Characterisation of Rh and Other Blood Group Systems Amongst the Maldivian Blood Donors

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SUMMARY

Objective: We here report the first study on the distribution of red cell antigens and phenotype frequencies of various blood group systems in Maldives.

Method: Randomly selected 123 regular blood donors of O group were phenotyped for seven blood group systems by direct tube agglutination and or indirect antiglobulin tests. Blood group systems studied were Rh, Kidd, Duffy, Lewis, Kell, P and MNS system.

Results: Rh blood grouping showed, 7.3% donors were Rh(D) negative, 92.7% were Rh(D) positive with the predominance of genotype complex of DCe/DCe (39.0%). The incidence of Jk(a+b+) phenotype was the most common in Kidd system. In Duffy system, the incidence of Fy(a+b+) phenotype was 50.4%. Lewis system was predominated by Le(a-b+) phenotype accounting to 80.5% of the donors. In the Kell system only two phenotypes were present, K+k- (5.7%) and k+k+ (94.3%), in the Maldivian blood donors. P system was represented by P_1, P_2 and P_2^k phenotypes with an incidence of 28.5%, 70.7% and 0.8% respectively. In the MNS system, MNss and MNSs phenotypes summed up to 48.8% of blood donors.

Conclusion: The detail knowledge of red cell antigen composition and their frequencies in the Maldivian population will be helpful in terms of population genetic perspectives, in establishing a donor data-bank for in-house production of indigenous screening and identification cell panels, and facilitate availability of antigen negative compatible blood for patients with previously identified multiple alloantibodies.

KEY WORDS:
Blood group antigens, phenotype frequencies, Maldives

INTRODUCTION

Human blood group systems are highly polymorphic. Over 300 blood group antigens that demonstrate polymorphism have been identified on the surface of human erythrocytes[1]. These integral, inherited antigens, which are expressed on the red cell membrane, are grouped into genetically distinct systems, collections and series of independent antigens based on their serological and biochemical characteristics[2]. A total of 27 well-defined blood group systems have been identified[3]. Some clinically important blood group systems include the ABO, Rh, Kell, Kidd, Duffy and MNSs systems. Of these, the first three systems are the most important in clinical transfusion practices because their antigens are more immunogenic and active at body temperature. The Rh system is the most polymorphic of all human blood group systems, comprising over 40 different antigens. D antigen in this system is the most complex and the most immunogenic of all non-ABO red cell antigens.

The genetically controlled red cell antigens may have variable frequencies in ethnically different population groups. A high-incidence antigen present in one ethnic group may be a low-incidence antigen in another. For example, Lewis, Ss and P blood group systems in ethnic Malays, Chinese and Indians from Malaysia have shown significant differences in their inter-ethnic distributions[4]. Similarly, incidence of the Fy(a-b-) phenotype in West Africa is reported to be greater than 80%, but the same incidence is very low in populations of Caucasians[5].

These polymorphic antigens, empanelled to different systems with variable degrees of immunogenicity, can stimulate antibody production of clinical significance. Some naturally occurring and immune antibodies that react with their corresponding red cell antigens can cause variable degrees of clinical conditions, including haemolytic transfusion reactions (HTR), haemolytic disease of foetus and newborn and autoimmune haemolytic anaemia[6].

In a standard setting, a safe transfusion can be assured by the correct typing of donors and recipients with respect to the ABO phenotype and the Rh(D) type, as well as a screening of patients' sera for the presence of unexpected, clinically significant antibodies directed against polymorphic antigens in the local population. In routine blood transfusion practices in the Maldives, compatibility testing includes ABO grouping, Rh typing and a serological cross-match with patient serum and donor red-cells using the indirect antiglobulin test (IAT). Screening for unexpected antibodies to red cell antigens has not yet been established in the country. This is partly due to the unavailability of screening panel reagent red cells and a lack of knowledge regarding phenotypic distribution of red cell antigens in the population. Therefore, in this study, we aim to characterise for the first time the phenotypes and frequencies of minor blood group antigens among the regular blood donors of O phenotype in

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the Maldives, which could be used to empanel indigenous in-house reagent red cells for screening and identification of unexpected immune antibodies.

