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Phenotypic and Allelic Frequency of ABO and Rh (D) Blood Group among Whole Blood Donors in a Teaching Hospital of Chhattisgarh

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Authors' contributions

This work was carried out in collaboration among all authors. Author WM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author LS and Author CR managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: ABO and Rh (D) blood groups are the most important in blood transfusion and are determined genetically. Although these blood groups are common to all humans, there is variation in their allelic frequency based on region and population. This study was performed to determine the allelic frequency of ABO & Rh (D) in the donor population in the Blood Center of Chhattisgarh located in Central India.
Place and Duration of Study: It is a cross-sectional study performed in the Department of Transfusion Medicine & Blood Bank of a teaching hospital from July 2021-February 2022.
Methodology: Only the accepted whole blood donors were included. ABO & Rh (D) blood grouping was performed by conventional tube technique and their allelic frequency was

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determined. We studied 4078 whole blood donors out of which 4055 were males and 23 were females.

Results: Phenotypic frequency of ABO blood group system was O>B>A>AB. Rh (D) positive was more prevalent than Rh (D) negative. Allele frequency of ABO system was 0.1545 for I^A, 0.2351 for I^B, and 0.6105 for I^O. In Rh system, allele frequency of I^D was 0.8441 and I^d was 0.1559.

Conclusion: Phenotypic & allelic frequency of ABO & Rh (D) shows heterogeneous distribution in different parts of the world. Our study showed blood group O & allele I^O as the most common. This data is of utmost importance in the planning of transfusion services, especially during a healthcare crisis in low-resource area like ours.

Keywords: Allelic frequency; ABO; RH; blood group; prevalence; phenotye.

1. INTRODUCTION

Human blood group antigens are genetically determined. ABO blood group determined first by Karl Landsteiner in 1900s has revolutionized the blood transfusion. Since then, around 43 blood group systems have been discovered and listed by the International Society of Blood Transfusion (ISBT) [1]. However, ABO & Rh (D) are the most important for blood transfusion for which routine testing is performed globally. By forward grouping, blood group is determined by detecting A and B antigens on the red cells by specific antisera. Reverse grouping is performed by known red cells to detect anti-A and anti-B in the serum. Depending on the presence of red cell antigens, ABO blood groups can be classified as A, B, AB and O. There are only two phenotypes as Rh (D) positive and Rh (D) negative, depending on presence or absence of Rh (D) antigen on red cells [2].

ABO gene is present on Chromosome 9 (9q) and determined as autosomal co-dominant and Rh gene on Chromosome 1 (1p). There are three alleles of ABO genes which are represented as I^A, I^B, and I^O and the four ABO phenotypes as blood group A (I^AI^A & I^AI^O), B (I^BI^B & I^BI^O), AB (I^AI^B), and O (I^OI^O). The genotype I^DI^D or I^DI^d represent as Rh (D) positive while I^dI^d as Rh (D) negative [3].

Although all humans share the same blood groups, their frequencies differ in different races, ethnic, and socio-economic groups across the world [4]. Knowing the distribution of ABO and Rh blood groups at local and regional levels is helpful in effective and safe blood transfusion services. Knowledge of their frequency is essential for effective management of blood bank inventory, be it a local, regional or national transfusion service. So, it is of utmost importance to have the information of these blood groups in any population [5].

Along with their role in blood transfusion, they are also useful in organ/ tissue transplantation, population genetic studies, researching population migration patterns as well as resolving medico-legal issues, like disputed paternity cases. The medical practitioners should have information about blood group frequency of the population of their region as there is genetic association between blood groups and various diseases like cardiovascular diseases, duodenal ulcers, gastric carcinoma, etc. [6]. So, keeping these facts in mind we conducted this study to determine the frequency of various blood group phenotypes and their alleles in the whole blood donors at our center.

2. MATERIALS AND METHODS

This was a cross-sectional study conducted from July 2021 to February 2022 among the whole blood donors in the blood center of a teaching hospital of a tribal state in India. Both the replacement & in-house/ out-door voluntary donors but not the Apheresis donors were included. Criteria for donor selection as per NBTC/ NACO guidelines were followed. The advisories regarding donor deferral related to COVID-19 infection/ contact/ travel/ vaccination were followed. We had 4078 accepted whole blood donors whose samples were studied. We ensured to take only one sample data in case of repeat donors so as to avoid duplication of data.

