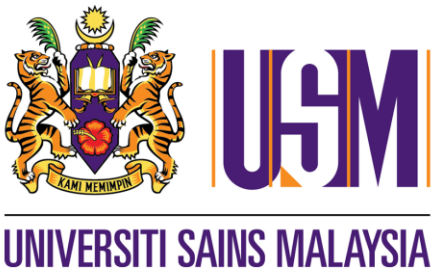
RED BLOOD CELL ALLOIMMUNIZATION AMONG RHESUS D NEGATIVE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

DR NOORAZLINA BINTI SHAARI

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (HEMATOLOGY)



UNIVERSITI SAINS MALAYSIA

2022

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ACKNOWLEDGEMENT

It was very satisfactory for me to be able to complete this dissertation. Firstly, I would like to express my gratefulness and praises to Allah S.W.T for giving me strength and ability to complete this dissertation. In the process of completing this project, I am indebted to many people of their helping and supports.

My deepest appreciation goes to Dr Mohd Nazri bin Hassan, my supervisor and lecturer from Hematology Department, Hospital USM for his continuous and unrequited advices, ideas, supervision, guidance and encouragement for me to conduct this research and successfully complete my dissertation.

I am also very grateful to my co-supervisor, Dr Marini bt Ramli, lecturer from Hematology Department, Hospital USM for the ideas and continuous support in finishing this dissertation.

Many thanks to blood bank staff, especially Puan Salamah binti Ahmad Syukri, senior MLT and Puan Faizatul Syima binti Abdul Manaf, Science Officer for their important work with regard to extracting data from the laboratory information system.

I would like to express my gratitude to Encik Kamarudin, senior staff in Unit Rekod Hospital USM in helping me in collecting the patients' documents. Finally, I would like to thank my family, especially my husband, Encik Mohd Fadli Mohd Zain for his unlimited support and understanding throughout this study.

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LIST OF ABBREVIATIONS

Anti	antibody
Anti-D	antibody D
Anti-Le	antibody Lewis
FMH	fetomaternal hemorrhage
Hb	hemoglobin
HDFN	hemolytic disease of fetus and newborn
HTR	hemolytic transfusion reaction
HUSM	Hospital USM
Ig	immunoglobulin
IgG	immunoglobulin G
Le	Lewis
LISS	low ionic strength saline
PDN	National Blood Centre of Malaysia
PC	pack cells
RBC	red blood cell
Rh	rhesus
RhD	rhesus D

ABSTRAK

Alloimunisasi Sel Darah Merah di kalangan Pesakit Rhesus D Negatif di Hospital Universiti Sains Malaysia.

Latar belakang: Pesakit Rhesus D (RhD) negatif dianggap sebagai pesakit yang mempunyai kumpulan darah yang jarang berlaku di negara Asia termasuk Malaysia. Oleh itu, aloimunisasi sel darah marah (SDM) di kalangan pesakit RhD negatif dianggap signifikan kerana ketersediaan dan kekurangan bekalan darah itu sendiri menjadikannya lebih sukar untuk mencari darah yang serasi. Tujuan kajian ini adalah untuk menentukan kelaziman, kekhususan aloantibodi, dan faktor-faktor berkaitan dengan aloimunisasi SDM di kalangan pesakit RhD negatif yang dimasukkan ke Hospital USM.

Bahan dan Kaedah: Ini adalah kajian keratan rentas yang melibatkan 562 pesakit RhD negatif yang dimasukkan ke Hospital Universiti Sains Malaysia (USM), dari Januari 2011 hingga Disember 2019. Data demografi, klinikal dan transfusi dikumpulkan dari rekod pesakit dan sistem maklumat makmal secara retrospektif. Ujian saringan dan pengenalpastian antibodi SDM menggunakan sistem mikrotip gel Diamed ID dijalankan ke atas sampel darah mengikut prosedur imunohematologi yang standard. Ujian Pearson Chi square dan Fisher Exact digunakan untuk analisis statistik dan nilai p kurang dari 0.05 dianggap signifikan.