MATERIALS AND METHODS

An audit of O phenotype blood donors registered at the Indira Gandhi Memorial Hospital blood bank, Maldives, were taken from June 2002 to June 2004. After elimination of repeat donors, 200 unrelated donors were randomly selected. Out of the 200 donors, 123 subjects gave written informed consent to participate in this study while 77 (38.5%) declined. All the recruited donors declared no history of previous transfusion. They were 121 males and 2 non-pregnant females. Five millilitres (5mL) of venous blood were obtained from each participant into EDTA anticoagulant vaccutainer tubes.

All the 123 donors were phenotyped for Rh, MNS, P, Lewis, Kidd, Duffy and Kell blood group system antigens on the red cell surface. The following anti-sera were used: anti-D, anti-C, anti-E, anti-c, anti-e for Rh system; anti-M, anti-N, anti-S and anti-s for MNS system; anti-P1 and anti-Pk for P system; anti-Lea and anti-Leb for Lewis system; anti-Jka and anti-Jkb for Kidd system; anti-Fy^a and anti-Fy^b for Duffy system; anti-K and anti-k for Kell system.

Approximately 5% suspension of donor red cells in normal saline were used for blood group phenotyping either by direct tube-agglutination method or indirect antiglobulin test (IAT) used in accordance with manufacturer's directions. Agglutination or haemolysis were interpreted as a positive test result indicating the presence of antigen. A smooth suspension of red cells after re-suspension of the cell button constitutes a negative result and indicates negative for the antigen. Ethical approval for this study was obtained from the Ministry of Health, Maldives.

RESULTS

The incidence of Rh(D) negative individuals amongst the Maldivian blood donors were 7.3%. As shown in Table I, antigen e of the Rh system has a ubiquitous distribution amongst the donors with an incidence of 97.6%. Phenotypically R1R1 (CDe/CDe) has the highest incidence of 39%, as summarised in Table II. One donor had weak D positive cells that could only be demonstrated with monoclonal anti-D reagent incubated with added antiglobulin serum.

Table I: Distribution of Rh antigens amongst blood donors

Reagent	Number of positive	Percentage
Anit-D	114	92.7
Anti-C	103	83.7
Anti-E	29	23.6
Anti-c	75	61
Anti-e	120	97.6

The study of Kidd systems showed Jk(a+b+) was the predominant phenotype amongst the blood donors, and the two major antigens, Jk^a and Jk^b, occurred at an incidence of 86.2% and 68.3% respectively. Gene frequency calculated using direct counting methods showed Jk^a =0.553 and Jk^b =0.447.

Of the Duffy system, half of the donor population (50.4%) had Fy(a+b+) phenotype. The other half had phenotypes Fy(a+b-) and Fy(a-b+) with an incidence of 35.8% and 13.0% respectively. Duffy null phenotype, Fy(a-b-), was present in one donor. Fy^a antigen was seen in 86% of donors, while Fyb was demonstrated in 63.4% giving a gene frequency of Fy^a =0.615 and Fy^b =0.385.

The Lewis blood group system showed 94.3% of the donors were positive for Le^b antigen, while only 18.6% of donors carrying Le^a on their red cell membranes. Thus, Le(a-b+) made 80.5% of the Lewis system phenotypes, as shown in Table II. A single donor had Le(a-b-) phenotype.

In the Kell system, none of the donors exhibited homozygosity for K antigen. However, seven donors showed heterozygosity for Kk antigens, while majority (94.3%) of the them had double dose for k antigen.

Table II: Phenotype and frequencies of blood group systems

Phenotype	No of individuals	Phenotype frequencies
Rh system		
R1R1	48	39.0
R1r	37	30.1
R1R2	18	14.6
rr	9	7.3
R2r	8	6.5
R2R2	3	2.4
Kidd System		
Jk(a+ b+)	58	47.2
Jk (a+ b-)	39	31.7
Jk (a- b+)	26	21.1
Duffy System		
Fy(a+ b+)	62	50.4
Fy(a+b-)	44	35.8
Fy(a-b+)	16	13.0
Fy(a-b-)	1	0.8
Lewis system		
Le(a+ b-)	06	4.9
Le(a-b+)	99	80.5
Le(a-b-)	01	0.8
Le(a+b+)	17	13.8
Kell system		
K+K+	0	
K+k-	7	5.7
k+k+	116	94.3
P system		
P_1	35	28.5
P_2	87	70.7
P_2^k	1	0.8
MNS System		
M+N-	36	29.3
M+N+	67	54.5
M-N+	20	16.3
S+s-	13	10.6
S+s+	46	37.4
S-s+	63	51.2
S-s-	01	0.8

The study of P system revealed that red cells of 70.7% of the donors had P_2 phenotype indicating the absence of P_1 antigen, and the rest were P_1 phenotype except for a single donor who had P_2^k phenotype.

