A STUDY OF Rh POSITIVE PHENOTYPES / GENOTYPES FREQUENCY AMONG REGULAR BLOOD DONORS OF DIFFERENT ETHNIC GROUPS IN HOSPITAL USM, KELANTAN

BY

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CERTIFICATE

This is to certify that the dissertation entitled

"A Study Of

Rh Positive Phenotypes / Genotypes Frequency Among Regular

Blood Donors Of Different Ethnic Group In Hospital Usm, Kelantan"

is a bonafide record of research work done by

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TABLE OF CONTAIN

LIST OF TABLE

LIST OF FIGURES

ABSTRACT

This study was perform in the Transfusion Medicine Unit of HUSM among 200 individuals from two major ethnic groups of Malay and Chinese regular blood donors of both sexes and different of age groups. The aims of this study was to determine the frequency of Rh positive phenotypes and genotypes among regular blood donors of Hospital University Sains Malaysia (HUSM). The frequency of Rh genotypes were determined by standard serological techniques. In this study nine phenotypes were found to occur in the Malay and Chinese populations. In the Malay population (n=100), there were seven phenotypes detected. The commonest phenotype in the Malay population was *CCDDee (R1R1)* with the frequency of 62%. This is followed by *CcDDEe (R1R2) 19%, CcDdee (R1r) 8%, CCDDEe (R1Rz) 8%, ccDDEe (R2r) 3%, CcDDEE (R2Rz) 2%* respectively. The rarest phenotype in Malay population is *ccDdee (Ro r)* with the frequency of only *1%.* The findings were different with the Chinese population where the rarest phenotype is *CCDDEE (RzRz)* with the frequency of 1% which was not present in the Malay population, like wise the *ccDdee (Ro r)* phenotype, it not present in the Chinese population. This was followed by *ccDDEe (R2r) 2% , CcDdee (R1r) 4%, CCDDEe (R1Rz) 5%, and ccDDEE (R2R2) 9%* respectively. The *R2R2* phenotype was found to be present only in the Chinese population with quite high frequencies and was absent in the Malay population. The commonest phenotype in Chinese, *CCDDee (R1R1)* with the frequency of *55%* and followed by *CcDDEe (R1R2)* with frequency of *24%* respectively. This kind of study should be on going and should be also extended to other categories of blood donors among other ethnic groups in Malaysia in view of keeping proper record of regular blood donors.

ABSTRAK

Kajian ini dilakukan di Hospital Universiti Sains Malaysia (HUSM) dengan subjek yang terdiri daripada 200 orang penderma darah dari dua kumpulan etnik iaitu Melayu dan Cina. Mereka ini terdiri daripada pelbagai peringkat umur dan kedua jantina. Matlamat atau objektif kajian ini dijalankan adalah untuk mengenalpasti frekuensi genotip dan fenotip Rh positif di kalangan penderma darah di HUSM. Frequensi genotip dan fenotip Rh positif ini ditentukan dengan menggunakan teknik ujian serologi. Daripada keputusan ujian yang telah dijalankan, sembilan fenotip telah dijumpai berada di kalangan populasi ini. Di dalam populasi Melayu (n=100), tujuh fenotip telah pun dikenal pasti. Fenotip yang paling kerap dijumpai adalah *CCDDee (R1R1)* dengan frekuensi *62%.* Ini diikuti dengan *CcDDEe (R1R2)19%, CcDdee (R1r) 8%, CCDDEe (R1Rz) 8%, ccDDEe (R2r) 3%, CcDDEE (R2Rz) 2%* dan fenotip yang paling jarang dijumpai di kalangan populasi Melayu ini adalah *ccDdee (Ro r)* dengan frekuensi *1%.* Penemuan ini amat berbeza dengan populasi Cina yang fenotip paling jarang dijumpai adalah *CCDDEE (RzRz)* dengan frekuensi *1%.* Fenotip ini tidak dijumpai pada populasi Melayu. Fenotip yang jarang di jumpai ini di ikuti dengan *ccDDEe (R2r) 2% , CcDdee (R1r) 4%, CCDDEe (R1Rz) 5%, and ccDDEE (R2R2) 9%* .Fenotip *ccDDEE (R2R2)* di jumpai hanya pada populasi Cina dengan frekuensi yang paling tinggi dikalangan fenotip yang jarang dijumpai. Bagi populasi Cina, fenotip yang paling kerap adalah sama dengan populasi Melayu iaitu *CCDDee (R1R1*) dengan frekuensi *55%* dan *CcDDEe (R1R2)* dengan frekuensi *24%*.. Kajian ini seharusnya diteruskan dan diperluaskan kepada beberapa katogori umur dan jantina dan dilakukan pada penderma darah dari pelbagai etnik lain di Malaysia untuk melengkapkan rekod dikalangan penderma tetap.

