RED BLOOD CELL ALLOANTIBODY DEVELOPMENT AMONG MULTI-TRANSFUSED PATIENTS IN A SINGLE TERTIARY CENTRE

By
DR. HANNAH BINTI ISMAIL

Submitted to the Department of Clinical Medicine
Advanced Medical and Dental Institute,
Universiti Sains Malaysia
in partial fulfilment of the requirement
for the Degree of
MASTER OF MEDICINE
(TRANSFUSION MEDICINE)

November 2023

TABLE OF CONTENTS

DISCLAIMER…… iv

ACKNOWLEDGEMENT……..…….…… v

LIST OF TABLES…….…… vi

MANUSCRIPT TABLES…….…….. vii

LIST OF FIGURES…...…….... viii

MANUSCRIPT FIGURES…….…… ix

LIST OF ABBREVIATIONS OR NOMENCLATURES…..……..…….. x

ABSTRAK…….…….. xi

ABSTRACT…….…… xiii

CHAPTER 1 INTRODUCTION…….…….. 1

1.1 Overview…….……... 1

1.2 Background of Study……. 1

1.3 Literature Review…….. 3

1.4 Problem Statements……... 19

1.5 Research Justifications…….…….. 22

1.6 Research Questions…….……... 23

CHAPTER 2 OBJECTIVES…….…….…… 24

2.1 General Objective...……...……...…… 24

2.2 Specific Objectives……...…… 24

2.3 Alternative Hypothesis……...……... 24

2.4 Null Hypothesis……...…….. 24

CHAPTER 3 METHODOLOGY……..……..……..... 25

3.1 Study Background…….……... 25

3.2 Study Design…….……... 25

3.3 Study Area…….……...……..... 25

3.4 Study Population…….……...…….…..... 26

3.5 Subject Criteria…….……..... 27

3.5.1 Inclusion Criteria……..... 27

3.5.2 Exclusion Criteria……... 27

3.6 Sample Size…….…… 28

3.7 Sampling Method and Subject Recruitment…….…..... 32

3.8 Research Tools...…….……. 32

3.9 Data Collection Method…….…….. 33

3.10 Statistical Analysis…….…….…… 35

3.11 Operational Definition……....…… 36

3.12 List of Variables…….…….…… 37

3.13 Variables Definition…….…….……... 38

3.14 Ethical Consideration…...…….…….. 39

3.15 Study Flowchart…….……..... 40

CHAPTER 4 MANUSCRIPT…….…….……..... 41

REFERENCES…….……..... 70

APPENDICES……. 76

DISCLAIMER

I hereby certify that the work in this dissertation is my own, except for the quotations and summaries, which have been duly acknowledged. I declare that I have no financial interest in the instruments or materials used in this study.

HANNAH BINTI ISMAIL 17th November 2023
P-IPM0039/19

ACKNOWLEDGEMENT

Alhamdulillah, praise be to Allah S.W.T and blessings upon our Prophet Muhammad S.A.W. I am grateful for the opportunity, good health, courage, and strength to complete my master's degree by submitting my dissertation.

Firstly, I want to express my utmost appreciation to my supervisor, Dr Siti Salmah Noordin. Her exceptional and meticulous guidance throughout conducting this study as well as correcting my thesis has been very helpful. Her unwavering commitment to her role has inspired me to pursue excellence in all my future endeavours. A special thanks to Dr Rohayu Hami for the statistical input and Dr Sabariah Mohd Noor for the assistance in data collection.

I am incredibly grateful to my dear mother, Hajjah Haminah binti Abdullah for her immense contribution in supporting me to complete this thesis. Her unwavering emotional and spiritual encouragement throughout my life has been indispensable. My deepest appreciation for the irreplaceable contributions to both mother and my beloved late father, Haji Ismail bin Mokhtar, also to my dearest siblings and family members.

Special thanks to my batchmates for our stimulating discussions, consistent teamwork, working tirelessly up to the deadlines, and all of the bittersweet experiences - both positive and challenging - we shared over the last four and a half years.

Finally, I want to express my appreciation to everyone who contributed to my thesis, directly or indirectly. Their invaluable support had tremendously contributed to the successful completion of my research, and for that, I am deeply honoured.

LIST OF TABLES

Table	Title	Page
Table 1.1	Clinical significance of RBC alloantibodies and recommendation for the selection of blood……	9
Table 1.2	Major blood group systems and their main antigens……	10
Table 3.1	Sample size calculation for Objective 2……..	30
Table 3.2	Operational Definition…….	36
Table 3.3	Variables Definition……	38

MANUSCRIPT TABLES

Table	Title	Page
Table 1	Multi-transfused patients in HRPB in the year 2020-2021.....	63
Table 2	Demographic and clinical characteristics of multi-transfused patients.....	64
Table 3	Frequency and specificity of RBC alloantibody among multi-transfused patients.....	65
Table 4	Distribution of the total number of RBC alloantibody identified based on the blood group systems.....	66
Table 5	Association between patients' demographic and clinical characteristics with RBC alloantibody development by simple logistic regression.....	67
Table 6	Association between patients' demographic and clinical characteristics with RBC alloantibody development by multiple logistic regression.....	68

LIST OF FIGURE

Figure	Title	Page
Figure 3.1	Flowchart of the study.....	40

MANUSCRIPT FIGURES

Figure	Title	Page
Figure 1	Prevalence of RBC alloantibody among multi-transfused patients in HRPB…….	69
Figure 2	Frequency of RBC alloantibody among multi-transfused patients with positive RBC alloantibodies.....	69

