International Journal of Multidisciplinary and Current Research

Research Article

ISSN: 2321-3124
Available at: http://ijmcr.com

RHD and RHCE frequencies and gene complexes among Major Tribes of Turabah Province, Saudia Arabia

Malik H. Ibrahim Mustafa [1,2], Tariq Elfatih Elmisbah [1,2,] Abdel- Moneim M.Salim[1], Mukhtar A. Mohammed Ahmed [1], Sawsan Babiker [1,3] and Omaima Nasir [1]

[1] Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Turabah, Taif University, Kingdom of Saudia Arabia.
[2] Department of Hematology, College of Medical Laboratory Sciences, Sudan University of Science and Technology, P. O. Box 407, Khartoum, Sudan.
[3] Departments of Mathematics, Faculty of Sciences, Gezira University, P.O.Box 20, Wad Medeni, Sudan.

Accepted 20 May 2014, Available online 01 June 2014, **Vol.2 (May/June 2014 issue)**

Abstract

Background: *The RH blood group system is one of the most polymorphic and immunogenic systems known in humans. In the past decade, intense investigation has yielded considerable knowledge of the molecular background of this system. The aim of the present study was to determine the types and frequency of D and/or RHCE variants among major trips in Turabah Province, Saudia Arabia, using the immunodiffusion gel technique.*
Methodology: *RHD blood grouping was performed on 400 samples. Blood group antigens were determined using an immune-deficient gel technique (Bio-Rad, USA), and the genotype frequencies were expressed as percentages.*
Results: *The distribution of the variant RHD alleles found among the 400 samples RHD positive were 346 (86.5%) and RHD negative 54 (13.5%) and the most common probable RH-genotypes present in our samples in decreasing order of frequency were for DcE^{1+}, DcE^{2+}, dcE^{1+}, dcE^{2+}, dcE^{3+}, dcE^{4+}, DcE^{3+}, dice and DCE^{4+} and the DcE^{4+} (29%) were the commonest genotype 116 out of 400 samples.*
Discussion: *RHD and RHCE variants performed on blood samples of participants from Turabah Province, Saudi Arabia to provide transfusion-relevant information because of the frequency of variant RH alleles. RHD molecular analysis may improve transfusion therapy of patients by allowing better donor and recipient matching based not only on phenol-typically matched red blood cell units, but also on units that are genetically matched with regards to RHCE variants.*
Conclusion: *The most common phenotype was DcE^{4+}. Phenotype and probable genotype showed a wide range of variations in different races and religion. Reliable population based frequency data of the RH antigens study has a vital role in population genetic study, in resolving medico legal issues and most importantly in transfusion practice. This study is the first small step to create a rare donor data bank and to prepare indigenous cell panels to provide compatible blood to all multi-transfused alloimmunized patients.*

Keywords: *RH system, RHCE, Immunodiffusion Gel Technique, Turabah, KSA.*

Introduction

Red blood group antigens include various cellular and soluble components of whole blood that interact with specific antibodies, Red blood cell phenotype can be achieved using antibodies and reagents of known specificities. Blood groups have been the subject of research because of the importance of blood transfusion in surgery and other hemorrhagic catastrophes.

Since the discovery of the ABO blood group by Landsteiner, different blood typing systems have been devised. Blood group antigens are integrated parts of the red blood cell (RBC) membrane and have many essential functions (membrane transporters and protein canals, ligand receptors, adhesion molecules, enzymes, and structural proteins). These surface antigens also have different biochemical compositions [(1)]. According to The International Society of Blood Transfusion (ISBT), there are 287 antigens within the 33 blood group systems and 42 antigens in Collections (low and high incidence antigens) [(2)].