1.0 INTRODUCTION

An adult human has about 4-6 liters of blood circulating in the body. Among others, blood transports oxygen to various part of the body. Blood consist of several types of cells floating around in fluid, called plasma. The red blood cell contains hemoglobin. A protein that binds oxygen. Red blood cells transport oxygen to the body tissues and remove carbon dioxide from the tissues. White blood cells are for fighting of infection and for body defense mechanisms. Platelets help the blood to clot if there any injury to the blood capillaries or tissues and plasma, the fluid that contain salts and various kind of proteins.

Human blood groups are difference from one individual to the other due to the presence or absent of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cell and antibodies are in the plasma.

Each of individual has a difference type and combination of these antigens and antibodies are unique. The blood group that the individual belongs to depend on what they have inherited from their parent. More than 20 genetically determined blood group systems are known today. But ABO and Rh system are still the most important ones in blood transfusion practice. Not all blood groups are compatible with each other. Mixing of incompatible blood group may leads to blood clumping or agglutination. Nobel Laureate Karl Landsteiner was involved in the discovery of both the ABO and Rh blood groups.

1.1 ABO Blood Group System

There are different kinds of blood types in this system. They are groups A, B, AB, and O.

1.1.1 Blood group A

Individual with type A blood have A antigen on the red blood cell surface and produce B antibody in their serum or plasma.

1.1.2 Blood group B

An individual with type B blood have the opposition arrangement to type A with B antigen on the red blood cell surface and A antibody in their serum or plasma.

1.1.3 Blood group AB

Type AB people have on their red blood cells are both antigen A and antigen B and they do not produce any antibody against their substance in the serum. Therefore in theory a person with type AB blood can safely receive any ABO types blood and is called a 'universal recipient '. But unfortunately the blood of this AB person cannot be given to others except to the corresponding AB type subjects.

1.1.4 Blood type O

Blood type O people have red blood cell with neither A nor B antigens but produce antibody against both types of antigen. Because of this arrangement, in the old transfusion practice, type O blood can be safely given to any person with any ABO blood types. A person with blood type O is said to be a 'universal donor' but can only receive blood from the corresponding O type people. This concept are no longer accepted in modern transfusion practice where the blood will be given only based on the specific blood type and specific component that are required by the recipient only will be transfused.

1.2 Rh (Rhesus) system

Rh system is one of the most polymorphic and immunogenic system known in human. It is the most important compared to the other commonly utilize blood group system because of its capacity to cause sensitization, immune hemolysis and hemolytic disease of the newborn (HDN). Rh system can be subdivided into Rh positive and Rh negative based on the present or absent of D antigen. A more complete phenotype includes determination of the presence of Rh C, c, E and e antigens.

1.3 Other known blood group systems

- The MNSs System
- The P System
- The Kell System
- The Lewis System
- The Duffy System
- The Kidd (Jk) System
- The Lutheran (Lu) System

2.0 Rh SYSTEM

The Rh system is the most important to the other commonly used blood grouping systems. Rh blood types were discovered in 1940 by Karl Landsteiner and Alexander Wiener. Their experiment was to produce an antibody to the red cells of the Rhesus monkey in rabbits and guinea pigs, but they have discovered that not only did the antibody in the rodents' serum agglutinate the Rhesus monkey red cells, but it also agglutinated the red cells of 85% of the Caucasean population. If an individual's red cells were clumped together by this antiserum, they were said to have the Rhesus factor on their red cells (Rhesus positive: Rhesus +). If an individual's cells were not agglutinated by the antiserum, they were said to lack the Rhesus factor (Rhesus negative: Rhesus -). Chemically, Rhesus antigens are proteins (polypeptides). They are not glycosylated and are hydrophobic.