2 ml of blood was collected in EDTA vial. ABO & Rh (D) grouping was performed by conventional tube technique. Forward grouping for ABO blood groups was performed by using commercially available monoclonal antisera anti-A, anti-B, anti-AB manufactured by Tulip Diagnostics Pvt. Ltd. Anti-A1 and anti-H lectins were also used to determine subgroups of blood group A. Reverse grouping was performed by testing with in-house prepared pooled A-cells, B-cells & O-cells as per

standard operating procedure. For Rh (D) grouping, anti-D IgM and IgM/ IgG blend was used. D^u test was performed in Rh (D) negative cases and samples tested positive were considered Rh (D) positive. All the reagents used were subjected to daily quality control and pooled cells were prepared freshly daily.

The collected data was entered in Microsoft excel and analyzed. The frequency of ABO and Rh (D) blood groups was reported in simple numbers and percentages. The allelic frequencies were calculated by assumption of Hardy Weinberg equilibrium with Ceppilini correction.

Assumptions of Hardy Weinberg equilibrium include:

- ABO system is determined by three alleles of a single gene, namely A, B and O.
- A and B are co-dominant and both are dominant over O.
- This gene is in accordance to Hardy-Weinberg frequencies in the population.
- The data were a random sample from the population.
- Non overlapping generations.
- Sexual reproduction in population.

Static allele frequencies in a population across generations assume that there is no mutation, no migration or emigration, infinite population size and no selective pressure for/ against any genotypes.

Chi-square test for goodness of fit of observed and expected (using estimated values) phenotype numbers was also calculated.

3. RESULTS

During the study period, we received 4078 samples. Out of these, 4055 (99.4%) were males and 23 (0.6%) were females. Majority were replacement blood donors.

ABO & Rh (D) phenotypes: The distribution of ABO blood groups (Table 1) in decreasing order were O>B>A>AB and Rh (D) positive>Rh (D) negative. O Rh (D) positive was most common (36.1%) and AB Rh(D) negative was least common (0.2%).

ABO & Rh (D) allelic frequency: Based on Hardy-Weinberg equilibrium, the allelic frequency of the ABO and Rh (D) blood group genes was calculated as:

$p = 1 - \sqrt{(B + O)}$; $q = 1 - \sqrt{(A + O)}$; $r = \sqrt{O}$; $E = 1 - e$; $e = \sqrt{dd}$

where p, q, r, E, and e represent the allele frequencies of the genes for A, B, O, Rh (D) positive and Rh (D) negative respectively. A, B, O, and dd represent the phenotype frequencies of blood groups A, B, O, and Rh (D) negative respectively. Table 2 shows allelic, genotypic & phenotypic frequencies in our study.

Allele frequency of O (r/ I^O):
$r = \sqrt{O}$
$= \sqrt{0.372}$
$= 0.6105$

Allele frequency of A (p/ I^A)
$p = 1 - \sqrt{(B + O)}$
$= 1 - \sqrt{(0.3421+0.3727)}$
$= 1 - \sqrt{(0.7148)}$
$= 1 - 0.8455$
$= 0.1545$

Allele frequency of B (q/ I^B)
$q = 1 - \sqrt{(A + O)}$
$= 1 - \sqrt{(0.2124 + 0.3727)}$
$= 1 - \sqrt{(0.5851)}$
$= 1 - 0.7649$
$= 0.2351$

Allele frequency of RhD-ve (e/ I^d)
$e = \sqrt{dd}$
$= \sqrt{0.0243}$
$= 0.1559$

Allele frequency of RhD+ve (E/ I^D)
$E = 1 - e$
$= 1 - 0.1559$
$= 0.8441$

The Chi-square test for goodness-of-fit between the observed and expected phenotypes in the case of ABO blood groups was not found to be statistically significant at p-value≤0.05. A software program S2ABO estimator by Silva Square was used for statistical calculations and confirmation of manually calculated gene frequencies. This program is useful for estimation of allele frequencies of ABO blood group system, and performs some statistical tests, like comparison of estimates of allele frequencies, obtain maximum likelihood (ML) estimates of allele frequencies and perform goodness-of-fit tests of Hardy-Weinberg assumption [7]. The observed & expected frequencies as calculated by the S2ABO estimator is tabulated in Table 3.

