ORIGINAL RESEARCH ARTICLE

FREQUENCIES OF RH PHENOTYPES AND PROBABLE GENOTYPES IN RHD NEGATIVE BLOOD DONORS IN OMAN: A CROSS-SECTIONAL STUDY

ABSTRACT

Aims: Knowledge of Rh phenotype frequencies guides in assessing the probability of Rh alloantibody formation and finding Rh-negative antigen donors. There is limited information on the RhCE phenotypes and probable genotype frequencies among D negative Omani population, hence this study was conducted to determine that and compared it with other populations.
Study design: Cross-sectional study
Place and Duration of Study: The study was conducted in Oman College of Health Sciences between July 2016 and April 2017.
Methodology: A total of 205 RhD negative blood samples were collected from Omani blood donors. Haemagglutination methods were used for Rh typing. The data was collected to determine the Rh phenotype, probable genotype and haplotype frequencies.
Results: The most prevalent Rh antigen was e followed by c , C and E. The most common probable genotype/phenotype and haplotype were *rr* / *rr* and *r* with frequencies of 0.766 and 0.887 respectively.
Conclusion: There was no difference in Rh phenotype / probable genotype distribution from other populations. However, a significant difference was found in the frequencies of Rh antigens C, E, and c with some of the other populations. Likewise, haplotype *r* and probable genotype *rr* frequencies were found to be significantly different from some other populations.

Keywords: *Haplotype, Genotype, Oman, Rh Blood group*

ABBREVIATIONS

CI: Confidence Interval; HDFN: Haemolytic disease of the fetus and newborn; K2EDTA: dipotassium Ethylenediaminetetraacetic acid; RBC: Red blood cell

1. INTRODUCTION

The Rh blood group system is considered to be the second most important blood group in transfusion medicine after ABO groups. It consists of more than 50 antigens, however, the most important and significant are D, C, E, c, and e [1]. Rh antibodies can only be produced after foreign red blood cells (RBCs) exposure through transfusion or pregnancy. It is the main cause of hemolytic disease of the fetus and new born (HDFN) and is of primary importance in obstetrics [2].

Due to the immunogenicity of Rh antigens, RhD testing was made compulsory in pre-transfusion testing [3]. ABO/RhD blood testing is mandatory in pre-transfusion testing and pregnancy in Oman as well; however, Rh phenotyping (C, E, c and e) for the blood donors is not routinely performed in most of regional blood banks in Oman. Different blood group antigens frequency knowledge can be of great help in risk assessment of alloantibody formation and probably to know how easy is to find the antigen- negative donor, particularly for thalassemia major and sickle cell disorders' patients. In 2013, the estimated number of patients with thalassemia major and sickle cell disorders in Oman was 400 and 3000, respectively [4] which urges the necessity to perform Rh phenotyping in regional blood banks for possible safer transfusions.

The immunogenicity of Rh antigens differs, with RhD being the most immunogenic followed by c, E, C and e antigens [5]. The majority of cases of alloimmunization following blood transfusion or pregnancy are due to these major antigens [6]. The frequency of these antigens determines frequency of corresponding antibodies occurrences. Therefore, implementing a program that ensures complete Rh phenotyping of donor and recipient for the common five Rh antigens will reduce the risk of Rh alloimmunization due to these antigens.

The distribution and frequencies of RhD and RhCE vary between races and ethnic groups in different parts of the world. The majority of Rh studies have concentrated on Caucasian populations. A study on D negative donors in West Africa showed 100% with e and c antigens and 19.57% with C antigen [7]. Whereas, a similar study in Iran showed that 100% of the population pose e and c, 2% with E and 13.7% with C antigens [8].
Very little is known about the frequencies of Rh of the South Eastern Arabic Peninsula. A retrospective study on D negative pregnant women attending Sultan Qaboos Hospital in Oman showed a prevalence of 7.3% [9], however it did not include other Rh antigens frequency. A study conducted by Danubio and Anelli, 1987 on RHD locus frequency among Omanis showed RHD allele (D) exhibited the frequency estimate of 0.738 whereas the remaining 0.262 without D locus (d) [10]. However, RHD allele frequency calculations in the previous study were based on the conventional serological tube method with limitations to detect D variants that would have raised the D locus frequency among Omanis if observed. To our best knowledge, no other Rh phenotypes & haplotypes studies have been reported for the Omani population. This propelled us to study Rh frequencies on D negative Omani blood donor and compare it with those of other populations.