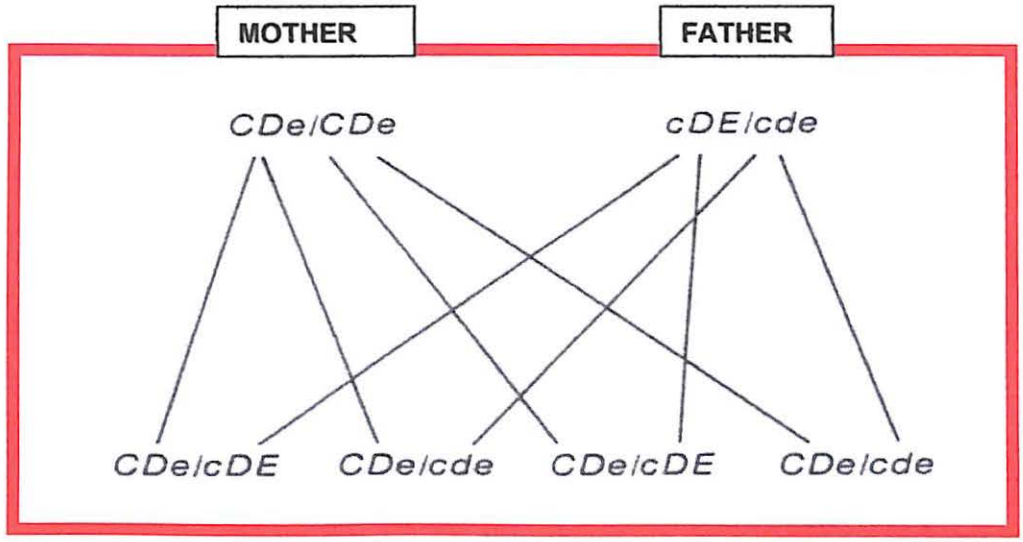
2.1 Inheritant

The Rh blood group system may be the most complex genetically of all the blood group systems since it involves approximately 40 different antigens on the surface of red cells that are controlled by 2 closely linked genes on chromosome 1. Rh (or the *D* antigen) is inherited on one locus (on the short arm of the first chromosome, 1p36.2-p34) with two alleles, of which individuals who are homozygous dominant (DD) or heterozygous (Dd) are Rh positive. Those who are homozygous recessive (dd) are Rh negative.

These genes code for a polypeptide (Rh antigens) on the red cell membrane. Rh negative individuals (*dd* genotype) were said to be absent of this gene thus cannot produce Rh

antigens, but this individual (Rh negative) can develop Rh antibodies in the blood plasma if he or she receives the blood from a person with Rh positive blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh positive blood can receive blood from a person with Rh negative blood without any problems. Rh antigens are only present on red cells and are well developed at birth. The antigens appear to develop very early in fetal life. After D antigen or Rh antigen, the most important antigens are C, c, E and e, which appear to be closely related to Rh system.

Figure 2.1: Example inherited Rhesus genotypes from maternal and paternal



(Compatibility testing, Ortho Diagnostic Raritant New Jersey 08869)

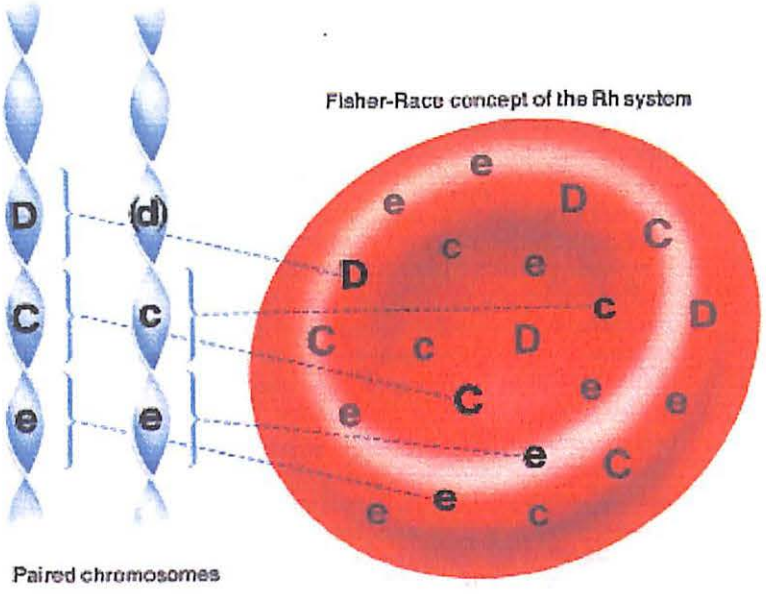
2.2 NOMENCLATURE

There are 4 different Rh nomenclatures exist. Two of them are best on the postulated genetic mechanism of the Rh system. Third is describing only the presence or absence of a given antigens. The forth is result of the effort of the International Society of Blood Transfusion (ISBT). The existing nomenclatures are Fisher-Race, Wiener, Rosenfield and ISBT. In routine works Fisher-Race and shorthand is mostly used.