Hasil: Purata umur pesakit adalah 41 tahun dengan majoriti adalah wanita (71.4%), Melayu (87.5%), dan kumpulan darah O (40.2%). Sebab utama kemasukan adalah disebabkan oleh kehamilan (48.5%) dan trauma (18.1%). Sebilangan besar pesakit adalah rr (67%). Kelaziman aloimunisasi SDM adalah 3.6% (n = 20). Sebilangan besar pesakit aloimunisasi

dengan aloantibodi tunggal (n = 18), dan tergolong dalam antibodi Rh (n = 16). Aloantibodi khusus yang paling biasa adalah anti-D (n = 14) diikuti oleh anti-Le (n = 4). Faktor yang berkaitan dengan aloimunisasi SDM adalah sejarah pemindahan darah (p=0.049) dan fenotip Rh (p=0.047).

Kesimpulan: Kadar aloimunisasi SDM pada pesakit RhD negatif adalah rendah. Walaupun begitu, adalah satu kewajipan untuk mengikuti standard protokol sejagat bagi mengenal pasti pesakit RhD negatif dan pemeriksaan antibodi terutama anti-D, yang merupakan signifikan secara klinikal.

ABSTRACT

Red Blood Cell Alloimmunization among Rhesus D Negative Patients in Hospital Universiti Sains Malaysia

Background: Rhesus D (RhD) negative patients are considered rare blood group in Asian country including Malaysia. Thus, the red blood cell (RBC) alloimmunization among RhD negative patient is considered significant because of the availability and rarity of the blood itself make it more difficult to find compatible blood. The aim of the study is to determine the prevalence, specificities of alloantibodies, and associated factors of RBC alloimmunization among RhD negative patients admitted to Hospital USM.

Materials and Methods: This cross-sectional study involved 562 RhD negative patients admitted to Hospital USM, from January 2011 to December 2019. Demographic, clinical and transfusion data were collected from patients record and laboratory information system retrospectively. The blood samples were subjected to the standard immuno-hematological procedure for RBC alloantibody screening and identification using Diamed ID gel microtyping system. Pearson's Chi square and Fisher Exact test were used for statistical analysis and p value less 0.05 is considered significant.

Result: The mean age of patients is 41 years old with majority were female (71.4%), Malay (87.5%), and blood group O (40.2%). The main reason of admission is due to pregnancy related (48.5%) and trauma (18.1%). Majority of the patients were rr (67%). The prevalence of RBC alloimmunization was 3.6% (n=20). Most of alloimmunized patients were with single alloantibody (n=18), and belong to rhesus antibody (n=16). The commonest alloantibody specificity is anti-D (n=14) followed by anti-Le (n=4). The

significant associated factors with RBC alloimmunization were history of blood transfusion (p=0.049) and Rh phenotype (p=0.047).

Conclusion: The rate of RBC alloimmunization in RhD negative patients was low. Nevertheless, it still mandatory that there should be one standard universal protocol to identify RhD negative patients and screening for antibody especially anti-D which is clinically significant.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The Rhesus (Rh) blood group is one of the most complex blood groups known in humans. Since its discovery, it has become the second most important after the ABO blood group in transfusion medicine. Furthermore, it has remained of primary importance in obstetrics, as the leading cause of hemolytic disease of the fetus and newborn (HDFN) (1). The Rh antigens are highly immunogenic compared to other RBC antigens. The immunogenicity of Rh antigens listed in the order of decreasing immunogenicity are D > c > E > C > e (2). The new, corrected ranking of antigen immunogenicity was: D > K> Jka > Lua > E > P1 > c > M > Leb > C > Lea > Fya > S (3).

In terms of RhD group which is the most immunogenic in Rh system, individuals can be divided into RhD positive and RhD negative, by the presence or absence of D antigen on RBC surface respectively (4, 5). RhD negative was prevalent in Caucasians (15%), but less common in Blacks (8%) and Asians (1%) (1). Meanwhile, based on study done by National Blood Centre of Malaysia found that the prevalence of RhD negative was around 2.5% from the study population, and about 0.5% is RhD negative based on average population in Malaysia (6). It can be considered as rare blood group in Malaysia. This has led to limited RhD negative blood products in our center. Alloantibody in RhD negative patients results in difficulty in finding the compatible lead to delay in blood transfusion to them.

