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Phenotype Frequencies of "O" Blood Group (Rh, Kell, and MNS) among Donors in Transfusion Medicine Department of a Training Institute of Bangladesh

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Abstract:

Background: Although blood transfusions can be lifesaving but it is not without risk. It is a necessity to have knowledge about the red cell antigen phenotype frequencies in a population to provide antigen-negative compatible blood to patients with multiple alloantibodies. This is the first study on extended phenotype of blood group systems in blood donors at AFIP, Dhaka.

Aim: Present study is aimed to determine the antigen frequencies of "O" blood group phenotype among donors at AFIP, Dhaka.

Methods: An observational study was carried out at AFIP, Dhaka from July 2019 to December 2019. A total of 40 "O" blood group donors from blood bank of AFIP were typed for D, C, c, E, e, K, k, M, N and S antigens either by adding antisera to 3-5% of donor RBCs suspension or by using Indirect Antiglobulin Test (IAT) according to manufacturer's instruction. Coomb's control cells, positive and negative control cells were used as quality control. Antigens and phenotype frequencies were expressed as percentages.

Results: Forty (40) blood donors belonging to "O" blood group were included for extended phenotyping. D antigen was found in 100% donors, followed by e [42.5%], C [38.13%], c [11.88%], and E [7.5%] with DCe/DCe (R1R1, 55%) as the most common phenotype. k was found to be positive in 72% of donors. In the MNS system, 57.5% donors were typed as M+N+, 32.5% as M+N-, and 10% as M-N+. S-s+ was found amongst donors with 52.5% as the commonest phenotype.

Conclusion: This study was conducted to provide information about the antigen frequency of a common blood group "O" in local donors to provide antigen negative compatible blood units to multi transfused alloimmunized patients. D antigen was found in 100% donors with DCe/DCe (R1R1, 55%) as the most common phenotype.

Keywords: Blood group systems, Phenotype frequency, Red cell antigens and Alloantibodies.

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Introduction:

The primary goal of any blood transfusion is to provide the patient with donor red blood cells that optimally survive after transfusion and serve their function and to ensure that the patient actually benefits from the transfusion. To achieve this goal, donor red cells that are compatible with those of the patient's blood are selected for transfusion. The criteria for selection of donor cells focuses on absence of antigens on donor cells for the antibodies that are detected in the patient's serum needing transfusion during antibody detection and identification. Successful antibody detection and identification in patient's serum depends on the use of appropriate and comprehensive screening and panel

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red cells. Knowledge of phenotype frequencies of major blood group systems help in formulating in-house red cells for antibody detection and identification[1].

Because Bangladesh is an overpopulated country with several distinct population groups, there is an obvious need for phenotype frequencies to be determined in different parts of Bangladesh. Still, packed cells for blood transfusion are mostly matched for major antisera, ABO and D. Avoiding alloimmunization by addressing antigenic frequencies would be useful for supporting specific patient populations such as immunosuppressed patients, chronic transfusion recipients, haematopoietic cell transplant recipients, and neonates.

Methodology:

Selection of study participants

This was an observational study conducted in AFIP, Dhaka among 40 volunteer blood donors from July 2019 to December 2019. All 40 participants having "O" blood group were fulfilling the national blood transfusion guidelines.

Sample collection and extended red cell antigen typing

Total 4 ml of blood sample was collected in Ethylenediamine tetraacetic acid (EDTA) tubes from "O" blood group voluntary donors after taking their consent for using their blood samples in the present study. These samples were collected from blood bank; AFIP from different ages, genders and locality. Donors were selected to have representative samples in the study from urban area of Bangladesh. Then a 5% suspension was prepared for antigen profile.

All donor samples included in the current study were selected only after confirming that their Direct Antiglobulin Test (DAT) results were negative, because if DAT is positive due to IgG sensitized cells, typing reagents employing the indirect antiglobulin test (IAT) may give invalid results[2]. All collected "O" blood group samples were randomly selected for red cell antigen typing of the following blood groups Rh (D,C,E, c, e), Kell (K, k) and MNS (M, N, S, s).The blood grouping for the above antigen types was done by conventional tube technique following manufacturer's instructions.

Interpretation of results

Agglutination reactions in positive test results were recorded using agglutination viewer and were graded as 1+ to 4+. The antisera used for the study were from Diamed, Switzerland and Immucor Gamma, USA. The antihuman globulin reagent used in the study was from Diamed, Switzerland. The typing of D,C,c,E, e, K, M, and N antigens was done using monoclonal antisera, while that of k antigen was done using polyclonal antisera employing IAT. Positive and negative control cells and coomb's control cells were used in quality control.

Procedure of collecting data

Data were collected in data collection sheets on the spot randomly in Transfusion Medicine Department of AFIP and total 40 patients were included for the study.

Statistical analysis

a. After completion data were checked, verified, edited, and coded. The data were analyzed with the help of statistics.

b. Data were process and analysed using SPSS (Statistical Package for Social Sciences) software version 23. The summarize data were presented in the tables.

Ethical measures

a. All data were collected from volunteered source of blood donation.

b. Data taken from the participants were coded and regarded as confidential.

c. Ethical permission was taken from Institutional review board (IRB) of AFIP, Dhaka accordingly.

