ABO, Rhesus, and Kell Antigens, Alleles, and Haplotypes in West Bengal, India

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Keywords

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Summary

Background: Few studies have documented the blood group antigens in the population of eastern India. Frequencies of some common alleles and haplotypes were unknown. We describe phenotype, allele, and haplotype frequencies in the state of West Bengal, India. **Methods:** We tested 1,528 blood donors at the Medical College Hospital, Kolkata. The common antigens of the ABO, Rhesus, and Kell blood group systems were determined by standard serologic methods in tubes. Allele and haplotype frequencies were calculated with an iterative method that yielded maximum-likelihood estimates under the assumption of a Hardy-Weinberg equilibrium.

Results: The prevalence of ABO antigens were B (34%), O (32%), A (25%), and AB (9%) with *ABO* allele frequencies for O = 0.567, A = 0.189, and B = 0.244. The D antigen (RH1) was observed in 96.6% of the blood donors with *RH* haplotype frequencies, such as for CDe = 0.688809, cde = 0.16983 and CdE = 0.000654. The K antigen (K1) was observed in 12 donors (0.79%) with *KEL* allele frequencies for K = 0.004 and k = 0.996. **Conclusions:** For the Bengali population living in the south of West Bengal, we established the frequencies of the major clinically relevant antigens in the ABO, Rhesus, and Kell blood group systems and derived estimates for the underlying ABO and KEL alleles and RH haplotypes. Such blood donor screening will improve the availability of compatible red cell units for transfusion. Our approach

using widely available routine methods can readily be applied in other regions, where the sufficient supply of blood typed for the Rh and K antigens is lacking.

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Introduction

The antigens of the ABO blood group system are most important in transfusion and organ transplantation. The common antigens of the Rhesus and Kell blood group systems are also frequently tested in blood donors and patients because of their clinical relevance. Several studies from the 1960s addressed ABO and Rh antigens in various West Bengal populations [1], and several current studies [2–6], conducted in other parts of India, documented the antigen prevalence for the 3 blood group systems. The available data using current technology is limited for the Bengali population in eastern India [7, 8] although approximately 91 million people live in the state of West Bengal, bordering Bangladesh to the west and north. The Bengali is one of the largest populations inhabiting one of the most densely populated areas worldwide and has a literacy rate of only 77%. No data have recently been published on blood group allele or haplotype frequencies for this large population.

Blood group data in donors and patients is important for effective management of blood bank inventory. Apart from their relevance in blood transfusion practice and pregnancy management, blood group antigens can inexpensively be applied to medico-legal issues, such as disputed parentage. Blood group data are also useful in guiding population genetic studies for rare alleles that are not easily captured by current mass scale molecular methods. Using current serologic technology, we tested for common ABO, Rhesus,

Fig. 1. Blood donor demographics in West Bengal. The 1,528 donors tested in the study (black line) averaged 33.01 years old with a standard deviation of ± 9.06 years. A quarter of the donors were 26 years or younger (25% percentile), half of the donors 32 years or younger (mean, 50% percentile) and three quarters of the donors 39 years or younger (75% percentile). Female donors (gray line) represented 11% of all donors.

and Kell blood group antigens and calculated the allele and haplotype frequencies in the Bengali population.

Material and Methods

Blood Samples

EDTA-anticoagulated blood samples were collected between January 2014 and June 2015. The blood donors came from Kolkata and adjacent areas in the south of the state of West Bengal. The regular whole blood donations were collected by the Department of Immunohematology and Blood Transfusion of the Medical College Hospital at its main site or nearby outdoor voluntary blood donation drives. Among the approximately 10,000 whole blood donations during the collection period, 1,528 samples from different blood donors were chosen at random. Blood donation camps have been established since, and whole blood donations approximated 30,000 in 2016.

Informed written consent to blood group typing as part of their blood donation was obtained from each donor. Whole blood donations comprised 350 ml. Per Government of India regulations, the minimum interval was 3 months. The study was approved by the ethics committee of the West Bengal University of Health Sciences (no. MC/Kol/IEC/128/11–2013).

Immunohematology

The serologic testing was done as part of the routine standard operating procedure at the Medical College Hospital. Blood group serology was done with conventional tube methods [9–11], mostly using monoclonal antibodies (see Supplementary Material; available at <http://content.karger.com/ProdukteDB/produkte.asp?doi=475507>) [12].

