0.1852	124	32.12	36.80 - 27.45	8.54
c	RH4	0.8148	367	95.08	92.91 - 97.25	100
E	RH3	0.0816	62	16.06	12.38 - 19.74	3.66
e	RH5	0.9184	385	99.74	99.23 - 100.2	100
p-value *						Chinese/Indian $p < 0.05$

ISBT - International Society of Blood Transfusion.
*** p-value calculated by chi square test of independence.**
**** - 95% confidence intervals.**

RESULTS

Study population

During the period of our study, there were 88,856 units at the Blood Transfusion Department. All of the units were tested for ABO and Rh D antigens. Only Rh D negative samples, except the newborn and the outpatient units, were further typed for extended Rh (C, c, E, e) antigens. The Rh D negative units were 433, including 24 newborns, which were excluded from the Rh antigens (C, c, E, e) study. Besides, 23 out of the 433 Rh (D) negative patients, which belong to the outpatients, were not tested for the Rh antigens (C, c, E, e) either. A total of 386 patients were, therefore, included in the study and phenotyped for the common Rh antigens (C, c, E, e). When referring to the percentage prevalence of various Rh phenotypes, the study number is 88,809.

ABO and Rh D phenotypes

Of the total 88,856 individual samples, 88,423 typed as Rh D positive: A Rh D positive 23,776 (26.76%), B Rh D positive 29,825 (33.57%), O Rh D positive 25,935 (29.19%,), and AB Rh D positive 8,887 (10.00%); 433 were typed as Rh D negative. A Rh D negative, B Rh D negative, O Rh D negative, and AB Rh D negative were found to be rare in our population with 116 (0.13%), 145 (0.16%), 126 (0.14%), and 46 (0.05%), respectively (Table 1). One Rh D - - phenotype was found. The Rh D negative rate in the total populations studied was 0.49%. Among the 433 Rh D negative patients, 28.18% were male and 71.82% were female, including 12 male newborns and 12 female newborns.

Rh (C, c, E, e) phenotypes

Overall, seven phenotypes were found to be present in our population, the most common Rh negative phenotype observed was ccdee, followed by Ccdee. The frequencies of Rh phenotypes in the study population are shown in Table 2: ccdee (0.2466%) > Ccdee (0.1002%) > ccdEe (0.0473%) > CCdee (0.018%) = CcdEe (0.018%) > CCdEe (0.0034%) > ccdEE (0.0011%).

The distribution of the various phenotypes among males, females, and ABO blood group was comparable, with the ccdee being the most common phenotype observed in both sexes and ABO blood group, followed by Ccdee and ccdEe.

Antigen and allele frequency

Observed and expected values of genotype frequencies were compared using the chi-squared test. Genotype frequencies obeyed the Hardy-Weinberg equilibrium ($p > 0.05$) and allele frequencies were stable.

Table 4 shows the results of our tests on 386 Rh D negative blood samples. Of the four antigens (C ,c, E, e) that were phenotyped by serological methods, the "e" antigen was found to have the highest frequency (99.74%), followed by the c and C antigen (95.08% and 32.12%, respectively). The lowest prevalence being observed was the E antigen (16.06%).

DISCUSSION

Only a few studies, mostly in non-Asian patients, have investigated the frequency of Rh phenotype and allele. In the present study we examined these elements and defined the common Rh-negative phenotypes among Chinese, which have not been previously described.

This observational study was conducted on 88,856 patients to determine the prevalence of Rh phenotypes and the distribution of Rh antigens in our population.

A significant difference in the Rh blood group distribution was found in populations of different regions of the world. The worldwide incidence of D antigen is different in different ethnic groups it being 85% in Caucasians, 92% in Blacks [12,13] and 93.39% in Indians [11]. The rate of Rh D positivity in the total population in our study was 99.51%, while the Rh-negative rate was 0.49% (Table 1). The prevalence of Rh D negativity seemed to be slightly higher in women than in men

(0.35% and 0.14%, respectively), which is similar to that in an Indian study [11].

The phenotype frequencies of the study were compared with that of Indians, Caucasians and Black population (Table 2 and 3) [12,13]. Wherever applicable, we compared our results with that of the other study from India by Beenu Thakral and Karan Saluja [11]. A considerable difference was noted in the prevalence of the various Rh phenotypes between our population and other populations. Rh phenotype distributions differ in various populations. In our population ccdee was found to be the most common Rh-negative phenotype, which is similar to that in Indians and Caucasians.

In all populations the "e" antigen was found to be the most common. Whereas the prevalence of C antigen was higher, that of c was found to be lower in our study than in Indians (Table 4). Since we did not test the Rh (C, c, E, e) antigens in Rh D positive patients, the presumed Rh haplotype frequencies in our population cannot be observed. We report for the first time, the frequencies of other antigens in the Rh system (C, c, E, e) in Chinese population. We found a marked difference in the frequency of C and c antigens in our population as compared to Caucasians and Blacks.

Rare blood is defined, on the basis of the blood group characteristics, as being found at a frequency of $\leq$ 1:1,000 random samples in a given population [14,16]. The prevalence of Rh phenotypes ccdEe, CCdee, CcdEe, CCdEe, and ccdEE were found to be rare in our population with percentages of 0.0473%, 0.018%, 0.018%, 0.0034%, and 0.0011%, respectively (Table 2). As known to all, when we get a transfusion of blood, the most important procedure is to test the ABO and Rh blood group systems. The transfusion of ABO and Rh compatible but unknown phenotype blood for clinically significant antigens may result in alloimmunization. In our hospital, only the Rh D negative patients are required to test Rh (C, c, E, e). In the present study, we provide Rh antigen frequencies in our population. This would help if the patient is badly in need of blood. In addition to all that, if we test Rh (C, c, E, e) on a large scale, we could establish a huge database. That would help to maintain supplies of rare blood types.

CONCLUSION

Knowledge of red cell antigen phenotype frequencies in a population is helpful in terms of their ethnic distribution. We presented the prevalence of common Rh antigens in a large population of China, and the Rh D negative phenotypes are revealed. The Rh blood group distribution in this population was different from that in other populations.

Data availability:

All of the data are available.

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Declaration of Interest:

None.

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