2.2.1 FISHER-RACE

This theory is named after the two British workers who proposed it in 1940's. Fisher and Race were investigating the antigens found on human red blood cells. They postulated that the antigens of the system are produced by three closely linked sets of alleles. On chromosome of a homologous pair, each gene was responsible for producing an antigen on the red blood cell surface. They name the antigens of the system are D, d, C, c, E and e. no 'd' antigen has been found and it is considered as an amorph (silent alleles) or the absent of D antigen. The phenotype of a given red blood cells is defined by the presence or absence of D , C, c, E,and e. Each person inherited a set of Rh genes from each parent.

Figure 2.2.1 Fisher- Race concept of the Rh system



(D.Hermening Pittiglio,(1983). The Rh Blood Group System. In: Modern Blood Banking And Transfusion Practiced. , F.A,Davis Company,Philadelphia. pp 123-157.)

Rh genes are codominant. Each inherited gene expresses its corresponding antigen on the red blood cell. The combination of maternal and paternal halotypes determines one's genotype and phenotype. The term ' d ' indicates the absence of D antigen: the ' d' gene antigen does not exist.

2.2.2 WEINER: The Rh-Hr terminology

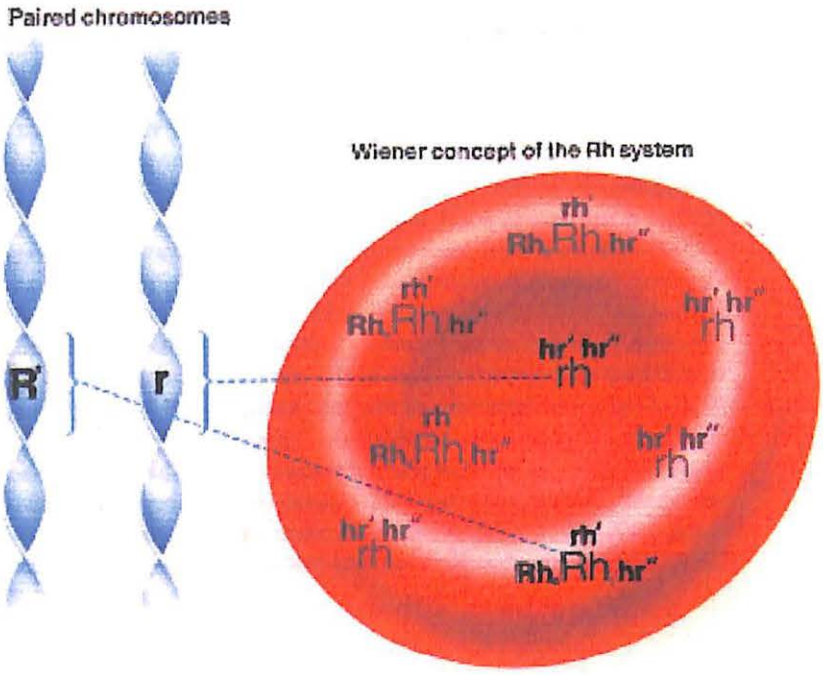
Another early theory was that of the American Dr. Alexander Wiener. Wiener's theory is that Rh inheritance is controlled as follows:

- There is one Rh locus at which occurs one Rh gene, but this gene has multiple alleles.

Wiener's believed that the gene responsible for defining Rh actually produce agglutinogen that contain a series of blood factors. According to the Rh-Hr terminology Rh gene produce at least three factors within an agglutinogen. The agglutinogen may be considered the phenotypic expression of the halotype. For example, one gene R^1 produces one agglutinogen (antigen) Rh_1 which is composed of three "factors": Rh', Rh (o), and hr". The three factors are analogous to C, D, and e respectively in the Fisher-Race nomenclature. Alleles of the one gene could be R^2 (cDE in Fisher-Race), R^o (cDe in Fisher-Race), etc. The *d* gene does not exist in Wiener's theory. Each factor is an antigen recognized by antibody. Antibody can recognized single or multiple factors (antigen)

Note: The main difference between the Fisher-Race and Wiener theories is that in the Fisher-Race theory has three closely linked loci, the Wiener theory has only one gene locus at which multiple alleles occur.

Figure 2.2.2 Wiener concept of the Rh system



(D.Hermening Pittiglio,(1983). The Rh Blood Group System. In: Modern Blood Banking And Transfusion Practiced. , F.A,Davis Company,Philadelphia. pp 123-157.)