Statistical Description

Phenotype and haplotype frequencies were calculated using the counting method of Ceppellini et al. [13], which yields maximum-likelihood estimates in an iterative process [14], as described previously [15]. An alternative method was also applied for ABO and KEL allele frequency calculation (available at <http://content.karger.com/ProdukteDB/produkte.asp?doi=475507>) [16]. A two-sided chi-square test was performed to compare the frequency distribution of weak D observations between 2 populations [15]. The 95% confidence interval (CI) was calculated using the Poisson distribution.

Results

All 1,528 individuals tested in the study were voluntary, unpaid donors for non-directed and non-family whole blood donations. They were between 18 and 65 years old (fig. 1), comprising 1,358 male (89%) and 1,70 female donors (fig. 1). The data from these donor samples, collected at random and representing approximately 15% of all, were considered representative of our total donor cohort.

Antigen Frequencies for the Three Major Blood Group Systems

We determined the ABO, RHD, and Kell antigens (table 1) and calculated maximum-likelihood estimates for the ABO and KEL allele frequencies (table 1) using the counting method [13]; very similar results could be calculated with an alternative, more conventional algorithm [16] (available at <http://content.karger.com/ProdukteDB/produkte.asp?doi=475507>).

Antigen and Haplotype Frequencies for the Rhesus Blood Group System

We determined the 5 major antigens of the RH blood group system (table 2). Because of the larger number of antigens and concatenated RHCE and RHD alleles (RH haplotypes), there is no straightforward algorithm to calculate the 8 common RH haplotypes from these phenotype data (table 2). However, the counting method [13] allowed us to determine maximum-likelihood estimates for the RH haplotypes (table 3), as described previously (supplemental fig. 1 available at <http://content.karger.com/ProdukteDB/produkte.asp?doi=475507>) [15].

Weak D in India

No sample of serologic weak D phenotype was observed (table 2), and the prevalence of the serologic weak D phenotype was calculated to be 0.215% or less (upper 95% limit of the expected frequency) among Bengali blood donors. Compared to a German population (mean of 0.44% among all donors) [15], the difference is statistically significant ($p = 0.0192$, chi-square test, two-sided).

Discussion

The present study was conducted to obtain blood group data for volunteer blood donors in West Bengal, India, using standardized current technology. We determined the phenotype frequencies of the common ABO, Rhesus, and Kell blood group antigens. The prevalence of alleles and haplotypes were calculated using a counting method. The data corroborated the prevalence of the common blood group antigens for the large Bengali population and aided in creating a donor database. Now we can more readily supply antigen-negative blood that is compatible for patients with multiple alloantibodies.

The B antigen was most prevalent in the eastern parts of India (table 1), similar to reports from Northern India [3, 4]. Large co-

Table 1. Frequencies of ABO, RHD, and Kell phenotypes

Blood group	Serology			Molecular genetics	
	phenotype	frequency, %	observations, n	allele	frequency (fraction)
ABO	A	25.13	384	A	0.189
	B	33.77	516	B	0.244
	O	32.07	490	O	0.567
	AB	9.03	138	n.a.	
Rhesus	D positive *	96.60	1476	n.a.	
	D negative	3.40	52	n.a.	
	ccdde	2.75	42	n.a.	
	dd with C and/or E	0.65	10	n.a.	
Kell	KK (K positive)	0	0	n.a.	
	Kk(K positive)	0.79	12	K	0.0039
	kk (K negative)	99.21	1516	k	0.9961

n.a. = Not applicable.
 *No weak serologic reactivity was observed for D antigen testing among the 1,528 samples. The upper limit of the 95% confidence interval for a serologic weak D phenotype is 1 in 465.14 among all blood donors (0.215%).

horts from other parts of India had a slight preponderance of blood group O [2, 6]. The prevalence of the Rhesus antigens was similar to previous reports for Delhi, India [4]. The CCDEE phenotype was rare but more frequently observed ($n = 4$, table 2) than was predicted by our calculations of the *CDE* haplotype ($n = 0$, table 3); the 4 donors were unrelated based on the donors' residence, name, religion, location, and date of blood donation (data not shown). The prevalence of Kell antigens varied markedly among Indian populations: Thakral et al. [4] reported for Northern India a K prevalence of 5.68%, which almost resembles the European situation, for instance 7.82% in Germany [11]; in West Bengal the K prevalence was 0.79% (table 1), more typical for most non-Caucasian populations [5].

