PREVALENCE OF RH ANTIGENS, ITS PHENOTYPE AND MOST PROBABLE GENOTYPE AMONG THE REGULAR BLOOD DONORS IN SARAWAK

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UNIVERSITI SAINS MALAYSIA

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KONG HUNG CHUO

Dissertation Submitted In Partial Fulfillment of The Requirements For The Degree of Master of Science (Transfusion Science)

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DECLARATION

I hereby declare that I am the sole author of this thesis entitled 'Prevalence of Rh Antigens, Its Phenotype and Most Probable Genotype among Regular Blood Donors in Sarawak'. I declare that this thesis is being submitted to University Science Malaysia (USM) for the purpose of the award of Master of Science in Transfusion Science. This dissertation is the result of my own research under the supervision of Dr. Abdul Rahim Hussein and Dr. Mohammad Masrin Bin Md Zahrin except as cited in the references. The dissertation has been accepted for the study performed and is not concurrently submitted in candidature of any other degree.

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KONG HUNG CHUO

P-IPM0022/14

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LIST OF ABBREVIATIONS AND SYMBOLS

AABB	American Association of Blood Banks
AHG	Anti-human Globulin
AMDI	Advanced Medical and Dental Institute
Anti	Antibody
BB	Blood Bank
BBIS	Blood Bank Information System
Cl	Confidence Interval
Ctl	Control
CPD	Citrate-Phosphate-Dextrose
DAT	Direct Anti-globulin Testing
Df	Degree of freedom
EC	Expected Count
EDTA	Ethylenediamine tetra-acetic acid
et al.	and others
etc	et cetera
HA	Hemolytic Anemia
HDFN	Hemolytic Disease of the Fetus and Newborn
HgbS	Sickle-cell Hemoglobin
HIV	Human Immunodeficiency Virus
HTR	Haemolytic Transfusion Reaction
HUS	Hospital Umum Sarawak
IAT	Indirect Anti-globulin Test
ID-Card	Identification Card
i.e.	id est

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IS	Immediate Spin
ISBT	International Society of Blood Transfusion
kD	Kilodalton
MOH	Ministry of Health
NBC	National Blood Centre
p	Probability
PCR	Polymerase Chain Reaction
PIN	Personal Identification Number
QC	Quality Control
RBCs	Red Blood Cells
RhoGAM	RhoD Immune Globulin
Rhlg	Rh Immunoglobulin
rpm	Revolutions per minute
SCA	Sickle Cell Anemia
SD	Standard Deviation
SGH	Sarawak General Hospital
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
SMLT	Senior Medical Laboratory Technologist
USM	University Science Malaysia
χ^2	Pearson Chi-square test

^{0}C	degree Celsius
µl	microliter
min	minute
ml	millilitre
%	percentage
/	or
>	greater than
<	less than
≥	greater than/equal to
=	equality
±	plus minus

ABSTRAK

Sejak penemuan awal antigen D pada tahun 1939, 50 antigen berkaitan telah diberikan kepada sistem kumpulan darah Rh oleh Persatuan Antarabangsa Transfusi Darah (ISBT). Di antaranya lima antigen utama iaitu D, C, c, E dan e adalah yang paling penting. Ujian antigen RhD telahpun diwajibkan sebelum proses mengeluarkan darah yang serasi dalam perubatan transfusi disebabkan oleh immunogenisitas antigen D bersama-sama dengan kumpulan darah ABO. Uniknya Sarawak dengan berbilang kaum dan perkahwinan campur mempunyai kesan yang besar ke arah taburan Rh fenotip dan genotip paling mungkin di kalangan penderma darah tetap di Sarawak. Namun sehingga masa kini, tiada sebarang kajian tentang antigen utama Rh, fenotip dan genotip paling mungkin yang telah dilakukan di kalangan penduduk Sarawak. Kajian ini telah dijalankan di Tabung Darah, Jabatan Patologi, Hospital Umum Sarawak (HUS) dari bulan Januari hingga Februari 2015 yang melibatkan 155 Iban, 128 Cina, 123 Melayu, 43 Bidayuh, 32 Orang Ulu dan 27 Melanau. Sampel darah daripada 508 subjek telah diuji untuk kumpulan darah ABO dengan menggunakan kaedah aglutinasi tiub dan lima antigen utama Rh sistem kumpulan darah diuji melalui kaedah kad gel. Untuk sistem kumpulan darah ABO, didapati kumpulan darah O (37.80%) dan B (32.28%) adalah kumpulan darah yang paling biasa ditemui di kalangan penduduk Sarawak. Kejadian RhD adalah 99.80% dan 0.20% sampel negatif untuk antigen D. Insiden antigen Rh yang lain seperti C, E, c dan e adalah sebanyak 95.87%, 31.89%, 35.43% dan 97.05% masing-masing. Antigen yang paling kerap ditemui di antara lima antigen utama adalah RhD dan yang paling jarang dijumpai adalah antigen E. Fenotip yang paling biasa dijumpai dalam RhD sampel positif adalah DCCee (60.24%) dan di RhD sampel negatif adalah dccee (0.20%). Genotip paling mungkin mengikut urutan kekerapan adalah