RBC alloantibodies are the antibodies that develop in people who had been exposed to foreign RBC antigens either from a blood transfusion or fetomaternal hemorrhage (FMH) during pregnancy or delivery. These RBC alloantibodies can be either clinically significance or insignificance. Thus, identifying RBC alloantibody and their significance is crucial in transfusion practice as it provides information regarding the alloantibody that can cause harmful effects to the

recipients.

The Rh alloantibodies are IgG, which are clinically significant as they have been implicated in HDFN and hemolytic transfusion reactions (HTR). In particular, anti-D and anti-c are most frequently implicated in HDFN as they have the most (7). In the case of RhD negative individual, it will give rise to the development of anti-D if they encounter the D antigen on transfused RBCs (causing HTR) or on fetal RBCs (driving HDFN). For this reason, the determination of RhD status is routinely required in cases involving blood donors, transfusion recipients, and pregnancy.

To circumvent anti-D formation in RhD negative pregnant women, Rh immunoglobulin (eg: rhogum) is practically given at 28 weeks, at delivery, and cases of FMH. Anti-D alloimmunization has decreased tremendously because of strategy of matching RBC transfusions for RhD and because of the introduction of Rh immunoprophylaxis in the late 1960s (8). Till today, there are no prophylaxis available for prevention from other Rh (c, E, C, e) and other RBC antigen (Kell, Kidd, Duffy, MNS) alloimmunization. On top of that, the current transfusion practice is not mandatory to provide all RhD negative patient with a similar Rh or other RBC phenotype due to limited RhD negative blood product. Thus, the chance to develop other alloantibodies other than anti-D among RhD negative patient is feasible (8, 9). Meanwhile, other previous study worldwide which focused on child bearing women showed prevalence of RhD negative range from 7.7%-15.35%, whereas the prevalence of alloimmunized range from 2.2%-10%. The most common alloantibody was anti-D range 2.2%-6.46% (12, 13, 14).

This study aims to determine the prevalence and specificities of RBC alloantibody among RhD negative patients in this center. The association of selected factors with RBC alloimmunization among these subjects were also evaluated. To the best of our knowledge, this is the first study on RBC alloimmunization detection among RhD negative patients in Malaysia, which involved large

numbers of RhD patients. There was a previous study on RBC alloimmunization in Malaysia, focused mainly among transfused patients.

CHAPTER 2

OBJECTIVES OF THE STUDY

2.1 General Objective

To study RBC alloimmunization in RhD negative patients in Hospital USM (HUSM)

2.2 **Specific Objectives**

1. To determine the prevalence of RBC alloimmunization among RhD negative patients in Hospital USM.
2. To determine the RBC alloantibody specificities among alloimmunized RhD negative patients in Hospital USM.
3. To determine the association of selected factors (patient's factor- age, gender, race, medical condition- comorbid, pregnancy, history of transfusion, laboratory factor- blood group, Rh phenotype, type of transfused blood product) with RBC alloimmunization among RhD negative patients in Hospital USM.

CHAPTER 3

MANUSCRIPT

COVER PAGE

Type of article: ORIGINAL ARTICLE

Title: Red blood cell alloimmunization among Rhesus D negative patients in a Teaching Hospital Southeastern Malaysia

Running title: RBC alloimmunization among RhD negative

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Total page: 24

Word count: 1426 words

Source of support: GPSP (1001/ PPSP/807001)

Conflicts of interest: there are no conflicts of interest.

Abstract

Title: Red Blood Cell Alloimmunization among Rhesus D Negative Patients in a Teaching Hospital Southeastern Malaysia

Background: Rhesus D (RhD) negative patients are considered rare blood group in Asian country including Malaysia. Thus, the red blood cell (RBC) alloimmunization among RhD negative patient is considered significant because of the availability and rarity of the blood itself make it more difficult to find compatible blood. The aim of the study is to determine the prevalence, specificities of alloantibodies, and associated factors of RBC alloimmunization among RhD negative patients admitted to Hospital USM.

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(n=14) followed by anti-Le (n=4). The significant associated factors with RBC alloimmunization were history of blood transfusion (p=0.049) and Rh phenotype (p=0.047).

Conclusion: The rate of RBC alloimmunization in RhD negative patients was low. Nevertheless, it still mandatory that there should be one standard universal protocol to identify RhD negative patients and screening for antibody especially anti-D which is clinically significant.