2. MATERIALS AND METHODS

The study was performed on 205 blood samples from D negative Omani donors over 18 years old (180 male and 25 female) presenting at central and regional blood banks. Blood was collected in 3 ml BD Vacutainer dipotassium Ethylenediaminetetraacetic acid (K2EDTA) tubes and mixed immediately for 5 minutes on a tube roller. Samples were either tested immediately or stored at 4°C for up to 5 days.
All reagents were purchased from Bio-Rad (Cressier FR, Switzerland) unless indicated otherwise. Samples were tested by hemagglutination using tube and gel card techniques. For the tube method, 5% red blood cells (RBCs) suspension was used for phenotyping and indirect Coombs' test (ICT). Monoclonal IgM antibodies were used for Rh phenotyping: anti-c (cell line: MS-33), anti-e (cell line: MS-16, MS-21, MS-63), anti-C (cell line: MS-24) and anti-E (cell line: MS-260) according to the manufacturer's instructions. The samples were further tested by ICT using anti-human globulin (Atlas, UK) for weak D detection. For gel card technique, 5% and 0.8% RBCs suspension were used for Rh phenotyping (suspended in ID-Diluent 1 and tested using human RHD + phenotype card) and ICT (suspended in ID-Diluent 2, and tested using ID-Coombs anti-IgG card with the addition of ID-Diaclon IgG anti-D monoclonal antibody – cell line ESD1), respectively, according to the manufacturer's instructions.
Frequencies of Rh Phenotypes (C, c, E and e) were calculated as a percentage by dividing total positive cases for the particular antigen by a total number of samples. Rh haplotype frequency (for RhD negative donors) was calculated using the Hardy-Weinberg equation. Chi-square test of independence was performed using GraphPad Prism 6 to calculate *P*-value.

3. RESULTS AND DISCUSSION

This observational cross-sectional study was conducted on 205 RhD negative Omani blood donors to determine the frequency of Rh common antigens and phenotypes. To the best of our knowledge, there are no published data on Rh common blood group antigens and haplotypes frequencies for D negative Omani individuals, hence this is the first description. ABO distribution among Omanis were as follows: O (n = 115, 56.7%) > A (n = 49, 24.1%) > B (n = 30, 14.8%) > AB (n = 9, 4.4%).

All direct serological tests showed the following Rh phenotypes and probable genotypes: C-c+E-e+ (rr, n = 157, 76.6%), C+c+E-e+ (r'r, n = 38, 18.5%), C-c+E+e+ (r″r , n = 5, 2.4%), C+c-E-e+ (r'r', n = 2, 1%), C-c+E+e- (r″r″, n = 1, 0.5%) [Table 1]. The frequency of Rh phenotype and probable genotypes for males and females was also compared [Table 2]. Weak D testing by ICT was negative

for all samples except two (001 and 209) which showed +1 agglutination by gel but not tube technique with following phenotype and probable genotype: C-c+D+E-e+ (R0r, n = 2, 0.98%) [Figure 1]. The frequency of C, c, E and e were 20%, 99%, 3% and 99% respectively [Figure 2]. Haplotype frequencies were: (dce, 0.89), (dCe, 0.1) and (dcE, 0.01).

The D negative ratio for Omani males to females was 87.7:12.3. This ratio was similar to 80.65:19.35 ratio observed in another study from Ghana (*P* = .172) [11]. However, it showed significantly different (*P* < .001) from the observed ratio 56:44 in a study conducted by Rahmani et al, 2016 in Pakistan [12]. The current study targeted blood donors only and not the general population, which could explain such a significant difference compared to Rahmani et al study where individuals visiting blood banks for blood group determination were also included. In addition, about 30% of current study samples were collected from blood campaigns at armed forces and navy where male donors were dominant.

The current study showed no significant difference between D negative male and female Omani blood donors concerning Rh phenotyping (*P* = .4611). Out of 203 samples, 178 (87.7%) were males compared to 25 (12.3%) females [Table 2]. However, due to a low number of female donors, possible bias cannot be ruled out. The commonest phenotype and probable genotype noticed was C-c+E-e+ (rr). This finding is similar to the result observed in two Arabian countries, Tunisia [13, 14] and Saudi Arabia [15].