CDe/CDe (R_1R_1) – 60.24%, CDe/cDE (R_1R_2) – 23.23% dan CDe/cde (R_1r) – 7.28%. Sementara itu, genotip kemungkinan yang paling kerap ditemui adalah cde/cde (rr) dalam sampel Rh negatif. Fenotip kurang biasa termasuk CDE/CDE (R_zR_z) dan cDe/cde (R_0r) – 0.20% setiap satu, telah ditemui di kalangan subjek Iban dalam kajian ini. Fenotip luar biasa yang jarang ditemui iaitu -D-/-D- (Rh17) telahpun dikesan dalam salah seorang penderma darah Bidayuh (0.20%). Kajian ini juga membuat kesimpulan bahawa gen Rh dan fenotip adalah berbeza-beza secara meluas di kalangan bangsa berlainan dan sempadan geografi. Secara ringkasnya, kajian ini telah berjaya mewujudkan satu sistem pangkalan data dan membandingkan taburan antigen Rh, fenotip dan genotip paling mungkin untuk enam kumpulan etnik utama di Sarawak. Sistem pangkalan data yang ditubuhkan daripada kajian ini akan memberikan manfaat yang besar dan amat penting untuk perkhidmatan pemindahan darah tempatan pada masa akan datang.

ABSTRACT

Since the initial discovery of the D antigen in 1939, 50 related antigens have been assigned to the Rh blood group system by the International Society of Blood Transfusion (ISBT), among which the antigens D, C, c, E and e are the most essential. RhD antigen testing was made compulsory before issuing a compatible blood in transfusion medicine due to its potent immunogenicity along with ABO grouping. Uniquely Sarawak with multi-racial and intermarriage have substantial effect towards the distribution of Rh phenotype and most probable genotype among the regular blood donors in Sarawak. Until now, there is no study presented in major Rh antigens, its phenotype and most probable genotype in the Sarawak's population. This study was carried out at Blood Bank, Department of Pathology, Sarawak General Hospital from January to February 2015 involving 155 Iban, 128 Chinese, 123 Malay, 43 Bidayuh, 32 Orang Ulu and 27 Melanau. Blood samples collected from 508 subjects were tested for ABO blood group by using the tube agglutination method and five major Rh antigens via gel technology. For the ABO system, blood group O (37.80%) and B (32.28%) are the most common blood group found among the Sarawak's population. Incidence of RhD was 99.80% and only 0.2% samples negative for D antigen. The incidence of other Rh antigens such as C, E, c and e was 95.87%, 31.89%, 35.43% and 97.05% correspondingly. Antigen D was the most frequent antigen amongst five major antigens and the least common was antigen E. Most common phenotype in D-positive samples was DCCee (60.24%) and in D-negative was dccee (0.20%). Most probable genotype in order of frequency was CDe/CDe (R_1R_1) - 60.24%, CDe/cDE (R_1R_2) - 23.23% and CDe/cde (R_1r) - 7.28%. Meanwhile, the most frequent probable genotype was cde/cde (rr) in Rh negative samples. Less common phenotypes found in this study were

CDE/CDE (R_zR_z) and cDe/cde (R_0r) - 0.20% each, which was predominant in Iban subjects. Unusual rare phenotype –D-/-D- (Rh17) was revealed in one of the Bidayuh subject (0.20%). This present study concluded that Rh genes and phenotypes vary widely across races and geographical boundaries. In conclusion, this study has managed to establish the database and compare the distribution of Rh antigens, its phenotype and most probable genotype among six major ethnic groups in Sarawak. The established database will be of great benefit and importance in the future local blood transfusion service.

CHAPTER 1

INTRODUCTION

1.1 The Rh blood group system and problem

Up to now, ABO and Rh blood group systems are the most vital elements in transfusion science and medicine among other blood group system and antigens discovered (Sharma et al., 2010). Since the early discovery of the D antigen in 1939, 50 related antigens have been assigned to the Rh system by the International Society of Blood Transfusion (ISBT). The major, and the most important antigens are D, C, c, E, and e are which account for most clinical transfusion issues (Blaney and Howard, 2013).

The presence or absence of the D antigen determines whether an individual is Rh positive and Rh negative refer to; more accurate terms are D-positive and D-negative. Rh blood group systems play an important role in blood transfusion practice. Additionally, the D antigen has serious clinical effects, causing hemolytic disease of the fetus and newborn (HDFN) as prevention is the best approach to the management of this disorder. In 1940, Levine and Stetson had linked the cause of HDFN to the Rh blood group due to the similarity of this antibody obtained from guinea pigs and rabbits immunized with rhesus monkey red cells, and so the blood group system was named Rh for rhesus monkey (McCullough, 2011).

In order to elucidate the inheritance and to classify the most complex Rh blood group system, four theories have been recommended. There are two nomenclatures that are used interchangeably: the Weiner 1939 and Fisher-Race 1940. Two added systems: Rosenfield 1960 and International Society of Blood Transfusion (ISBT) were established because of a need for a universal language compatible with computers (Daniels et al., 1995).