MNS system showed that M+N+ phenotype was present in 54.5% of the donors (Table II). The other half of the donors had M+N- and M-N+ phenotypes with an incidence of 29.3% and 16.3% respectively. Similarly, 51.2% of the donors had S-s+ phenotype while the other half had S+s+ and S+s- phenotypes on their cell surfaces. In addition, one donor presented with a rare phenotype of S-s-. As presented in Table III, MNss and MNSs phenotypes summed up to 48.8% of blood donors.

Table III: Phenotype counts and frequencies of MNs system

Phenotypes	Counts	Per centage
MNss	35	28.5
MNSs	25	20.3
NNSS	16	13
MMSs	15	12.2
MMss	10	8.1
NNss	9	7.3
MMSS	7	5.7
MNSS	4	3.3
NNSs	2	1.6

DISCUSSION

A well defined blood group system includes those red cell antigens encoded by alleles at a single genetic locus or those produced by a epistasis of two or more linked homologous genes. In some systems the gene directly encodes for blood group determinant of protein in nature, whereas in others, genes do not code for membrane proteins but encodes a glycosyltransferase enzyme that catalyses the transfer of sugar units on various components on the red cell surface. Polymorphisms in the respective blood group system genes complemented with population genetic principles drive the phenotype frequencies in a given ethnic group or population. In this study we have used serological methods to detect the polymorphism in the blood group antigens.

Rh blood group system is highly polymorphic and is the second most important in transfusion medicine after ABO. Incidence of Rh(D) positives in Maldives was 92.7% with the phenotype R1R1 being the most frequent. Amongst Rh positive donors, 56.1% were homozygous (DD) and 36.5% were heterozygous (Dd). The incidence of Rh negative in Maldives was found to be 7.3%. A relatively lower pattern was seen in the neighbouring Sri Lanka, and in Pakistan the frequency was reported to be 5%[7,8]. In contrast, its frequency in Caucasians was reported to be much higher (35%)[5]. The frequency of other Rh antigens in our study population were as follows: C, 83.7%; c, 61%; E, 23.6%; and e, 97.6%. Though antigen D is the most potent immunogen, the other antigens in the system can sensitise the immune system to give rise to clinically significant antibodies when Rh D compatible units are transfused with red cells incompatible for C, c, E or e antigens. Antibodies specific to this system are the most frequent antibodies encountered in pretransfusion testing, and is the main cause of haemolytic disease of newborn (HDN)[9].

The Kidd blood group system has two major antigen Jk^a and Jk^b. Besides being antigenic, these functional transmembrane glycoproteins are the major urea transporters on erythrocytes. Frequency of Jk^a in the Maldivians (86.2%) showed a marginal elevation when compared to that of Asians (73%) in general and Caucasians (77%). However, Jk^b frequency amongst the Maldivian blood donors (68.3%) has shown to be comparatively lesser than other Asians (76%) and Caucasians (74%)[5]. The most common Kidd phenotype (Jk^a+Jk^b+) observed in the Maldives has a frequency of (47.2%), comparable to that of Caucasians (50%), Asians (49%) including ethnic Chinese (50.7%)[4,10]. The anti-Jka and anti-Jkb antibodies, produced when the immune system is challenged, causes delayed HTR and mild HDN.