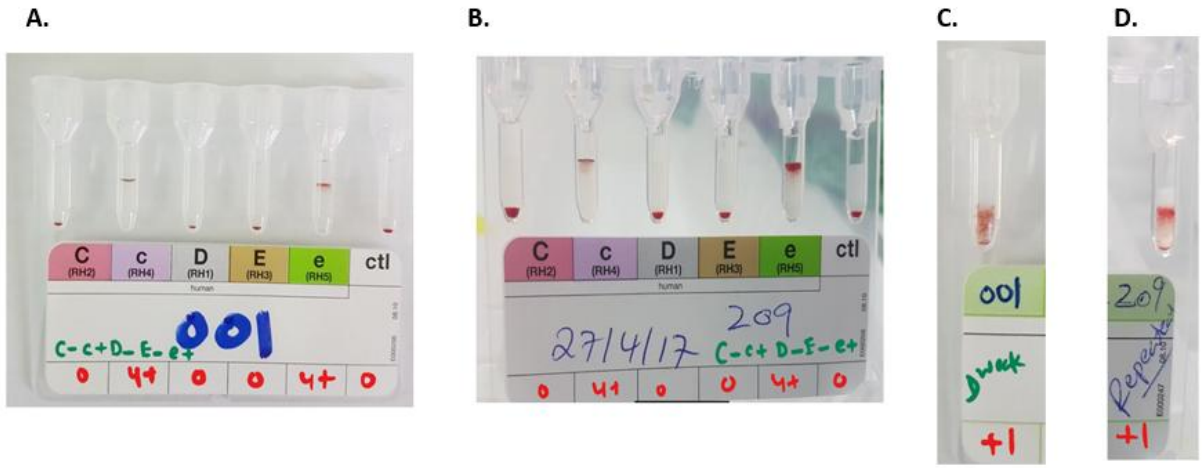

Fig. 1. Rh phenotype and ICT agglutination pattern. Rh phenotyping using BioRAD RhD + phenotype card for two D negative samples 001 & 209 both with C-c+D-E-e+ phenotype (A & B). Weak D testing using BioRad ID-Coombs anti-IgG showed +1 agglutination in both samples (C & D).

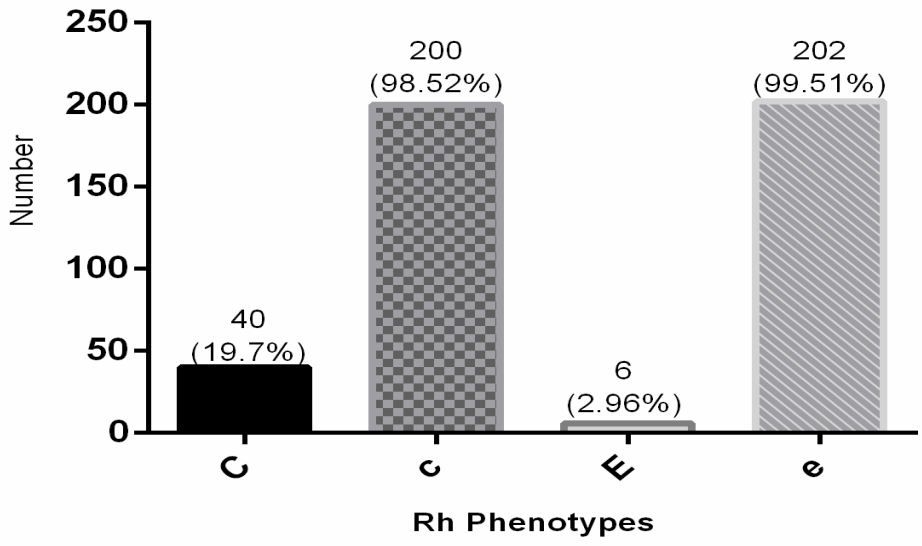

Fig. 2. Rh phenotype frequencies in D negative Omani blood donors. The most common antigen was found to be e followed by c, C & E.

Table 1. Rh phenotypes and probable genotype frequencies in D negative Omani donors (*n* = 203)

		Genotype				
Antigens	Phenotype	Wiener	Fisher race	Number	Frequency %	95% CI[a]
D-C-E-c+e+	rr	*rr*	dce/dce	157	76.6	72.2 – 80.8
D-C+E-c+e+	r'r	*r'r*	dCe/dce	38	18.5	14.3 – 22.7
D-C-E+c+e+	r"r	*r"r*	dcE/dce	5	2.4	< 6.8
D-C-E+c+e-	r"r"	*r"r"*	dcE/dcE	2	1	< 5.2
D-C+E-c-e+	r'r'	*r'r'*	dCe/dCe	1	0.5	< 4.7

[a] Confidence interval

Table 2. Distribution of Rh phenotype and probable genotype between D negative male and female blood donors

Gender	*r''r''*	*rr*	*r'r*	*r''r*	*r'r'*	Total
Male	1 (0.49%)	137 (67.49%)	34 (16.75%)	5 (2.46%)	1 (0.49%)	178
Female	0 (0%)	20 (9.85%)	4 (1.97%)	0 (0%)	1 (0.49%)	25
P value		***P* = .461**				**203**