The Malaysian blood donor population is composed of three major ethnic groups (Malay, Chinese and Indian) and other minor ethnic groups (e.g. native people from East Malaysia). As the donor population is quite heterogeneous, the distribution of Rh phenotypes and genotypes to diverge as well. Known as Bumi Kenyalang, Sarawak is one of two Malaysian states on the island of Borneo. It is the most diverse, in terms of its physical features, traditional cultures, population as well as its modern history (Douglas and Douglas, 1999).

The population in Sarawak is very varied; consisting of 27 ethnic groups and 40 sub-ethnic groups, each with its own different language, culture and custom, and lifestyle system. This unique characteristic makes Sarawak demography very distinct compared to its peninsular counterpart (Kenyalang and Negeri). Intermarriage between ethnic groups in Sarawak has been common for generations. But until lately most of the mixed marriages have been between indigenous groups with parallel backgrounds.

Undoubtedly, multi-racial and intermarriage have significant effect towards the distribution of Rh antigens, its phenotype or most probable genotype among the regular blood donors in Sarawak based on unpublished BBIS (Blood Bank Information System) Enteral DarahLink system. As yet, there is no published data in prevalence of Rh antigens, its phenotype or

most probable genotype among the population of Sarawak. It is therefore timely to conduct a related study to establish local database on Rh phenotype/genotype among Sarawak's population.

In this study, prevalence of Rh antigens, phenotype or probable genotype of the regular blood donors will only be done confined to Sarawak General Hospital (SGH) and Hospital Miri due to the restricted time frame and budget constraint. This research is going to be conducted in SGH because of its status as a regional referral centre, and provide a wide range of inpatient and outpatient care in addition to the high number of blood donations that it receive daily. Hospital Miri is a secondary referral hospital in the northern region of Sarawak.

The established database from this study will be of great benefit and importance in the future local blood transfusion service. This is particularly important in instances where blood for patients with certain antibody requiring urgent transfusion can be quickly phenotyped from prospective donors from the ethnic group with the highest prevalence of that particular blood group. Apart from that, established database from the study can be used to support the National Donor Data Registry Program which will be held by National Blood Centre (NBC). Well established data which will be reported yearly is important to help more people who have difficulty in finding a matching blood donor when urgent blood transfusion is obligatory. The growth in numbers of voluntary blood donors, plus the diversity of races and ethnicities on the registry, is improving all patients' chances of finding the matched blood they need.

1.2 Objectives

General objective: To determine the prevalence of Rh antigens, its phenotype or most probable genotype among regular blood donors in Sarawak

Specific objectives:

i. To classify the frequency of Rh antigens, phenotype or most probable genotype based on 6 major ethnic groups in Sarawak's population

ii. To assess the D--/D-- among regular blood donors in SGH and Hospital Miri including 6 major ethnic groups in Sarawak's population

iii. To compare the prevalence of Rh antigens, phenotype or probable genotype among regular blood donors in Sarawak's population with major ethnic groups (Malay, Chinese and India) in other studies

1.3 Hypothesis

- Null hypothesis, H_0: There is no difference between Sarawak regular blood donors and Peninsular Malaysian in term of Rh antigens, its phenotype or probable genotype

- Alternative hypothesis, H_1: Regular blood donors in Sarawak have different Rh antigens, its phenotype or probable genotype compared to its peninsular counterpart due to multi-racial, multi-ethnics and intermarriage in Sarawak

1.4 Literature review

1.4.1 The Rh blood group system

The highly complex and polymorphic Rh system is the second most essential red cell antigen system after the ABO system. At present, the Rh blood group system is comprised of some 50 different blood-group antigens. Five are of particular importance including antigens D, C, c, E, and e (Blaney and Howard, 2013). The presence or absence of the D antigen determines whether an individual is Rh positive or Rh negative. An individual whose red cells express the D antigen are termed Rh positive and those whose red cells do not express the D antigen are termed Rh negative.

C, c, E and e are the other four principal antigens identified that seemed to be embedded in the cell membrane bilayer in unique configurations and became part of the Rh blood group system. These five major antigens, along with their corresponding antibodies, account for most of the Rh-related clinical transfusion issues. In contrast to the ABO blood group, Rh blood group system antibodies are usually made by exposure to Rh antigens through blood transfusion or during pregnancy in women (Blaney and Howard, 2013).

Clinically, the Rh factor, like ABO factors, can cause potential clinical effects, initiating an adverse transfusion reactions or hemolytic disease of the fetus and newborn (HDFN). The greatest problem with the Rh blood group occurs when a mother's blood type conflicts with that of her newborn child. In addition to the clinical importance of the Rh system, determination of Rh blood group has been useful in population genetic studies and

migration pattern as well as resolving medico-legal issues. Thus, it is essential to have information on the distribution of these blood groups in any population groups.

1.4.2 History of discoveries

The first case of Rh incompatibility was reported in 1939 by Levine and Stetson. They published their case of a woman who delivered a stillborn fetus affected by hemolytic disease of the newborn. She developed a severe transfusion reaction upon receiving a blood transfusion from her husband though both of them were of blood-type O. Further tests indicated that the woman had become immunized specifically to a factor that she lacked which her child had inherited from the father. However, no name was specified to this agglutinin when described for the first case report (Simon et al., 2011).