Table 1. ABO & Rh (D) blood group distribution

S. No	ABO	Rh (D)	Total No.	Prevalence (%)
1.	A	Rh (D) positive	842	20.7%
2.		Rh (D) negative	24	0.6%
3.	B	Rh (D) positive	1369	33.6%
4.		Rh (D) negative	26	0.7%
5.	AB	Rh (D) positive	288	7.1%
6.		Rh (D) negative	9	0.2%
7.	O	Rh (D) positive	1480	36.1%
8.		Rh (D) negative	40	1%
			4078	100%

Table 2. Allelic & genotypic frequency of ABO & Rh blood groups

Blood group system	Allele	Allelic frequency	Genotype	Genotype frequency	Frequency	Phenotype	Frequency
ABO	A	0.1545	I^AI^A	p^2	0.0239	A	0.2124
			I^AI^O	2pr	0.1886		
	B	0.2351	I^BI^B	q^2	0.0553	B	0.3421
			I^BI^O	2qr	0.2871		
			I^AI^B	2pq	0.0726	AB	0.0728
	O	0.6105	I^OI^O	r^2	0.3727	O	0.3727
Rh	D	0.8441	I^DI^D	E^2	0.7463	RhD+ve	0.9757
			I^DI^d	2Ee	0.2632		
	d	0.1559	I^dI^d	e^2	0.0243	RhD-ve	0.0243

Table 3. Intrinsic goodness-of-fit test for observed & expected (Hardy-Weinberg) frequencies of ABO by S2ABO estimator program

	A	B	AB	O
Observed	866	1395	297	1520
Expected	866.67	1395.64	296.26	1519.44
2 O ln(O/E)	-1.330	-1.274	1.482	1.125
(O-E)2/E	0.001	0.000	0.002	0.000

4. DISCUSSION

The present study was conducted prospectively among whole blood donors in Department of Transfusion Medicine & Blood Bank of a teaching hospital situated in a state with large tribal population of 31.76% as compared to rest of India [7]. The region is also endemic for different hemoglobinopathies like sickle cell anemia, thalassemia, etc. We included both the replacement & voluntary donors so as to get proper representative sample of the region. However, Apheresis donors were excluded as they donated only specific blood group as per patient's demand & only slide blood grouping was performed in them.

In our study majority were males (99.4%). It is similar to several other studies conducted in different parts of India [3, 8]. Some of the reasons behind less female donors include anemia, low weight, lack of motivation, and fear of blood donation. Improving nutritional status of females and education & motivation can be helpful [8]. As blood group of an individual remains same throughout the life and only the accepted healthy blood donors as per NBTC/ NACO guidelines were included; so the age-group wise categorization was not done. However, certain conditions like malignancies, auto-immune disorders can alter blood group [9].

We compared ABO & Rh (D) distribution across different states of India [3, 10-20] (Table 4). Our study showed blood group O (37.27%) to be commonest followed by B (34.21%), A (21.24) & AB (7.28%). Although, we conducted study in Chhattisgarh in central region, yet our findings

Table 4. ABO & Rh (D) phenotype distribution in various states of India

Study		State	A	B	AB	O	Rh(D)+ve	Rh(D)-ve
Present study	Central region	Present study, Chhattisgarh	21.24	34.31	7.28	37.27%	97.57	2.43
Mehta et al. [10]		Madhya Pradesh	25.63	39.25	6.50	28.63	94.88	5.12
Kumar S et al .[11]	Northern region	Uttarakhand	30.39	31.68	11.70	26.24	93.51	6.49
Chandra et al. [12]		Uttar Pradesh	21.38	39.92	6.64	29.27	95.71	4.29
Mahroo RN et al. [13]		Delhi	23.98	35.40	9.65	30.96	95.63	4.37
Singh et al. [14]	Eastern region	Jharkhand	22.09	35.15	8.03	34.73	96.46	3.54
Nag et al. [15]		West Bengal	23.9	33.6	7.7	34.8	94.7	5.3
Giri et al. [16]	Western region	Maharashtra	28.38	31.89	8.72	30.99	95.36	4.64
Barot et al. [17]		Gujarat	21.79	35.96	8.43	33.82	93.44	6.55
Kumar et al. [18]		Rajasthan	15.46	39.95	8.30	35.68	91.17	8.82
Anushree et al. [19]	Southern region	Karnataka	21.4	34.8	5	38.8	97.1	2.9
Sooham J. [20]		Kerala	26.27	29.10	6.77	37.86	90.48	9.52
Sigamani et al. [3]		Tamil Nadu	22.03	30.92	7.88	39.17	95.96	4.04