Keywords: Rhesus D negative, alloantibody, alloimmunization, immunoprophylaxis

4.1 Introduction

The International Society of Blood Transfusion recognized 302 blood group antigens, most of which belong to 1 of 29 genetically discrete blood group systems [1]. These red blood cell (RBC) antigens have different prevalence among different populations and ethnic groups worldwide. The ABO and Rhesus (Rh) blood groups are among the most important blood groups system. The presence of Rh system (D, C, E, e, and c) was recognized in 1939, and it was confirmed within a few years [2]. An individual will be considered RhD positive if they possess D antigen on the RBC surface, while the absence of D antigen will be considered to be RhD negative. Rh system appears as the second most clinically important blood group system after ABO since their antibody was able to cause hemolytic disease of fetus and newborn (HDFN) and hemolytic transfusion reaction (HTR) [3]. RhD negative is considered a rare blood group in Malaysia since the prevalence of RhD negative among the overall Malaysian population and blood donors is only 0.5% and 2.5%, respectively and this is much lower than in other countries [4].

RhD incompatibility can give a significant problem when the RhD negative mother is pregnant with RhD positive fetus or RhD negative patients was transfused with RhD positive blood that causes HDFN or HTR, respectively [3]. Prior to anti-D prophylaxis, the perinatal mortality rate from HDFN was approximately 40%-50%. Immunoprophylaxis has drastically reduced the cases of RhD-induced HDFN, where the rate of HDFN after anti-D prophylaxis implemented was reported as low as 0.04% rate [5]. Malaysia has a standard practice in managing RhD negative patients, including pregnant mothers, giving blood products or immunoprophylaxis during pregnancy and postnatal [6].

Besides anti-D, RhD negative patients are also potential to develop other Rh antibodies (anti-c, -C, -E and -e) and antibodies to other RBC antigens, which have the potential to be clinically

significant. These antibodies can facilitate and accelerate destruction of RBC carrying the corresponding antigen and causing HDFN and HTR. There is no previous study on the prevalence of RBC alloimmunization in Malaysia involving many RhD negative patients. Two local studies reported on RBC alloimmunization, but the number of RhD negative patients involved were minimal. One local study involving pregnant women reported that three out of 20 RhD negative pregnant women were alloimmunized [7]. Another study involving transfused patients reported that only one RhD negative patient was alloimmunized [8]. Thus, the study aims to determine the prevalence and associated factors of RBC alloimmunization among RhD negative patients admitted to our center. We hope this study can give better understanding regarding the importance of antibody screening for RhD negative patients in relation to blood transfusion therapy and pregnancy.

4.2 Methods

This cross-sectional study was conducted at Hospital Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, involving RhD negative patients admitted from January 2011 to December 2019. The study was approved by the Human Research Ethics Committee USM (HREC) using protocol code USM/JEPem/19120947. A total of 562 RhD negative patients were included in this study. Those patients who were less than 12 years old were excluded from this study. Patients sociodemographic (age, race, gender, occupation), clinical data (the reason for admission, history of anti-D immunoglobulin prophylaxis), and laboratory data (ABO blood grouping, rhesus phenotype, transfusion history, type of blood product transfused, antibody screening, identification, and antibody specificity) were included for analysis. The RBC alloantibody can be either clinically significant or insignificant. A clinically significant alloantibody is defined as an antibody that can cause shortened survival of RBCs, leading to HDFN or HTR [9]. Examples of

alloantibodies classified under clinically significant are antibodies towards Rhesus, Kidd and Duffy antigen groups.

The blood samples were subjected to the standard immunohematological procedure for RBC antibody screening and identification using the Diamed ID gel microtyping system. A three-cell screening panel (Diamed ID- Dia cell Asia) from Switzerland was used. Sample with positive antibody screening were proceeded with antibody identification using an eleven-cell panel (Diamed ID-Dia panel, including the enzyme-treated cells (papain) at 37^{o}C and AHG phase) and antibody titer. Antibody titration was determined by preparing serial doubling dilutions of plasma and testing them by IAT using reagent red cells showing heterozygous expression of the corresponding antigen(s). RBC phenotyping of the respected antibodies specificity was done after antibody identification to confirm the underlying alloantibodies. Among those with anti-D specificity, to differentiate between immune anti-D from the passive immunization by anti-D immunoglobulin, we considered immune anti-D based on these criteria:

a) No history of recent anti-D prophylaxis given on this current sample

b) Anti-D titer >1:8 despite on recent anti-D prophylaxis [10, 11]

c) Increasing anti-D titer after follow-up [12]

Data was analyzed using Statistical Package for Social Sciences (SPSS) Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Mean and standard deviations were calculated for numerical statistical analysis, descriptive data expressed as a percentage for categorical data while Pearson's Chi-square and Fisher Exact test were used for association in categorical data. The prevalence of RBC alloimmunization and their specificities were described using descriptive statistics.