In early 1940, Levine and Stetson linked the cause of hemolytic disease of the newborn to the Rh blood group system. Rh was the name given to the system because of the similarity of this antibody to one made from stimulating guinea pigs and rabbits with rhesus monkey red cells (*Macaques mulatta*). This serum had a pattern of reactivity similar to that of Levine and Stetson's patient. Besides agglutinated the monkey red cells, this Rh antibody also agglutinated about 85% of white people red cells tested and was nonreactive with 15% (Smart and Armstrong, 2008). The population was characterized as Rh-positive or Rh-negative from this discovery.

Later work established that the Rh antibody made from the guinea pigs and rabbits was similar, but not identical, to the human Rh antibody. The Rh antibody specificity was actually directed toward another different red cell antigen. However, the nomenclature was established by that time, and this antigen was named LW in honor of the discoverers (Scott, 2004). The name of Rh blood group system had been established by then and was not changed.

1.4.3 Rh nomenclature

1.4.3.1 Fisher Race: CDE Terminology

The Fisher-Race system postulated three closely linked loci, each with its own gene and gene product. D gene is inherited at one locus, C or c are inherited at the second locus, and E or e genes are inherited at the third locus. Each parent contributes one haplotype or set of Rh genes. Each gene with the exception of 'd' codes for a specific antigen that can be detected on the red cell membrane. It is possible that 'd' is a silent allele, and no anti-d has been found so far (Smart and Armstrong, 2008). The 'd' is still sometimes written to denote the absence of the D antigen. According to the Fisher-Race theory, the order of the genes on the chromosome is DCE to represent the co-location of the C and E encoding on the RhCE gene accurately. However, it is often written alphabetically as CDE (Blaney and Howard, 2013). The Fisher-Race theory is most widely used in transfusion practice since they can fit easily with most of the serologic reaction obtained.

1.4.3.2 Wiener: Rh-Hr Terminology

In contrast to the Fisher-Race theory, Wiener system assumes the gene product was a single entity comprising a variable number of blood factors. Each parent contributes one Rh gene. Each gene product produced a multiple serologic specificities agglutinogen which could react with various antibodies. The gene complex or agglutinogen is made up of factors that are identifiable as separate antigens (Smart and Armstrong, 2008).

According to Wiener system, there are eight alleles: R^1, R^2, R^o, R^z, r', r'', r and r^y exist at the Rh gene locus. The gene codes a structure on the red cells called agglutinogen, which can be recognized by its factors. These factors are identified with the same antisera that agglutinate the D, C, c, E and e antigens which had been mentioned in the Fisher-Race system. The dissimilarity between the Wiener and Fisher-Race theories is the inheritance of the Rh blood group system on a single gene locus rather than three separate genes (Blaney and Howard, 2013).

Wiener terminology can be straightforwardly translated to Fisher-Race terminology when the following points are kept in mind: R is the same as D; r indicates no D antigen; the number 1 and the character ' denote C; and the number 2 and the character '' denote E (Blaney and Howard, 2013). Nevertheless, Wiener: Rh-Hr terminology is unwieldy for routine use due to its complexity.

1.4.3.3 Rosenfield: Numeric Terminology

Both the Fisher-Race and the Wiener terminologies are based on genetic concepts. The Rosenfield system was developed later to communicate phenotypic information more appropriate for computerized data entry. It does not address genetic information (Blaney and Howard, 2013).

In 1962, a third terminology for the Rh system was introduced by Rosenfield in which each antigen was assigned a number, and the serologic reactions were used. The phenotype of a cell is expressed by the Rh followed by a colon and the numbers corresponding to the tested antigens. If a red cell sample is negative for the D antigen, a minus sign is written before the number as Rh:−1 (minus one) (Smart and Armstrong, 2008). This numeric designation for Rh antigens is an anticipation of the computer era. The numeric terms are not far and wide used in the clinical laboratory, except for Rh17, Rh29, Rh32 and Rh33 (Simon et al., 2011).

1.4.3.4 International Society of Blood Transfusion (ISBT)

In an effort to develop and maintain standardized guidelines for blood group antigen and allele nomenclature, the ISBT, assigned a six-digit number to each specific blood group. The first three numbers symbolize the system and the remaining three characterize the antigen specificity. The assigned number of the Rh blood group system is 004 (Table 1.1) and the remaining three numbers correspond to the Rosenfield system. An ISBT alphanumeric designation similar to the Rosenfield terminology is used to mention to a specific antigen. The

term Rh is written in uppercase letters and the antigen number immediately follows the system designation. The ISBT number for the C antigen is 004002 and the ISBT symbol for C is RH2 (Blaney and Howard, 2013).