Among D negative blood donors, the most common antigen found was e followed by c, C and E. This is similar to that in other Arab countries UAE, Saudi Arabia, Morocco and India [15-18]. Predominantly, there are no significant differences between these frequencies compared to the most other Arab countries, Iran and India nonetheless; significant differences have been observed in C and E antigens [14-16]. Rate of RhC positive in the population was 19.7% and showed significant difference compared to 9.52% in Saudi Arabia, 33.5% in India and 6.7% in Tunisia ($P < .001$) whereas the rate of RhE positive was 3% and determined significant difference in comparison to India ($P < .001$). Rhc antigen positivity found to be 98.5% compared to 93.6% in UAE with significant difference ($P = .05$) [14-16]. Frequencies of major Rh antigens in D negative Omani population compared to other populations are provided in Table 3.

Table 3. Rh major antigens frequencies in different D negative ethnic populations

Rh antigen	Current study	Saudi Arabia [15]	India [16]	UAE [17]	Morocco [18]	Iran [8]	Tunisia [14]
C	40 (19.7%)	62 (9.52%)***	1269 (33.5%)***	13 (21%)	20 (15.2%)	7 (13.7%)	30 (6.7%)** *
E	6 (3%)	12 (1.8%)	68 (1.8%)***	2 (3.2%)	1 (0.8%)	1 (2%)	16 (3.6%)
c	200 (98.5%)	639 (98.2%)	3736 (98.7%)	58 (93.6%)*	131 (100%)	51 (100%)	448 (100%)* *
e	202 (99.5%)	647 (99%)	3762 (99.3%)	61 (98.4%)	131 (100%)	51 (100%)	448 (100%)
Total	*n = 203*	*n = 651*	*n = 3773*	*n = 62*	*n = 131*	*n = 51*	*n = 448*

* $P = .05$ ** $P < .01$ *** $P < .001$

The incidence of weak D varies between populations worldwide. In our study we observed possible two cases of weak D (0.98%) which is exactly equally observed percentage in Pakistan [12]. A similar weak D frequencies study in India by Makroo et al, 2010 was reported to be 0.12% [19] while another study by Seema et al, 2011 reported that as 0.135% [20]. Both studies showed low weak D frequency compared to current study. In whites, weak D ranges between 0.2% and 1% [21]. In Albanian population, it was reported to be 0.14% [22]. It is common in Africans and blacks [11] and can be as high as 10% [23]. In studies conducted in Ghana [11] and Nigeria [24], the prevalence of weak D phenotype among D negative were 6.45% and 7.5% respectively. However, current study showed no significant difference between Oman and other populations on weak D frequency except Africans (P = .05) [Table 4]. Both cases in current study lack C phenotype, therefore effect of C trans as a cause is ruled out.

The most common haplotype observed in current study was r followed by r' and r″. The most frequent r haplotype reflected in the fact that rr is the most common phenotype. Rh haplotypes frequency order observed in current study was similar in other D negative populations. In our study, r haplotype frequency was 0.887 which is almost proportionate with frequency in UAE, Morocco and Iran, whereas it showed significant difference with frequency in Saudi Arabia and India (P = .05) [Table 5].

Table 4. D weak positivity among D negative individuals in Oman and other populations

Population	Current study	Pakistan [12]	India [20]	Albania [22]	Gana* [11]
Weak D status					
Positive	2	44	1	58	2
Negative	203	4410	738	4214	29
Total	205	4454	739	4272	31

*P = .05 **P < .01 ***P < .001

Table 5. Rh Haplotype frequencies in different RhD- ethnic populations

Haplotype	Current Study	Saudi Arabia* [15]	India* [16]	UAE [17]	Morocco [18]	Iran [8]
r	0.887	0.944	0.809	0.88	0.916	0.918
r'	0.1	0.046	0.178	0.071	0.08	0.072
r''	0.013	0.006	0.004	0.049	0.004	0.01
r_y	0	0	0.011	0	0	0

$^{*}P = 0.05$ $^{**}P < .01$ $^{***}P < .001$

4. CONCLUSION

We studied major Rh antigens, haplotypes and probable genotypes frequency among D negative Omani blood donors and compared that with other populations. A significant difference was observed in some populations in Asia. In future, we would include molecular investigations that possibly could rule out few donors from D negative pool.

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ETHICS APPROVAL AND CONSENT

Ethical approval obtained from ministry of Health Oman under approval number (MOH/DGPS/CSR/PROPOSAL_APPROVED 25 / 2016). All donors who participated in this study signed a written consent.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company.

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