The Rh blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to ABO, is the most clinically significant in transfusion medicine. The ability to clone complementary DNA (cDNA) and sequence genes encoding the Rh proteins have led to an understanding of the molecular bases associated with some of the Rh antigens. Serologic detection of polymorphic blood group antigens and of phenotypes provides a valuable source of appropriate blood samples for study at the molecular level [(3-5)]. The Rh blood group system, involved in

autoimmune responses after a blood transfusion and in hemolytic disease of the newborn, is of great clinical importance [6]. The system comprises more than 50 antigens referenced by the International Society of Blood Transfusion (http://www.isbt-web.org) [7]. The most common ones are the D (RH1), C (RH2), E (RH3), c (RH4), and e (RH5). The antigens of the Rh system are encoded by two homologous genes, the *RHD* gene encoding the D protein, and the *RHCE* gene encoding the protein carrying the C/c and E/e antigens. *RHCE* has four main alleles encoding the Ce, CE, ce and cE antigen combinations [8-10]. *RHD* and *RHCE* genes, each composed of ten exons, represent a cluster of genes5-10. Their respective alleles segregate as haplotypes, the frequencies of which vary according to ethnic group [11].

The common Rh antigens: D, C or c, and E or e, were originally written in alphabetical order (CDE) but later, when it was recognized that C and E antigens are inherited en bloc, the order was changed to DCE [12]. Although d antigen, which was thought to be antithetical to D, does not exist, the letter ''d'' is used to indicate the D-negative phenotype. The most frequently occurring forms of *RHCE* and *RHD* encode 8 haplotypes: Dce, dce, DCe, dCe, DcE, dcE, DCE, and dCE, known in short, respectively, as R_0, r, R_1, r',R_2, r'', R_2, and r_y. The uppercase ''R'' is used when the D antigen is expressed, lowercase ''r'' when it is not. This notation has practical value in transfusion medicine as a means to communicate the Rh phenotype of a patient or donor. Rare deletion phenotypes use dashes in the notation to indicate a lack of antithetical antigens; e.g., D2-. RBCs lack E and e antigens, and D-- RBCs lack C, c, E, and e antigens. RBCs with the Rh_{null} phenotype do not express any of the Rh antigens [13-15].

The knowledge of RBC antigen phenotype frequencies in a population is helpful in terms of their ethnic distribution, in creating a donor data bank for the preparation of indigenous cell panels and for providing antigen-negative compatible blood of patients with multiple alloantibodies [16]. Although blood transfusions can be life saving for a number of patients, they are not without risks. In addition to risks such as transfusion-transmissible diseases (TTD) caused by donor viruses, parasites, or bacterial contaminants of blood products, there is also a risk of alloimmunization due to donor-recipient antigen phenotype disparity [17] .

There are well-defined differences in the incidence of blood group antigens between people of different ethnic origins. The determination of Rh genotypes is useful to determine the incidence of these phenotypes among the population. The collected data will be baseline for other studies, e.g. association between phenotype and disease. So the aim of the present study was to determine the type and frequency of D and/or RHCE variants among majors Tribes in Turabah Province, Saudi Arabia, This work was performed in order to tabulate regional blood group antigenic frequencies

Materials and methods

Study design

A descriptive, prospective analytical study conducted during the period of 2013-2014, to determine the *RHD* and *RHCE* frequencies among the Major Tribes of Turabah Province, Saudia Arabia.

Samples

A total of 400 samples of volunteers from Major Tribes of Turabah Province, Saudia Arabia, 166 males (53%) and 150 females (47%). The blood donations were made in accordance with the Health Ministry of Saudi Arabia regulations. EDTA blood (5 ml) was drawn from these individuals for laboratory tests to determine the phenotype for D, C, E, c and e groups using the immunodiffusion Gel Technique. The consent of all participants was obtained before donation.

Serological typing

Participant's red blood cells were phenol-typed for D, C,E, c and e with two commercial monoclonal reagents, IgG and IgM for D antigen and IgM for C, E, c and e antigens (Diagast, Loos, France) on Beckman Coulter PK 7200 and PK 7300 automated systems (Beckman Coulter, Brea, CA, USA).Fy phenol-typing of the samples was performed using the particle Immunodiffusion (ID) gel card technique (DiaMed-ID Micro Typing System) All reagents from ID-Card "DiaClon Rh – subgroups; (Brussels, Belgium). The micro-card contains the following monoclonal antibodies: anti-C (cell line MS-24), anti-c (cell line MS-33), anti-E (cell line MS-260), anti-e (cell line MS-16, MS-21, MS-63) (cell line MS-56) within each gel matrix. Negative controls were included in each card. The Techno Twin Station (BioRad, Hercules, CA, USA) automated analyzer for gel cards with anti-Fya and anti-Fyb reagents (BioRad, Hercules, CA, USA) [18].