Table 1.1: Currently recognized antigens within blood group systems

System		Antigen number											
		001	002	003	004	005	006	007	008	009	010	011	012
001	ABO	A	B	A,B	A1	...							
002	MNS	M	N	S	s	U	He	Mi^a	M^c	Vw	Mur	M^g	Vr
003	P1PK	P1		P^k	NOR								
004	RH	D	C	E	c	e	f	Ce	C^w	C^x	V	E^w	G
005	LU	Lu^a	Lu^b	Lu3	Lu4	Lu5	Lu6	Lu7	Lu8	Lu9	...	Lu11	Lu12
006	KEL	K	k	Kp^a	Kp^b	Ku	Js^a	Js^b	...	...	Ul^a	K11	K12
007	LE	Le^a	Le^b	Le^{ab}	Le^{bH}	ALe^b	BLe^b						
008	FY	Fy^a	Fy^b	Fy3	...	Fy5	Fy6						
009	JK	Jk^a	Jk^b	Jk3									
010	DI	Di^a	Di^b	Wr^a	Wr^b	Wd^a	Rb^a	WARR	ELO	Wu	Bp^a	Mo^a	Hg^a
011	YT	Yt^a	Yt^b										
012	XG	Xg^a	CD99										
013	SC	Sc1	Sc2	Sc3	Rd	STAR	SCER	SCAN					
014	DO	Do^a	Do^b	Gy^a	Hy	Jo^a	DOYA	DOMR	DOLG				
015	CO	Co^a	Co^b	Co3	Co4								
016	LW	...	...	...	...	LW^a	LW^{ab}	LW^b					
017	CH/RG	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	WH				Rg1	Rg2

1.4.3.5 Determining the genotype from the phenotype

The term phenotype actually refers to the variety of antigens detectable on red cells using specific antisera; and the term genotype refers to the genetic makeup of an individual. Five antisera, used to determine the Rh blood group system phenotypes, include anti-D, anti-C, anti-c, anti-E and anti-e.

The genotype can be inferred precisely from the phenotype in many blood group systems. However, determination of genotype based on phenotype is not applicable in the Rh blood group system. In Rh system, any one antigen may derive from any of several genes, the genotype cannot be absolutely determined from the detecting antigens. It is necessary to do family studies or molecular testing to determine the genotype with more certainty, which are always unlikely. When the phenotype is known, the most probable genotype can be inferred based on the frequency of genes in a population (Table 1.2) (ME and C., 2004).

For example, if a red cell specimen is typed as D+, C+, E-, c+, e+, the phenotype (antigens detected on the red cells) would be CcDe. When the genotype is inferred generally in the white population, the most common D positive genotype is CDe/ce or R_1r. In the black population, the most probable genotype would be CDe/cDe or R_1R_0 because R_0 allele is more common than the r allele. Meanwhile, the most common D negative genotype is ce/ce or rr (Blaney and Howard, 2013, Knight, 2012).

Table 1.2: The most probable phenotype (% occurrence) based on gene frequency in the population

Phenotype (alternative)	*Caucasians*	*Blacks*	*Asians*	*D antigen copy*
D-positive				
R_1R_1 (R_1r')	18.5	2.0	51.8	14 500–19 300
R_2R_2 (R_2r'')	2.3	0.2	4.4	15 800–33 300
R_1r (R_1R_0; R_0r')	34.9	21.0	8.5	9900–14 600
R_2r (R_2R_0; R_0r'')	11.8	18.6	2.5	14 000–16 000
R_0r (R_0R_0)	2.1	45.8	0.3	12 000–20 000
R_zR_z (R_zr_y)	0.01	Rare	Rare	
R_1R_z (R_2r'; R_1r_y)	0.2	Rare	1.4	
R_2R_z (R_2r''; R_2r_y)	0.1	Rare	0.4	
R_1R_2 (R_1r''; R_2r'; R_zr; R_0Rz; R_0r_y)	13.3	4.0	30.0	23 000–36 000
D-negative				
$r'r$	0.8	Rare	0.1	
$r'r'$	Rare	Rare	0.1	
$r''r$	0.9	Rare	Rare	
$r''r''$	Rare	Rare	Rare	
rr	15.1	6.8	0.1	
$r'r''$ (r_yr)	0.05	Rare	Rare	
$r'r_y$; $r''r_y$; r_yr_y	Rare	Rare	Rare	
$r^{'S}r$	0	1–2	0	

Null: Rh_{null}
Unusual: Rh_{mod}; many variants

1.4.4 Rh system antigens

A major step forward in understanding the Rh system was the isolation of Rh antigen containing components of the red cell membrane (Cartron, 1999). The D antigen is a 30-kD erythrocyte membrane protein which is associated with the red cell membrane skeleton. The transmembrane proteins probably traverse the membrane several times, which structure suggests that they are ion channels. Rh antigens D, C, c, E and e are encoded by two adjacent gene loci, namely RHD and RHCE. The RHD gene encodes the RhD protein with the D antigen and the RHCE gene encodes both the Cc and Ee polypeptides. There are four

alleles at this locus: RHCE, RHCe, RhcE and Rhce (Figure 1.1). D-negative individuals do not have the RHD gene and thus that RhD polypeptide. This explains why there is no 'd' antigen (McCullough, 2011, Daniels, 2008).