4.3 Results

A total of 562 RhD negative patients were included in this study. The mean age of the patients is 41 (16.7) years old, with the majority were between 21-40 years. Most of the patients were Malay (84.0%), admitted due to pregnancy-related (48.6%), blood group O (40.2%), and predominantly were rr phenotype (64.1%). The majority of them did not have any history of transfusion (85.6%). For those with transfusion history, 30 out of 81 (37.0%) patients have a history of transfusions with RhD positive blood products. The details on descriptive data are illustrated in Table 1.

Prevalence and specificity of RBC alloantibody among RhD negative patients

Among 562 patients, 30.2% (170) were positive for antibody screening. Out of positive antibody screening, only 20 patients were alloimmunized. Patients with positive antibody screening due to passive immunization by anti-D immunoglobulin (n=150) were considered non-alloimmunized. Thus, the prevalence of RBC alloimmunization among RhD negative was 3.6% (20 out of 562 patients) (Table 2).

Most of the alloimmunized patients were with single alloantibody (n=20) and belonged to rhesus antibody (n=16), the clinically significant antibody. The commonest alloantibody specificity was anti-D (n=14) followed by anti-Le (n=4), anti-C (n=1) and anti-G (n=1), as described in Table 3. The rate of clinically significant antibodies among alloimmunized RhD patients was 80.0% (16 out 20 patients).

The details of clinical data for 20 alloimmunized RhD negative patients are summarized in Table 4. The majority of them had with anti-D alloimmunization (n=14), female (n=17), was admitted due to pregnancy-related (n=14) and with no history of transfusion (n=15). From this study, noted

that only patients with rr (n=17) and r'r (n=3) phenotypes were alloimmunized. Surprisingly, ten of the patients were alloimmunized with anti-D despite receiving anti-D immunoglobulin prophylaxis, either during pregnancy (n=7) or after RhD positive blood product transfusion (n=3). Seven patients who had a transfusion history developed RBC alloantibody. Of these patients, four patients had a history of transfusion with RhD positive cellular blood products and three female patients with anti-D alloimmunization had a very high anti-D titer; 1:1024, 1:512 and 1:256, respectively.

Factors associated with RBC alloimmunization in RhD negative patients

The factors associated with RBC alloimmunization among RhD negative patients that had been analyzed in this study include age, gender, history of transfusion, ethnicity, reasons of admission, blood group, Rh phenotype, and the number of transfusions. This study showed significant associations between transfusion history (p=0.049) and Rh phenotype (p=0.047) with RBC alloimmunization among RhD negative patients. There was no significant association of RBC alloimmunization with the other factors (Table 5).

Table 1 Demographic data and descriptive analysis of RhD negative patients (n = 562)

Variables	Total (n=562), n (%)	Alloimmunized (n=20), n (%)
Age (years)*	41 (16.7)	39 (mean)
12 – 20	33 (5.9)	0 (0.0)
21 – 40	312 (55.5)	15 (75.0)
41 – 60	132 (23.5)	2 (10.0)
> 60	85 (15.1)	3 (15.0)
Gender		
Male	180 (32.0)	3 (15.0)
Female	382 (68.0)	17 (85.0)
Ethnicity		
Malay	472 (84.0)	18 (90.0)
Chinese	16 (2.8)	0 (0.0)
Indian	25 (4.4)	1 (5.0)
Others	49 (8.7)	1 (5.0)
Reason of admission		
Trauma	105 (18.7)	2 (10.0)
Pregnancy related	273 (48.6)	14 (70.0)
Infection	68 (12.1)	0 (0.0)
Tumor	50 (8.9)	2 (10.0)
IHD	16 (2.8)	2 (10.0)
CKD	9 (1.6)	0 (0.0)
Surgical intervention	12 (2.1)	0 (0.0)
Stroke	5 (0.9)	0 (0.0)
Others	24 (4.3)	0 (0.0)

"(contd.)"