Table 5. ABO & Rh(D) phenotype distribution in different countries of the world

Study	Country	A	B	AB	O	Rh(D)+ve	Rh(D)-ve
Present study	Chhattisgarh	21.24	34.31	7.28	37.27%	97.57	2.43
	Neighbouring countries						
Shrestha et al. [21]	Nepal	29.73	27.01	8.21	35.05	97.3	2.7
Rahman GU. [22]	Pakistan	23.99	33.37	9.74	33.14	90.63	9.37
Dipta TF et al. [23]	Bangladesh	26.7	34.4	8.6	30.4	97.4	2.6
	Other countries						
Iyiola OA et al. [24]	Nigeria	22.4	20.7	3.6	53.3	97	3
Woldu et al. [25]	Ethiopia	26.44	21.71	4.81	47.04	94.24	5.76
Bashwari et al. [26]	Saudi Arabia	24	17	4	52	93	7
Mollison et al. [27]	USA	41	9	4	46	85	15
Firkin et al. [28]	Britain	42	8	3	47	83	17

Table 6. Allelic frequency of ABO & Rh in different countries of the world

Study	Country	I^A	I^B	I^O	I^D	I^d
Present study	India	0.1545	0.2351	0.6105	0.8441	0.1559
Zafar M et al. [29]	Pakistan	0.2330	0.1714	0.6062	0.9980	0.0900
Woldu et al. [25]	Ethiopia	0.1714	0.1433	0.6859	0.7599	0.2401
Iyiola OA et al. [24]	Nigeria	0.14	0.13	0.73	0.97	0.03
Allawati M et al. [30]	Oman	0.15	0.14	0.71	0.71	0.29
Amin MD et al. [31]	Malaysia	0.17	0.20	0.63	0.73	0.27
Moghrabi et al. [32]	Libya	0.2207	0.1377	0.6415	0.6293	0.3706

vary from Mehta et al [20] in Madhya Pradesh where blood group B was found dominant. This could mean that local population of Chhattisgarh is different from rest of central region and closer to southern & eastern region. Rh(D) distribution is similar to other states with Rh(D) positive being common.

The distribution of ABO blood group varies across neighbouring [21-23] & other countries [24-28] of the world as seen in Table 5. In India O & B phenotypes are common; whereas in America [27] & Europe [28] O & A phenotypes are common. The difference in distribution of blood group phenotypes could be attributed to pathogen-driven blood group antigen changes. Rh (D) positive is common across the world.

On comparing allele frequencies of different studies world-wide [24, 25, 29-32], we found that allele frequency of I^O in ABO & I^D in Rh blood group system is highest across the world (Table 6). Thus our findings are similar to other studies.

4. CONCLUSION

Phenotypic & allelic frequency of ABO & Rh (D) shows heterogeneous distribution in different parts of the world. Our study showed blood group O & allele I^O as the most common. Our data is of utmost importance for planning of blood transfusion services especially during healthcare crises especially in low-resource areas like ours and also valuable for genetic studies and practicing physicians. It will serve as a reference for further such studies. Further large scale population based genetic studies should be performed to add up to the national database.

CONSENT AND ETHICAL APPROVAL

The study data is extracted from an intramural project which was approved by the Institutional Ethics Committee of AIIMS, Raipur [AIIMSRPR/ IEC/ 2O21/ 699]. All the data was collected from the whole blood donors after their written informed consent and the information was handled with utmost confidentiality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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