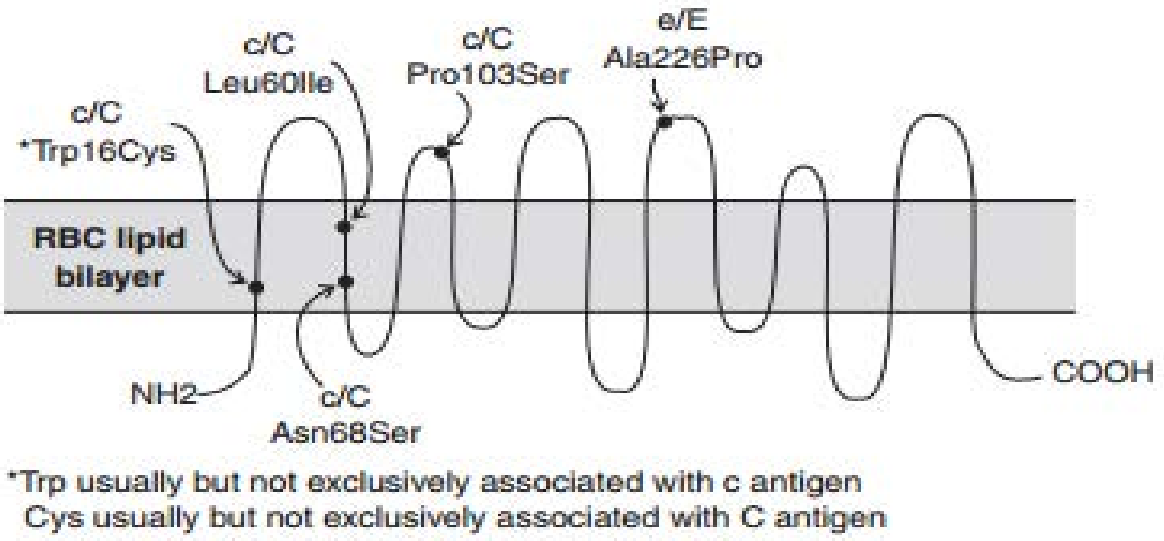

Figure 1.1: Molecular basis of RHCE antigens

1.4.4.1 D antigen

The D antigen is a potent immunogen. An immunogen is any antigen that is capable to elicit an immune response instead of immunological tolerance. From the clinical viewpoint, the D antigen is the most significant blood group polymorphism. Up to 85% of Rh-negative individuals will produce an antibody with anti-D specificity if given an Rh-positive blood. For that reason, a D-negative patient should receive D-negative RBC unit when blood transfusion is indicated (Kelton et al., 1984). Routine typing for C and E antigens is not performed as these antigens are not as immunogenic as D.

1.4.4.1.1 Weak D

Most red cells can be typed directly for the D antigen with certain anti-D reagents. Although the antibody to D is typically of the IgG class, reagent manufacturers have developed monoclonal anti-D antisera that can be used concomitantly with anti-A and anti-B testing.

Some individuals who inherit the quantitative weak D phenotype have red cells that react weakly, even though in most cases all immunogenic D epitopes are present (ME and C., 2004). Due to low avidity of the antibody, monoclonal anti-D could fail to react with weak D cells instead of complete loss of the epitope (Daniels, 2008).

Red cells that are negative for both anti-D reagent at immediate spin (IS) and after 37 °C incubation; but positive for D only by the IAT referred to as weak D phenotype. The D control tested at the IAT phase determines whether patient cells are already coated with IgG antibodies before testing. Reagent manufacturers specify the use of controls in their package inserts and it is vital to become familiar with these guidelines (Blaney and Howard, 2013). Weaker D expression can result from several different genetic circumstances.

1.4.4.1.2 Weak D: Genetic

Some RHD genes code for a weaker expression of the D antigen. The reason for the weakened expression of the D antigen in these individuals is unknown. The molecular basis of weakly expressed D gene is heterogenous and has been related with the presence of several point mutations in RHD (Simon et al., 2011). Hereditary weak D is characterized serologically as non-agglutinating RBCs antibodies with anti-D typing reagents. An indirect anti-globulin test (IAT) using anti-D is usually required to detect this form of D. This quantitative variation in the RHD gene is more common in blacks and is often part of the cDe (R_0) haplotype. Weak D donors are of greater concern because of their immunogenicity in stimulating D-negative recipients.

1.4.4.1.3 Weak D: Position effect

The Ce (r') gene paired with a CDe (R_1) or a cDe (R_0) gene weakens the expression of the D antigen due to the inheritance of C in the *trans* position to D. Genes inherited in *trans* are inherited on opposite chromosome (McCullough, 2011). This type of weak D antigen is usually detected by the indirect antiglobulin test (IAT) using anti-D without additional testing because of the increased sensitivity of monoclonal reagents. The occurrence of the Ce (r') gene is less than 2%.

Nevertheless, the expression of D is normal when the haplotype producing the weakly expressed D is partnered by another haplotype that encodes neither C nor D (dce or dcE) in a different family member (Daniels, 2008).