Blood Group		
O	226 (40.2)	6 (30.0)
A	167 (29.7)	3 (15.0)
B	125 (22.2)	8 (40.0)
AB	44 (7.8)	3 (15.0)
Rh Phenotype		
rr	360 (64.1)	17 (85.0)
r'r	144 (25.6)	3 (15.0)
r''r	33 (5.9)	0 (0.0)
r'r'	21 (3.7)	0 (0.0)
r'r''	2 (0.4)	0 (0.0)
r''r''	2 (0.4)	0 (0.0)
History of transfusion		
No	481 (85.6)	14 (70.0)
Yes	81 (14.4)	6 (30.0)
No. of transfusion (units) †		
< 10	76 (93.8)	5 (83.3)
≥ 10	5 (6.2)	1 (16.7)
RhD type of transfused product †		
RhD negative	51 (63.0)	2 (33.3)
RhD positive	30 (37.0)	4 (66.7)

*=mean (SD); †=among transfused patients (n=81 in total patients and n=6 in alloimmunized patients); O&G=obstetrics and gynecology; A&E=accident and emergency; IHD=ischemic heart disease; CKD=chronic kidney disease

Table 2 Prevalence of RBC alloimmunization among RhD negative patients (n=562)

Antibody screening	n (%)
Negative	392 (69.8)
Positive *	170 (30.2)
Alloimmunized	20
Passive alloimmunization due to anti-D Ig	150
Total	562 (100)

*=passive immunization by anti D Ig is considered non-alloimmunized; Ig=immunoglobulin.

Table 3 Specificity of RBC alloantibody among alloimmunized RhD patients (=20)

RBC alloantibody	n (%)	Clinically significant
Number		
Single	18 (90.0)	
Multiple	2 (10.0)	
Antibody specificity		
Single		
Anti-D	14 (70.0)	Yes
Anti-Le^b	2 (10.0)	No
Anti-C	1 (5.0)	Yes
Anti-G	1 (5.0)	Yes
Multiple		
Anti-Le^a & Anti-Le^b	2 (10.0)	No
Total	**20 (100)**	16 (yes), 4 (no)

Table 4: Details on the clinical data of alloimmunized RhD negative patients (n=20)

No.	Age (year)	Gender	Antibody	ABO, Rh	Parity	Reason of admission	Tx	RhD +ve product	anti-D Ig	Titer*
1	28	F	Anti-D	B, rr	G_3P_2	Delivery	No	-	Yes	1:16
2	31	F	Anti-D	B, rr	G_2P_1	Delivery	No	-	Yes	1:16
3	35	F	Anti-D	AB, rr	G_4P_3	Delivery	No	-	No	1:1024
4	34	F	Anti-D	O, rr	G_2P_1	Delivery	No	-	Yes	1:16
5	33	F	Anti-D	B, rr	G_4P_3	Delivery	Yes	No	Yes	1:64
6	40	F	Anti-D	B, rr	G_3P_2	Delivery	No	-	Yes	1:16
7	41	F	Anti-D	O, rr	G_3P_2	Delivery	No	-	Yes	1:16
8	29	F	Anti-D	B, rr	G_4P_3	Delivery	No	-	Yes	1:512
9	32	F	Anti-D	O, rr	G_1P_0	Delivery	No	-	Yes	1:16
10	40	F	Anti-D	O, r'r	G_2P_1	Delivery	No	-	Yes	1:16
11	62	F	Anti-D	O, rr	P_6	Trauma	Yes	Yes	Yes	1:64
12	32	M	Anti-D	O, rr	NA	Trauma	Yes	Yes[t]	No	1:64
13	58	M	Anti-D	B, r'r	NA	Colon ca	Yes	Yes[‡]	No	1:256
14	66	M	Anti-D	A, rr	NA	Gastric ca	Yes	Yes[§]	No	1:16
15	38	F	Anti-C	B, rr	G_4P_3	Delivery	No	-	Yes	1:2
16	30	F	Anti-G	AB, rr	G_4P_{2+1}	Delivery	No	-	Yes	1:4
17	67	F	Anti-Le^b	A, rr	Unknown	IHD	Yes	No	Unknown	ND
18	29	F	Anti-Le^b	A, rr	Nulliparous	Refractory HL	Yes	Yes[‖]	No	ND
19	25	F	Anti-Le^a, -Le^b	AB, r'r	G_1P_0	Delivery	No	-	Yes	ND
20	38	F	Anti-Le^a, -Le^b	B, rr	G_4P_{2+1}	Delivery	No	-	Yes	ND