Table 2.2 FISHER-RACE TERMINOLOGY

Gene	Agglutinogen	Blood Factors	Shorthand Designation	Fisher-Race Antigens
Rh^0	Rh_0	$Rh_0hr'hr''$	R_0	Dce
Rh^1	Rh_1	$Rh_0rh'hr''$	R_1	DCe
Rh^2	Rh_2	$Rh_0hr'rh''$	R_2	DcE
Rh^z	Rh_z	$Rh_0rh'rh''$	R_z	DCE
rh	rh	hr'hr''	r	dce
rh'	rh'	rh'hr''	r'	dCe
rh''	rh''	hr'rh''	r''	dcE
rh^y	rh_y	rh'rh''	r^y	dCE

(D.Hermening Pittiglio,(1983). The Rh Blood Group System. In: Modern Blood Banking And Transfusion Practiced. , F.A,Davis Company,Philadelphia. pp 123-157.)

Table 2.3 WEINER, FISHER-RACE and ROSENFIELD TERMINOLOGY

	Genotype			Frequency (%)
	Wiener	Fisher-Race	Rosenfield	(approx., White)
Common genotypes	R^1r	DCe/dce	Rh:1,2,-3,4,5	33
	R^1R^1	DCe/DCe	Rh:1,2,-3,-4,5	18
	rr	dce/dce	Rh:-1,-2,-3,4,5	15
	R^1R^2	DCe/DcE	Rh:1,2,3,4,5	11
	R^2r	DcE/dce	Rh:1,-2,3,4,5	9
	R^2R^2	DcE/DcE	Rh:1,-2,3,4,-5	2
Rarer genotypes	r^Yr	dCe/dce	Rh:-1,2,-3,4,5	1
	r^Yr^Y	dCe/dCe	Rh:-1,2,-3,-4,5	0.01
	$r^"r$	dcE/dce	Rh:-1,-2,3,4,5	1
	$r^"r^"$	dcE/dcE	Rh:-1,-2,3,4,-5	0.03
	R^0r	Dce/dce	Rh:1,-2,-3,4,5	2
	R^0R^0	Dce/Dce	Rh:1,-2,-3,4,5	0.1
	$r^{y"}r$	dCE/dce	Rh:-1,2,3,4,5	rare

(D.Hermening Pittiglio,(1983). The Rh Blood Group System. In: Modern Blood Banking And Transfusion Practiced. , F.A,Davis Company,Philadelphia. pp 123-157.)

2.2.3 ROSENFIELD: ALPHANUMERIC TERMINOLOGY

This system has no genetic basis but simply demonstrates the presence or absence of the antigen on the red blood cell. A minus sign preceding a number designates the absence of the antigen. If an antigen has not been typed for, its number will not appear in the sequence. An advantage of this nomenclature is that the red blood cell phenotype is thus succinctly described. For the five major antigens, D is assigned Rh1, C is Rh2, E is Rh3, c is Rh4 and e is Rh5. For red blood cell that typed: D+ C+ E+ c negative and e negative, the Rosenfield designation is Rh: 1,2,3, -4, -5.

2.3 CLINICAL SIGNIFICANT.

The Rh blood group system is the most complex of all blood group systems and comprises about more than 40 antigens and numerous phenotypes. From a clinical viewpoint, the D antigen is the most important blood group polymorphism encoded by a protein and very importance for transfusion medicine because of the high immunogenicity of its antigens. The Rh antigen, which is highly immunogenic, can cause:

- Severe transfusion reaction
- Hemolytic disease of the newborn
- Autoimmune hemolytic anemia.

2.3.1 SEVERE TRANSFUSION REACTION

The D antigen is the most immunogenic antigen outside the ABO system. When anti D is detected, a careful medical history will reveal red cell exposure through pregnancy or transfusion of product containing red cells. Circulating antibody appears within 120 days after a primary exposure and within 2-7 days after a secondary exposure. Rh mediated hemolytic transfusion reaction whether due to primary sensitization or secondary immunization; usually result in an extravascular destruction of immunoglobulin coated red blood cell. The transfusion recipient may have an explained fever, a mild bilirubin elevation, and decrease in hemoglobulin and heptoglobin.

2.3.2 HEMOLYTIC DISEASE OF THE NEWBORN

Hemolytic disease of the newborn occurs when the mother is Rh negative (dd) and the father is Rh positive (DD or Dd). Maternal antibodies can cross the placenta and destroy the fetal red blood cells. . After the first pregnancy, the mother develops antibodies against Rh positive red blood cells, which cross the placenta and hemolyses the blood of the second child. The risk increases with each pregnancy. Nutrients and the mother's antibodies regularly transfer across the placental boundary into the fetus, but her red blood cells usually do not (except in the case of an accidental rupture). Normally, anti-Rh antibodies do not exist in the first-time mother unless she has previously come in contact with Rh positive blood. Therefore, her antibodies are not likely to agglutinate the red blood cells of her Rh positive fetus.