1.4.4.1.4 Weak D: Partial D

Weak D must be properly distinguished from partial D. A weak D reflects a quantitative variation of D antigen, while partial D reflects a qualitative variation of D antigen. The D antigen is a structure consisting of at least four parts. If one or more parts of the antigen is missing, the remaining antigen can be weakly expressed due to the altered protein structure. These individuals can produce an alloantibody to the part they are missing, if alloimmunized to D antigen. For this reason, partial D individuals should receive Rh negative blood when transfused (Kelton et al., 1984). Conversely, partial D individuals should be labeled as D-positive when they are donating their blood.

Older terminology classified partial D as D *mosaic* or D *variant*. Different part of D on the outer surface of the red cell membrane will determine the different phenotypes of partial D. In the past, only seven partial D phenotypes are established. These reaction patterns were then expanded to nine patterns which are classified by their parts or epitopes (Lomas et al., 1993).

Apparent gene conversion occurs between RHCE and RHD polypeptide results in chimeric Rh protein. Some replacements produce partial D phenotypes with strongly expressed D epitopes (e.g., D^{III}, D^{IVa}), while others have variable expression of the relevant epitopes (e.g., D^{Va}, D^{VII}). In contrast, weak partial D phenotypes (e.g., D^{VI} Type I, DAR) have extremely weakly expressed epitopes (ME and C., 2004).

1.4.4.1.5 Significance of testing for weak D

The AABB Standards entails testing for weak D on all donor red cells that do not directly agglutinate with anti-D antigens (Carson, 2011). Donor center typing procedures should detect and label weak D-positive units as D-positive and should be transfused only to D-positive recipients. An autocontrol is an important part of the weak D test, which verifies that a positive result is not due to red cells already coated with antibodies. It is an in vitro test which the RBCs and plasma of the patient are combined and tested under the same conditions. The test is invalid if red cells are coated with IgG antibodies before testing with anti-D at the anti-globulin phase and additional procedures are required to determine the D antigen status of the donor (Blaney and Howard, 2013).

Testing for weak D on potential transfusion recipient samples is not mandatory. Some facilities test for weak D on recipient samples. D-positive blood is provided if a weak D is detected. Although unlikely, a patient with a weak D because of the partial D phenotype can produce anti-D theoretically. The partial D phenotype is rare and with current monoclonal reagents usually does not require the anti-globulin test for detection.

From a clinical standpoint, weak D testing is performed on prenatal or antepartum evaluations and Rh immune globulin (RhIg) work-ups. RhIg is given when a D-negative mother has a baby that is D-positive. RhIg suppresses the immune response after exposure to D-positive fetal red cells and prevents the mother from producing anti-D. Though RhIg can help to prevent Rh sensitization, it cannot prevent damage to D-positive fetal red cells if the mother is sensitized to the Rh factor beforehand. RhIg given to D-negative mother at

28 weeks of gestation and again within 72 hours of delivery of n D-positive infant has a success rate of greater than 99% (Obstetricians and Gynecologists, 1999).

Alloimmunisation occurs in D-negative mothers when exposed to D-positive red cells during pregnancy and in D-negative individuals following blood transfusions of D-positive blood. For this reason, determination of strong immunogenic RHD alleles is of utmost important in clinical practices (Rizzo et al., 2012).

1.4.4.2 Other Rh group antigens

The Rh blood group system is comprised of some 50 different antigens. Other than antigen D, C, c, E and e, other antigens are alternate variations produced by the RHCE and RHD genes. These antigens and corresponding antibodies are seldom encountered or have rare clinical relevance. Each antigen is assigned a number, and CEST or RH57 is the highest number given according to ISBT standardized numeric terminology. Actually it is not a correct reflection of the antigens encountered since many antigens such as Rh38 have been combined, others have been relocated to other groups or else removed (Ouchari et al., 2013).

1.4.4.2.1 Compound antigens

Examples of compound antigens in the Rh blood group system include the following:

- Rh6 (ce or f)
- Rh7 (Ce)
- Rh27 (cE)
- Rh22 (CE) (Blaney and Howard, 2013)

When certain genes code for an additional protein, a compound antigen is the additional antigen product formed. Let's say: when c and e are inherited as RHce, f antigen is expressed in addition to the c and e antigens and absent if the person does not inherit that particular allele. This means that the Rh haplotypes R_0 and r are f-positive, since both haplotypes comprise the RHce allele. Since most D-negative people carry the rr haplotype, f is present in the infinite majority of D-negative individuals. The f antigen is not really 'compound', in that it is not formed by the mere presence of c and e on the same red cell, but rather by the action of the Rhce allele that also encodes both c and e (Rudmann, 2005).

Antibodies to compound antigens are rarely encountered. If they are identified, locating antigen-negative units would require the use of common antisera such as anti-C, anti-c, anti-E and anti-e. When transfusion of RBCs are required, red cell units that are negative for one of the antigens creating the compound antigen can be securely transfused (Blaney and Howard, 2013).

1.4.4.2.2 G antigens

Genes that code for RhD, RhCe and RhCE also code for a particular amino acid which results in an antigen called G. Unlike the five major Rh antigens, an antibody to G reacts with cells that are either D-positive or C-positive. It simply cells that are negative for both the D and the C antigen are negative for the G antigen. An individual who carry either one of the RhD, RhCe and RhCE alleles will have G antigen (Blaney and Howard, 2013).