F=female; M=male; +ve=positive; Tx=transfusion; Ig=immunoglobulin; NA=not applicable; ND=not done; HL=Hodgkin lymphoma; ca=cancer; *=antibody titer was done only for clinically significant antibody; [t]=2 pints pack cells; [‡]=13 units apheresis and 77 units random platelet; [§]=9 units random platelets, [‖]=2 units apheresis and 8 units random platelet

Table 5: Factors associated with RBC alloimmunization in RhD negative patients (n=562)

Independent variables	Alloimmunization		p value
	No	Yes	
	n (%)	n (%)	
Age (years)			
40 and below	331 (95.7)	15 (4.3)	0.209†
Above 40	211 (97.7)	5 (2.3)	
Gender			
Male	178 (98.3)	3 (1.7)	0.097†
Female	362 (95.0)	19 (5.0)	
Ethnicity			
Malay	452 (95.8)	18 (4.2)	0.755‡
Non-Malay	88 (97.8)	2 (2.2)	
Reason of admission			
Female			
Pregnancy	259 (94.9)	14 (5.1)	
Non pregnancy	106 (97.2)	3 (2.8)	0.415‡
Male			
Trauma	80 (98.0)	1 (1.2)	
Non trauma	97 (98.0)	2 (2.0)	0.097‡
H/o transfusion			
No	467 (96.9)	14 (3.1)	0.049‡
Yes	75 (91.4)	6 (8.6)	
No. of transfusion *			
Less than 10	71 (92.1)	5 (7.9)	0.326
10 and above	4 (80.0)	1 (20.0)	

"(contd.)"

Rhesus D type of blood product transfused*			
Negative	49 (96.1)	2 (3.9)	0.187‡
Positive	26 (83.3)	4 (16.7)	
Blood Group			
O	220 (97.3)	6 (2.7)	0.343‡
Non-O	322 (95.8)	14 (4.2)	
Rh Phenotype			
rr	343 (95.3)	17 (4.7)	0.047†
non rr	199 (98.5)	3 (1.5)	

*among transfused patients (n=81); H/o=history of transfusion; †=Pearson Chi Square (X^2); ‡=Fisher Exact Test

4.4 Discussion

In this study, most RhD negative patients in our center were blood group O with rr phenotype. This result was concordant to RhD negative donors in the Malaysian population as well as in Southwest Nigeria and Southern Ethiopia [4, 13, 14]. However, the results differed in other countries, which showed the highest prevalence of blood group B in Pakistan and Northern India among the RhD negative populations [13, 14]. Almost all countries showed rr the most phenotype of RhD negative patients [11, 17]. This indicates that although there are differences of ABO distribution within different countries and ethnicities, the common Rh phenotype distribution among the RhD negative population is almost similar.

This current study showed that the overall prevalence of RBC alloantibodies among RhD negative patients was considered to be low (3.6%). This finding was lower than reported in the previous local study (15% from 20 RhD negative pregnant women) and among Oman RhD negative

pregnant women (9.1%) [13-16]. This current study also showed a much lower prevalence compared to the study among pregnant women before anti-D immunoprophylaxis (18%) [5]. However, this study showed a slightly higher prevalence than the study done among pregnant women in Southeast Michigan and Southwest Nigeria (1.1% and 0%, respectively) [18, 19]. The alloimmunization with clinically significant alloantibodies was even much lower (n=16). The rate of clinically significant alloantibodies were almost the same in tertiary center Maryland, which was 3.3% [20].