The studied population was expected to be homogeneous, because the study area was restricted to Kolkata and adjacent districts in the state's south. Tribal ethnic groups, such as Rajbansi and Lepcha, with possibly different blood group frequencies are living in the state's north and adjoining mountainous areas of the Himalaya.

In our region, 5.6% of the population carries the β -thalassemia trait and 5.0% the hemoglobin E (HbE) trait [17]. Patients can be heterozygous for the HbE and β -thalassemia alleles or homozygous for either the HbE or β -thalassemia allele [17]. Because the majority of these patients is transfusion-dependent and prone to alloimmunization [18, 19], we detected red cell alloantibodies to blood group antigens in 5.6% of multiply transfused patients with β -thalassemia disease at our hospital in Kolkata [20]. Knowledge of the antigen prevalence allows us to predict the number of red cell units that need to be typed when searching for antigen-negative units. If all donors would eventually be typed [21], as is routine and cost effective in many Western health care systems [15], allocating an e-negative unit [11, 22], for instance, would be easy, because at any given time more than 10 such ccDEE units are expected to be on the shelf at our hospital.

Table 2. Frequencies of Rhesus phenotypes

Phenotype	Rh phenotypes observed, n	Frequency, %
CcDee	424	27.75
CCDee	749	49.02
CcDEe	210	13.74
ccDEe	52	3.40
ccDEE	11	0.72
ccDee	15	0.98
CCDEe	11	0.72
CcDEE	0	0
CCDEE	4	0.26
Subtotal	1,476	96.60
ccdde	42	2.75
Subtotal	42	2.75
Cdddee	8	0.52
CCddde	0	0
CcddEe	0	0
ccddEe	2	0.13
ccddEE	0	0
CcddEE	0	0
CCddEE	0	0
Subtotal	10	0.65
Total	1,528	100

Antibody screening and identification methods employ commercial red cell panels, often prepared from donors of Western countries who may have a different antigen profile relative to Indian patients. The Mi^a antigen, frequent in South East Asia but apparently rare in Delhi [23], could be investigated in the Bengali population along with the U, Mur, Di^a , In^a , and variant Rh antigens. Rare red cells, including these and other clinically relevant indigenous antigen features, could be included in panels for anti-

Table 3. Frequencies of Rhesus haplotypes

	Haplotypes							
	cde	CDe	cDE	cDe	Cde	cdE	CDE	CdE
Frequency	0.16983	0.688809	0.088024	0.027814	0.013743	0.003927	0.007199	0.000654

body detection. Antigen screening of the major blood group systems will also aid in selecting red cells for developing panels locally.

The age distribution of the blood donors (fig. 1) may be representative for a growing population of relatively low average age. These details contrasted to some Western populations and their donor age distributions [24]. The fluctuation in donor numbers per age cohorts (fig. 1) may be random, as most cohorts are small, but could also be explained by donors living in villages with lower than average literacy rates, where a few of them may not even know their exact age. While the exact reasons for the almost yearly alternating donor numbers (fig. 1) remain unknown, they should be explored to better understand the donor demographics and possible data reporting issues. Female donors were underrepresented, which may be typical for donors in India, perhaps based on cultural attitudes. However, the generally low weight and body mass index may more readily produce borderline low hemoglobin concentrations in repeat female donors preventing them from donating blood more frequently.

Our observation of 4 rare CCDEE phenotypes among only 1,528 donors (table 2) emphasizes that a fresh look at routine blood group data can be rewarding [25]. The discrepancy between the observation and CDE allele frequency calculation (table 3) should prompt further study of the donors' phenotypes and haplotypes. Methodically studying blood group data, particularly based on methods newly applied to a donor population, continues to be worthwhile, even when utilizing methods that are standard in other regions of the world. Implementation of such methods in the blood service routine in West Bengal has the potential to aid in clinical care and patient safety [26, 27].

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Authorship Contribution

DB and SSD designed the study, performed serologic testing, and wrote drafts. CM and WAF developed a spreadsheet for the counting method and calculated the allele and haplotype frequencies. PB and KM discussed data. All authors reviewed and edited the manuscript. WAF analyzed all data and wrote the manuscript.

Statement of Disclaimer

The opinions expressed in this review are those of the authors and do not necessarily represent the views or policies of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

Disclosure Statement

The authors declared having no competing financial interest relevant to this article.

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