Data Entry and Analysis

Data were entered and analyzed using STATA ISE 12.0 for Windows, Version 16. (Log Xact8, Crossover, USA). , Fisher test were used for as described in [19]. $P < 0$ 0.05 was considered statistically significant, the results were read as Positive agglutinated cells forming a discrete red line (++++) on the surface of the gel or agglutinates dispersed in gel (+++; ++; + depending on the size and position of the agglutinates on the gel and Negative compact button of red cells on the bottom of the micro-tube. For the test to be valid the negative control must always show a negative reaction.

Table-1: Incidence of RHD+/- among the gender

	Sex		Total	%
	Male	Female		
RHD Positive	182	164	346	86.5
RHD Negative	36	18	54	13.5
Total	218	182	400	100

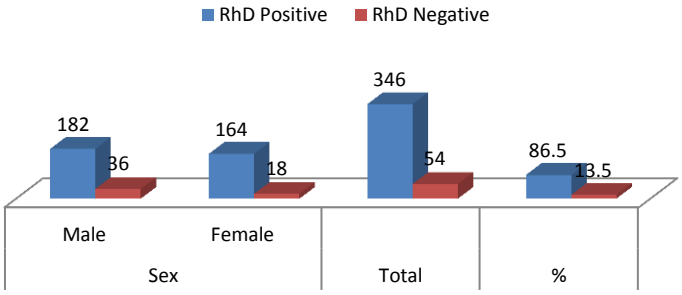

Table-2: Age Distribution of RHD +/- among studied population

Age group	RH		Total
	-	+	
Less than 25 years	16	109	125
25 – 29 year	8	47	55
30 – 39 year	1	71	83
40 – 49 year	8	49	57
50 – 59 year	7	25	32
60 – 69 year	2	23	25
70 – 79 year	0	12	12
80 years and above	1	10	11
Total	54	346	400

Table-3: Relationship among studied population

	Relation		Total
	Yes	No	
RH Positive	184	162	346
RH Negative	32	22	54
Total	216	184	400

Table-4: Distribution of RH and RHC

C	0	2+	3+	4+	Total	Fisher's exact p-value	OR 95% CI)
RH-	47 (87.04%)	1 (1.85%)	2 (3.70%)	4 (7.41%)	54		0.3021
RH +	112 (32.37%)	2 (0.58%)	52 (15.03%)	180 (52.02%)	346	0.011	(0.279,0
Total	159 (39.75%)	3 (0.75%)	54 (13.50%)	184 (46.00%)	400		.325)

Table-5: Distribution of RH and RHE

E	0	1+	2+	3+	4+	Total	Fisher's exact p-value	OR (95% CI)
RH-	47 (87.04%)	0 (0.00%)	2 (3.70%)	0 (0.00%)	5 (9.26%)	54	0.025	0.4293 (0.381,
RH +	182 (52.60%)	2 (0.58%)	8 (2.31%)	23 (6.65%)	131 (37.86%)	346		0.478)
Total	229 (57.25%)	2 (0.50%)	10 (2.50%)	23 (5.75%)	136 (34.0%)	400		

Table-6: Distribution of RH and RHe

e	0	1+	2+	3+	4+	Total	Fisher's exact p-value	OR (95% CI)
RH-	1 (1.85%)	0 (0.00%)	1 (1.85%)	16 (29.63%)	36(66.67%)	54	0.005	0.2004 (0.190 0.211)
RH +	28 (8.09%)	1 (0.29%)	3 (0.87%)	102 (29.48%)	212 (61.27%)	346		
Total	29(7.25%)	1(0.25%)	4(1.00%)	118(29.50%)	248(62.00%)	400		

Table-7: Distribution of RH and RHc

c	0	2+	3+	4+	Total	Fisher's exact p-value	OR (95% CI)
RH-	4(7.41%)	0(0.00%)	3(5.56 %)	47(87.04%)	54	0.006	0.2074 (0.195,0.219)
RH +	56(16.18%)	1(0.29%)	27(7.80%)	262(75.72%)	346		
Total	60(15.00%)	1(0.25%)	30(7.50%)	309(77.25%)	400		

Table -8: RH , C1, E1, contents (Freq.)