The prevalence of alloantibodies in this study was expected to be low in view of the widespread use of Rh immunoglobulin (RhIg) prophylaxis in the Malaysian population, which subsequently decreased anti-D alloimmunization. We observed that most patients developed single RBC alloantibody, and anti-D was the most frequently RBC alloantibody identified followed by anti-Le and other Rh antibodies. Surprisingly, the RBC alloantibodies specificity were quite different from the previous study among all blood recipients and pregnant women, in which they found the most common RBC antibody was anti-E followed by anti-D, anti-M and other Rh antibodies [8, 18]. We found that none of the patients in this recent study was alloimmunized by anti-E.

Although the prevalence of RBC alloimmunization among RhD negative patients was low, the alloimmunization continued to occur despite using RhIg prophylaxis. We found that 11 RhD negative women developed anti-D despite being given RhIg prophylaxis. The probably reasons are due to non-strict compliance to guidelines in managing RhD negative patients, an insufficient dose of RhIg prophylaxis given, and failure in early detection of RhD negative patients, which led to no initial RhIg prophylaxis given as shown in one of our reported cases. Another reason for RBC alloimmunization was the lack of immune globulins to other RBC antigens which gave additional attributed factors of developing other RBC alloantibodies such as anti-C, anti-G and

other clinical insignificant antibodies as shown in this study [21].

Our reported case was a 35-year-old G4P3 woman who was wrongly labeled as RhD positive during her previous pregnancies. Thus, antenatal and postnatal prophylaxis anti-D was not given during her last three pregnancies, which led to anti-D alloimmunization with a very high anti-D titer (1:>1024) in this current pregnancy. However, the causes of the failure to prevent anti-D alloimmunization in ten other RhD negative female patients was due to the following reasons: failure to administer an adequate dosage of antenatal or postnatal RhIg prophylaxis, failure to recognize clinical events that placed the patient at risk for alloimmunization during the pregnancy and inability to administer RhIg appropriately in post-transfusion with RhD positive blood product.

The other three cases of anti-D alloimmunization were male patients, and no RhIg was given despite being transfused with RhD positive platelet concentrate. This was because of a lack of awareness in using RhIg prophylaxis after RhD positive platelet concentrate transfusion in RhD negative patients. The guideline suggested to administer 50 g (250 iu) RhIg following every three adult doses of platelets [22].

Other than anti-D, we found that two of RhD negative pregnant women were alloimmunized with either anti-C or anti-G, which were also part of the Rh antibody. Anti-C and anti-G are less immunogenic than anti-D, but both can also cause HDFN [23]. RhD negative patients are less likely to be alloimmunized from other antibodies than anti-D because of less antigenicity of other RBC antigens. The Rh antigens are highly immunogenic compared to other RBC antigens. The immunogenicity of Rh antigens listed in order of decreasing immunogenicity are D > c > E > C > e [24]. However, this study showed that patients could also be alloimmunized by clinically insignificant naturally occurring alloantibodies such as anti-Le.

One RhD negative woman did not develop anti-D despite receiving RhD positive platelet, possibly due to this patient's non-responder status, perhaps related to underlying malignancy. A 'responder' is defined as any patient with at least one detectable clinically significant RBC alloantibody that could have been induced by transfusion or pregnancy. Meanwhile, a 'non-responder' is a patient with at least one RBC alloantibody screen completed 15 days or later after the recorded issue date of RBC transfusion at one of the study hospitals with no alloantibodies detected at that screen or at any other RBC antibody screen completed during the 3.5-year study period. [25].

This study demonstrated a significant association between RBC alloimmunization in RhD negative patients and blood transfusion. Blood transfusion was the most important and known independent risk factor for RBC alloimmunization. This result was similar to other studies that showed a significant association between RBC alloimmunization and history of transfusion [7,26]. Koelewijn et al reported that RBC transfusion was the most important and known independent risk factor for non-RhD alloimmunization in pregnancy, followed by parity, major surgery and hematological disease [21].

This study also showed a significant association between RBC alloimmunization and Rh phenotype. We found that patients with rr phenotype significantly associated with RBC alloimmunization and probably the first to report this finding since we could not find any other studies that reported the association of Rh phenotype with the RBC alloimmunization from the extensive literature that have been made.

In this study there was no significant association between RBC alloimmunization in RhD negative and the number of pack cell transfusions. This finding was consistent, as shown in the other previous studies [27-29]. However, this was in contrast with the previous studies done where they found a significant association between RBC alloimmunization and the number of RBC