Results:

Table-I

Distribution of Rh Antigens (C,c,E & e) in 40 blood donors (n=40×2=80).

Gene	Antigen	Number (n)	Percentages
D	D+	80	100
C	C+	61	76.25
c	C+	19	23.75
E	E+	12	15
e	e+	68	85

Table- I revealed that 40 (100%) were D+ and none was D- donors. The D antigen was found to have the highest frequency (100%), followed by e and C antigen (42.5% and 38.13%, respectively). However, the C antigen was found to be solely associated with presence of D antigen.

Table- II

Rh phenotypes in blood donors of AFIP (n=40).

Sl. no	Phenotypes	Donor no	Percentages
1	R_1R_1 (DCe/DCe)	22	55
2	R_1R_2 (DCe/DcE)	10	25
3	R_1r (DCe/dce)	08	20
4	rr (dce/dce)	—	—
5	R_2r (DcE/dce)	—	—
6	R_1R_Z (DCe/DCE)	—	—
7	R_0r (Dce/dce)	—	—
8	r'r" (dCe/dcE)	—	—
9	rr" (dce/dcE)	—	—
10	r'r' (dCe/dCe)	—	—

Table- II showed that R1R1 followed by R1R2 was the most common phenotype amongst Rh positive blood group.

Table-III

Phenotype frequencies of Kell blood group system (n=40).

Kell phenotype	Number	Percentage (%)
K- k+	29	72.5
K+ k-	02	5
K+ k+	07	17.5
K- k-	02	5

Table- III revealed that in the Kell blood groups system, 72.5% (29/40) donors were typed as K-k+ antigen positive and 17.5% as K+k+ antigen positive. K+k- and K-k- both phenotypes were found only in 02 persons.

Table-IV

Phenotype frequencies of MNSs blood group system (n=40).

MNSs phenotype	Number	Percentage (%)
M+ N+	23	57.5
M+ N-	13	32.5
M- N+	04	10
S- s+	21	52.5
S+ s+	11	27.5
S+ s-	08	20
s-s-	00	00

Table-IV showed that the most common phenotype observed in the MNSs blood group system was M+N+ (57.5%) and S- s+ (52.5%).

Discussion:

The data on incidence of antigens of various blood groups in the local donor population helps in routine blood transfusion practices of a blood centre[3]. Very few studies regarding the incidence of various blood groups in the blood donor population are published from Bangladesh and none has been reported from the blood donor population of AFIP, Dhaka. This is the first study reporting extended red cell antigen typing for various blood groups in 40 randomly selected "O" blood group donors. The results of our study are almost similar with two of such studies done in north India[4,5.]

The incidence of D antigen differs in different ethnic population, being 85% in Whites and 92% in Blacks[6-8]. In the present study, D antigen was found to be present in 100% of donors, which is comparable with other studies from India[9,10]. The frequency of C antigen in our study was 76.25%, which is high as compared to 68% (in Whites) and 27% (in Blacks), while this is comparable with the findings of study by Thakral (84.76%)[4]. c antigen was found to be positive in 23.75% of our samples, which is less than that of 80% (in Whites) and 96% (in Blacks); however, it is almost similar with the study by Thakral (32.82%)[4]. In the Kell system, k antigen was found in 72.5% of donors, which is nearer to the incidence (100%) of Thakral study[4]. The reason for the discrepancy is that the data in our study is skewed and biased because of small numbers (only 40) of samples.

In the MN blood group system, M+N- phenotype was found in 32.5% of sample, which is 15.8% in Thakral study [4]; however, this phenotype is much less in Whites and Blacks with a frequency of 28% and 26%, respectively[4]. The phenotypes M+N+ (23%) in our study were more common than 13.88% reported by Thakral[4]; however, it was comparable with that reported by Nanu and Thapliyal as 22.61% and 27.83%, respectively[5].

The phenotyping (blood grouping) of red cells depend upon interpretations of serological interactions between red cell antigens and antibodies. Failure to follow the

instructions provided by reagent manufacturers can lead to incorrect conclusions[11]. Tests employing IAT procedures must be interpreted accurately to avoid false positive and false negative results[12]. Outcomes of such studies can be used to formulate a" Blood Group Donor Registry" (donors lacking high frequency antigens) at national level, and patient's database (especially those requiring multiple transfusions) with antibodies against high frequency antigens.

Conclusion:

According to The International Society of Blood Transfusion (ISBT), there are 38 different blood group systems and the 900 series antigens in collections (low and high incidence antigens) [13]. Our results demonstrate that there exists antigenic variability as regards blood group system among our population. We observed that D antigen was found in 100% donors with DCe/DCe (R1R1, 55%) as the most common phenotype. The findings of this study can be used to provide antigen-negative blood for patients with unexpected antibodies. Antigenic frequencies in our donors differ from the widely available data of European and American countries for assessment of donor pool compatibility with chronic transfusion dependent populations. If this type of study conducted in a large population would play an integral role in the planning to address future health challenges relating blood transfusion and marriage counseling.

Limitations:

1. The frequency of the phenotypes according to our findings can not be generalized as a whole due to the lack of a national registry for blood donors
2. Another major limitation is the small sample size but the outcome of this study may be used as a basis of establishing local registries nationwide.
3. We did extended antigen typing in only 40 donor samples as the cost was a restraining factor for including large number of donor samples in study.

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