RH	E1 and C1			
	1		0	
	1	0	1	0
+	89	73	144	40
-	1	6	6	41

Table -9:- RH phenotype of the 400 blood samples

Gene	Freq.	Gene	Freq.	Gene	Freq.	Gene	Freq.	Gene	Freq.
D c e	38	D C^{1+}e	0	D C^{4+}e	62	D C^{2+} e	3	D C^{3+}e	9
D c E^{1+}	0	D C^{1+} E^{1+}	0	D C^{4+} E^{1+}	0	D C^{2+} E^{1+}	0	D C^{3+} E^{1+}	0
D c E^{2+}	0	D C^{1+} E^{2+}	1	D C^{4+} E^{2+}	1	D C^{2+} E^{2+}	0	D C^{3+} E^{2+}	0
D c E^{3+}	28	D C^{1+} E^{3+}	0	D C^{4+} E^{3+}	17	D C^{2+} E^{3+}	1	D C^{3+} E^{3+}	6
D c E^{4+}	116	D C^{1+} E^{4+}	1	D C^{4+} E^{4+}	51	D C^{2+} E^{4+}	4	D C^{3+} E^{4+}	8
d c e	41	d C^{1+} e	0	d C^{4+} e	4	d C^{2+} e	2	d C^{3+} e	0
d c E^{1+}	0	d C^{1+} E^{1+}	0	d C^{4+} E^{1+}	0	d C^{2+} E^{1+}	0	d C^{3+} E^{1+}	0
d c E^{2+}	1	d C^{1+} E^{2+}	0	d C^{4+} E^{2+}	0	d C^{2+} E^{2+}	0	d C^{3+} E^{2+}	0
d c E^{3+}	1	d C^{1+} E^{3+}	0	d C^{4+} E^{3+}	1	d C^{2+} E^{3+}	0	d C^{3+} E^{3+}	0
d c E^{4+}	4	d C^{1+} E^{4+}	0	d C^{4+} E^{4+}	0	d C^{2+} E^{4+}	0	d C^{3+} E^{4+}	0
Total	229		2		136		10		23

Results

In the present study the incidence of RHD positive were 86.5% (346) and 13.5% (54) samples belong to RHD negative. Out of 400 participants male was 218 (54.5%) and female 182 (45.5%) as shown in Table -1. Age of the participants varied from 18 to 85 years old (Table -2). From all studied populations almost 54.5% have family relation.

The systematic molecular analysis of the *RHCE* gene using Fisher's exact test showed a significant p-values for C (p= 0.011), and E (p=0. 025) and highly significant for c (p=0/005) and e (0.006), in Table4-7.

In our study the most common probable genotype was D c E^{+4} (116) were present in 29% of the whole samples followed DC^{4+}e ,(62) ,D C^{4+} E^{4+} (51) as shown in Table-9.

Discussion

The *RH* genes are a source of significant diversity favored by the opposite orientation of *RHD* and *RHCE* genes. Some variant Rh phenotypes are caused by exchange of genetic material between the two genes, resulting in hybrid *RH* genes. The Rh variants can weaken expression of the common antigens, produce partial antigens, generate low-prevalence antigens, and result in the absence of a high-prevalence antigen [20]. The D antigen is one of the most immunogenic blood group antigens. D variants have been classified at the molecular level. Based on *RHD* sequence variations, mutations changing the amino acid sequence predicted to be in the membrane-spanning or intracellular regions of the RhD protein were related to a feature of weak D, whereas mutations changing the amino acid sequence predicted to be in the extracellular regions were related [21] .