Duffy blood group antigens are glycoprotein in nature which function as receptors for cytokines and malaria parasite, *Plasmodium vivax*. Individuals with Duffy null phenotypes, Fy(a-b-), are resistant to invasion by *P vivax*[5]. This phenotype was seen in 0.8% of Maldivians and is very rare amongst Caucasians. However, this phenotype was seen in 68% of Blacks[5]. Similar to the Fya antigen frequency in Maldives (86%), it is extremely common in Asiatic ethnic groups such as Chinese (90.8%), Japanese (81.5%), and Thais (69%)[11]. In contrast, the frequency is significantly low in African populations (10%). Interestingly, the frequency of Fyb antigen in the Maldivian population was remarkably high (63.4%) when compared to the reported frequency for Asians (18.5%). The commonest Duffy phenotype Fy(a+b+) observed in the Maldivians and the Caucasians has the similar frequencies of 50.4% and 49% respectively. However, the phenotype is extremely rare in Africans (1%) and Chinese (9%). Antibodies specific to this system are mostly IgG and causes delayed transfusion reactions and HDN.

Lewis system antigens, Le^a and Le^b, are not intrinsic to erythrocytes but are adsorbed from the plasma. Similar to many populations, most common phenotype encountered in Maldives was Le(a-b+). Lewis phenotype with Le(a+b+) accounting for 13.8% of Maldivians are rare in the whites and blacks, but are more common in people of Asian origin (Malays, 7.0%; and Chinese, 12.0%) due to a deficiency in fucosyltransferase[12]. It is extremely rare for Le(a+) or Le(b+) red cells to cause HTR as specific naturally occurring antibodies are almost exclusively in the sera of Le(a-b-) individuals. However, pregnant women who transiently acquire Le(a-b-) may produce Lewis antibodies[2].

Kell system is composed of two major antigens, K and k, that are in antithetical relationships to each other, and is the third most potent after ABO and Rh systems at triggering an immune response. As observed here, the high frequency k antigen has a ubiquitous distribution around the world, while K antigen is a low frequency allele in all the populations studied. Though a greater proportion of Maldivian recipients are apparently at risk of developing immune anti-K antibodies, the probability of a prospective recipient receiving K positive blood would be uncommon as the K antigens were possessed by only 5.7% of the Maldivians. Contrary to this, recruiting a k negative compatible donor would be challenging as only 5.7% of the population were with the phenotype K+k. Anti-K antibodies are known to cause severe haemolytic transfusion reactions in numerous occasions, and cause HDN with severe foetal

anaemia[13]. Anti-k may cause a milder haemolytic transfusion reaction.

The low prevalence of P1 antigen observed in the Maldivian population is correspondingly low in many other Asian countries such as Vietnam, Cambodia and Thailand, and in some ethnic groups namely Malays and Chinese from Malaysia[4,5]. Extremely rare phenotypes P^2_k associated with P blood group system was demonstrated at a frequency of 0.8% in the Maldivian population. Such individuals consistently will produce IgM alloanti-P antibodies and is active at body temperature against P_1 and P_2 phenotype cells. Most P_2 phenotype individuals have naturally occurring IgM anti-P1 which is a weak cold agglutinin and does not cause HDN. However, unusual association with clinically significant transfusion reactions has been reported especially in P_1^K and P_2^K phenotype recipients[2].

The MNS system is another polymorphic system with over 40 antigens. The four major glycoprotein antigens M, N, S and s in the system are weak immunogens and may function as receptors for cytokines and *Plasmodium falciparum*[10]. Akin to Caucasians and Africans, donors expressing both M and N antigens predominate in the Maldivian population. It is shown here in this study that 0.8% of the donors were negative for S and s antigens. Individuals with this phenotype (S-s-) are likely to be negative for the high incidence U antigen; hence when transfused with U positive cells may respond with anti-U. Nearly 50% of Maldivian blood donors demonstrated MNss (28.5%) and MNSs (20.3%) phenotype, a finding consistent with Caucasians and Blacks. This is probably because of the considerable linkage disequilibrium between M,N and S,s. Antibodies produced against the system antigens are rare to causes transfusion reactions and HDN, but may be potentially severe.

CONCLUSION

The detail knowledge of red cell antigen composition and their frequencies in the Maldivian population will be helpful in terms population genetic perspectives and in establishing a donor data-bank for preparation of indigenous screening and identification cell panels that could be used nationwide. Extensive screening of regular blood donors to identify rare blood groups would be essential to facilitate swift availability of antigen negative compatible blood to patients with previously identified multiple alloantibodies.

It is recommended that the phenotypic status of minor blood group antigens such as E and K be determined routinely along with ABO and Rh(D) typing before the first transfusion to minimise the potential alloimmunisation, specially in multi-transfused thalassaemia majors.

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