Biochemically, the G antigen results when serine is found at position 103. In cells that are G-, proline is found at amino acid 103. In routine antibody identification studies, anti-G appears to be anti-D and anti-C. Adsorption and elution studies using r' and R_0 cells are necessary in distinguishing anti-G, anti-D and anti-C antibodies (Rudmann, 2005).

Exposure of D- and C- people to D-, C+ blood through transfusion or pregnancy could stimulate anti-C and anti-G antibodies. From previous experience, the stimulated G is quite antigenic and therefore clinically significant. It is important to test all Rh negative donors with anti-G antibodies (Allen and Tippett, 1958).

1.4.4.3 Unusual phenotype

Unusual phenotypes in the Rh blood group system are seldom encountered in routine blood bank testing. Unusual phenotypes include cells that have undetectable Rh blood group system antigen expression. Understanding the inheritance patterns and cell characteristics of unusual phenotypes provides insight into the genetics and biochemistry of the system (Blaney and Howard, 2013).

1.4.4.3.1 D-Deletion phenotype

Individuals who have the D-deletion phenotype may produce an antibody that reacts as a single specificity (anti-Rh17) or separable specifications such as anti-e and anti-C. An individual who produces anti-Rh17 would require D-deleted RBC units if transfusions are necessary. It will be difficult to find compatible blood but must be used for transfusion without untoward effects. The D-deletion phenotype is written as -D- or D-- (Blaney and Howard, 2013).

To identified the RHD deletion site, the most direct approach would be polymerase chain reaction (PCR) amplification spanning the RHD deletion site. In 2000, a model of the RH gene locus, identified the RHD deletion site in the prevalent D-negative haplotypes in whites. PCR methods was then developed and proved for the discrimination of RHD^{+}/RHD^{+} and RHD^{+}/RHD^{-} individuals. In consequence, direct testing for the presence of the RHD deletion is now routinely feasible (Wagner and Flegel, 2000).

1.4.4.3.2 Rh_{null} and Rh_{mod} phenotype

Individuals who do not express any Rh antigens on their red cells are termed Rh_{null}. Rare individuals lack all the Rh antigens with frequency of approximately 1 in 6 million individuals. Cells that typed as Rh_{null} are associated with deficient of LW and Fy5 and show markedly decreased expression of S, s and U antigens. The red cells of these individuals have impaired sodium and potassium ion transport. This results in hemolytic anemia of varying severity characterized stomatocytes, spherocytes, increased RBC osmotic fragility and elevated sodium potassium ATPase activity (Cartron, 1999, Qureshi et al., 2010). Null phenotypes are apparently disadvantageous, associated with diseases or in capacitating conditions, either obligatory or under stress factors (Flegel, 2010).

The Rh_{null} phenotype can be produced from at least two distinct genetic mechanisms. The inheritance of the Rh_{null} phenotype can result from a regulator gene or an amorph gene. A regulator gene, RHAG is inherited on chromosome 6 and codes for thirtieth named blood group system. Although the RHAG blood group system does not carry any Rh antigens, its presence is essential for the expression of the Rh system antigens. The amorph type of Rh_{null} phenotype is less well understood. The RHD gene is deleted, and there is molecular change in RHCE gene, causing neither protein to be produced. Both genotypes result in the same clinical syndrome (Qureshi et al., 2010).

The Rh_{mod} phenotype is analogous to the regulator Rh_{null}. In Rh_{mod} phenotype, red cells lack most of their Rh antigen expression as a result of the inheritance of a modified RHAG gene. Hemolytic anemia is also a characteristic of Rh_{mod} phenotype (Blaney and Howard, 2013).

1.4.5 Prevalence of Rh haplotypes

There are eight possible common Rh gene haplotypes with their diverse frequency in certain populations (Table 1.3) (Knight, 2012). Note that the gene combination Dce (R_0) haplotypes is seen much more common in Black population than the other population, whereas the DCE (R_1) haplotype is much more common in Asian population rather than the other populations. Haplotype dce (r) is more frequent in white people (37%) than in black people (26%). D- as a whole is rare in the Asian population comparing to other populations.

Table 1.3: Eight common Rh haplotypes together with their frequencies in certain population

Haplotype	White %	Black %	Asian %
DCe (R_1)	42	17	70
DCE (R_z)	<0.01	<0.01	1
Dce (R_o)	4	44	3
DcE (R_2)	14	11	21
dCe (r')	2	2	2
dCE (r_y)	<0.01	<0.01	<0.01
dce (r)	37	26	3
dcE (r")	1	<0.01	<0.01

1.4.6 Inheritance of Rh

Inheritance of the Rh genes is relatively straightforward. Nonetheless, rather in the same way that the MS, Ms, NS and Ns antigens are passed on as a 'block', Ce, CE, ce and cE are also passed on as a 'block'. This is not surprising when one considers that the genes for the antigens are mapped so closely to one another, and the antigens are formed by amino acids on the same polypeptide. Although the RHD gene is a separate entity to the RHCE gene and the D antigen is on a separate polypeptide, RHD and RHCE are almost always passed on as entity (Knight, 2012).