In our study, age of participants varied from 18- 85 years, Male and female and incidence of RhD positive was 86.5% and 13.5% belong to RhD negative .Similar result [22] of ABO and RHD blood grouping have been obtained from 400 Saudi adult male subjects, from Sakaka,Domaht Al-Jandal, Al-Qurayat and Sweer cities of the Al-Jouf Province of the Saudi Arabia blood group O was found highest (0.628), followed by A (0.193), B (0.179) and AB (7.3%) while the percentage of RHD Positive (0.705)was greater than RHD negative(0.295).

Four theories have been postulated to explain the inheritance and to classify the complex Rh system. Fisher and Race 1940 [23], Weiner 1939 [24], Rosenfield 1960 [25] and International Society of Blood Transfusion (ISBT) [26]. Fisher and Race [23] postulated that antigen of the Rh system is produced by three closely linked sets of allele genes i.e. D/d, C/c and E/e and each gene is responsible for producing the antigen D, C, c, E and e on the surface of RBC. No'd' antigen has been found on the RBC, so the d gene is considered an amorphous gene (silent allele) or the absence of D antigen. There are eight possible haplotype arrangements of Rh genes on the short arm of chromosome 1 i.e. *Dce, DCe, DcE, DCE, dec, dCe, dcE and dCE* results to 36 possible genotypes [27] .

The results of Jenan Y Taha, 2012 [28] showed the most frequently occurring antigen in Kalba Region, UAE was found to be e 643 (97.3%) followed by the D 602, C 484, c 407 and E 139, while in our study, it was found the most common probable RH-genotypes present in decreasing order of frequency were for DcE^{1+}, DcE^{2+}, dcE^{1+}, dcE^{2+}, dcE^{3+}, dcE^{4+}, DcE^{3+}, dice and DCE^{4+} and the DcE^{4+} (29%) was the commonest genotype (116 out of 400).

From our point of view, the present study is original in that, it is the first comprehensive study that documented the D and/or RHCE variants among major trips in Turabah Province, Saudi Arabia, using the immune-deficient gel technique. This study could have significant implications for the major blood banks; furthermore, the data generated in this study would be helpful to the researchers in the field of population genetics to explore the factors responsible for the observed distribution patterns of these genetic markers in this part of Saudi Arabia.

Conclusion

The RHD positive was found to be (86.5%) and RHD negative was (13.5%). The most common phenotype was DcE^{4+}. Phenotype and probable genotype showed a wide range of variations in different races n. Reliable population based frequency data of the RH antigens study has a vital role in population genetic study, in resolving medico legal issues and most importantly in transfusion practice.

Acknowledgements

The Authors would like to thank the staff and all the participants of Faculty of Applied Medical Sciences, Turabah, Taif University and Teaching Assistance Ayshai for her technical assistance in blood collections.

References

[1]. Cartron JP, Colin Y. Structural and functional diversity of blood group antigens. Transfus Clin Biol 2001;8:163-99.
[2]. Reid ME. ISBT scientific series - State of the art invited speaker. Protein blood group systems: Berlin and beyond. Vol. 7. ISBT science series, 2012:41-3.
[3]. Daniels GL, Anstee DJ, Cartron J-P, et al. Blood group terminology 1995: ISBT working party on terminology for red cell surface antigens. Vox Sang. 1995;69:265-279
[4]. Daniels G, Poole J, de Silva M, et al. The clinical significance of blood group antibodies. Transfus Med 2002; **12**: 287-95.
[5]. Neil D. Avent and Marion E. Reid :The Rh blood group system: a review .Blood, 15 January 2000; 95;2:375-387.
[6]. Daniels G, Poole J, de Silva M, et al. The clinical significance of blood group antibodies. Transfus Med 2002; **12**: 287-95.
[7]. Daniels G, Castilho L, Flegel WA, et al. International Society of Blood Transfusion Committee on terminology for red blood cell surface antigens: Macao report. Vox Sang 2009;**96**: 153-6.
[8]. Cherif-Zahar B, Le Van Kim C, Rouillac C, et al. Organization of the gene (RHCE) encoding the human blood group RhCcEe antigens and characterization of the promoter region.Genomics 1994; **19**: 68-74.
[9]. Mouro I, Colin Y, Cherif-Zahar B, et al. Molecular genetic basis of the human Rhesus blood group system. Nat Genet 1993; **5**: 62-5.
[10]. Cherif-Zahar B, Mattei MG, Le Van Kim C, et al. Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. Hum Genet 1991; **86**: 398-400
[11]. Colin Y, Cherif-Zahar B, Le Van Kim C, et al. Recent advances in molecular and genetic analysis of Rh blood group structures.J Med Primatol 1993; **22**: 36-43.
[12]. Rosenfield RE, Allen FH Jr, Swisher SN, Kochwa S. A review of Rh serology and presentation of a new terminology. Transfusion. 1962;2:287-312.
[13]. Rosenfield RE, Allen FH Jr, Rubinstein P. Genetic model for the Rh blood-group system. Proc Natl Acad Sci U S A. 1973;70:1303-1307.
[14]. Allen FH Jr, Rosenfield RE. Review of Rh serology: eight new antigens in nine years. Haematologia. 1972;6:113-120.
[15]. Rosenfield RE, Allen FH Jr, Swisher SN, Kochwa S. Rh nomenclature. Transfusion. 1979;19:487.
[16]. Aubin JT, Kim CL, Mouro I, et al. Specificity and sensitivity of RHD genotyping methods by PCRbased DNA amplification. Br J Haematol. 1997;98:356-364.
[17]. Gassner C, Schmarda A, Kilga-Nogler S, et al.RHD/CE typing by polymerase chain reaction using sequence-specific primers. Transfusion. 1997;37:1020-1026.
[18]. Neville JB (1994) An introduction to immunohematology. 3rd edition, Toronto: Congress L.March,8,1994.
[19]. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA: Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002, 70:425-434

[20]. Avent ND, Reid ME. The Rh blood group system: a review. Blood 2000; 95: 375-87.
[21]. Stratton F. A new Rh allelomorph. Nature 1946; 158: 25.
[22]. Mohamed Hassan Eweidah, Shaik Rahiman, MD Hassan Ali and Abdullah Mohamed Dbas Al-Shamary : Distribution of ABO and Rhesus (RHD) Blood Groups in Al-Jouf Province of the Saudi Arabia,© Kamla-Raj 2011 Anthropologist, 13(2): 99-102 (2011)
[23]. Weiner, Alexander S. Genetics and Nomenclature of the Rh- Hr Blood Types. Antonie van Leeuwenhoek (1 February 1949). (Netherlands: Springerlink) 1949;15(1):17–28. doi:10.1007/BF02062626. ISSN 0003-6072. Retrieved 6 November 2010.
[24]. Rosenfield RE, Allen FH Jr., Swisher SN, Kochwa S. A review of Rh Serology and presentation of a new terminology. Transfusion. 1962; 2:287-312.
[25]. Rosenfield RE, Allen FH Jr., Rubinstein P. Genetic model for the Rh blood-group system. Proc Natl Acad Sci U S A. 1973;70:1303-1307.
[26]. Rosenfield RE, Allen FH, Jr., Swisher SN, Kochwa S. Rh nomenclature. Transfusion. 1979; 19:487
[27]. Daniels GL, Anstee DJ, Cartron JP, W Dahr PD, Issitt J, Jørgensen L, Kornstad C,Levene C, Lomas-Francis A, Lubenko D, Mallory JJ, Moulds Y, Okubo M, Overbeeke ME, Reid P, Rouger S, Seidl P, Sistonen S, Wendel G, Woodfield T.Zelinski. Blood group terminology 1995: ISBT working party on terminology for red cell surface antigens. Vox Sang. 1995;69(3):265-279.
[28]. Jenan Y Taha : Rh Antigen and Phynotype Frequency in Kalba ,UAE.Bahrain Medical Bulletin , Vol.34,No.1,March 2012.