LIST OF ABBREVIATIONS OR NOMENCLATURES

AHG	Antihuman globulin
AHTR	Acute haemolytic transfusion reaction
BBISv2	Blood Bank Information System version 2.0
BCSH	British Committee for Standards in Haematology
BTS	Blood transfusion services
CAT	Column agglutination technique
CI	Confidence interval
CKD	Chronic kidney disease
DAT	Direct antiglobulin test
DHTR	Delayed haemolytic transfusion reaction
DSTR	Delayed serologic transfusion reaction
Fy	Duffy
HDFN	Haemolytic disease of foetus and newborn
HIS	Hospital Information System
HLA	Human leucocytes antigen
HRPB	Hospital Raja Permaisuri Bainun
HTR	Haemolytic transfusion reaction
IAT	Indirect antiglobulin test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IH	Immunohaematology
ISBT	International Society of Blood Transfusion
Jk	Kidd
K	Kell
Le	Lewis
Lu	Lutheran
PRBC	Packed red blood cell
RBC	Red blood cell
Rh	Rhesus
RhD	Rhesus D
SPSS	Statistical Package for the Social Sciences

ABSTRAK

PENGHASILAN ANTIBODI TERHADAP SEL DARAH MERAH DALAM KALANGAN PESAKIT-PESAKIT YANG MENERIMA TRANSFUSI DARAH BERULANG DI HOSPITAL TERTIARI

PENGENALAN

Penghasilan antibodi terhadap sel darah merah adalah salah satu komplikasi yang sering berlaku kepada pesakit yang kerap menerima transfusi darah berulang. Insidens alloantibodi adalah berlainan mengikut populasi dan etnik serta bergantung kepada perbezaan genetik antara penerima dan penderma darah. Selain itu, kekerapan terdedah kepada antigen yang berbeza, diagnosis penyakit, umur dan status imun juga mempengaruhi penghasilan antibodi.

OBJEKTIF

Tujuan utama kajian ini dijalankan adalah untuk mengenalpasti prevalens, kekerapan dan jenis alloantibodi terhadap sel darah merah dalam kalangan pesakit yang menerima transfusi darah berulang dan juga menghubungkait faktor-faktor risiko yang menyumbang dalam penghasilan alloantibodi.

BAHAN DAN METODOLOGI

Kajian keratan lintang ini melibatkan 348 pesakit yang telah menerima transfusi darah berulang di Jabatan Perubatan Transfusi, Hospital Raja Permaisuri Bainun, Ipoh, Perak mulai 1 Januari 2020 sehingga 31 Disember 2021. Data telah dikumpul secara retrospektif dari rekod klinikal pesakit, rekod ujian pre-transfusi darah atau ujian imunohematologi. Data sosiodemografik dan klinikal telah dianalisa secara deskriptif dan inferens.

KEPUTUSAN

Hasil kajian mendapati prevalens alloantibodi terhadap sel darah merah dari keseluruhan 5,675 pesakit yang telah menerima transfusi darah berulang adalah 16.3%. Seramai 155 (44.5%) dari 348 orang pesakit mempunyai alloantibodi. Jenis alloantibodi yang paling kerap adalah anti-E (39.6%), diikuti anti-Mia (26.2%), anti-M dan anti-Le^a (6.3%). Terdapat hubungan yang signifikan di antara penghasilan antibodi terhadap sel darah merah dengan pesakit buah pinggang kronik (p<0.001) dan pesakit talasemia (p=0.032).

KESIMPULAN

Kajian ini menunjukkan prevalens alloantibodi yang tinggi dalam kalangan pesakit yang menerima transfusi darah berulang, terutamanya pesakit buah pinggang kronik dan pesakit talasemia. Justeru itu, transfusi darah berdasarkan profil fenotip antigen sel darah merah adalah penting bagi mencegah alloimunisasi dan mengurangkan risiko komplikasi transfusi darah.

Kata kunci: Alloimmunisasi, antibodi, sel darah merah, transfusi darah

ABSTRACT

RED BLOOD CELL ALLOANTIBODY DEVELOPMENT AMONG MULTI-TRANSFUSED PATIENTS IN A SINGLE TERTIARY CENTRE

BACKGROUND

Red blood cell (RBC) alloantibody development is one of the complications of blood transfusion, especially among multi-transfused patients. The risk depends on the frequency of exposure to the foreign RBC antigen, the antigen's immunogenicity, as well as the recipient's gender, age, underlying therapy or disease, and geographic ancestry.

OBJECTIVES

This research aimed to determine the prevalence, frequency, and specificity of RBC alloantibody and to evaluate the factors associated with its development among multi-transfused patients in a single tertiary hospital.

MATERIALS & METHODS

This was a cross-sectional study involving retrospective data collection from 348 multi-transfused patients who underwent pre-transfusion or immunohaematology testing from 1st January 2020 until 31st December 2021. Demographics and clinical characteristics were analysed using descriptive and multiple logistic analysis.

RESULTS

The prevalence of RBC alloantibody among 5,675 multi-transfused patients was 16.3%. Of the 348 multi-transfused patients studied, RBC alloantibodies were detected in 155 (44.5%) patients. Anti-E was the most common single alloantibody (39.6%), followed by

anti-Mia (26.2%), as well as anti-M and anti-Le^a (6.3% each). Multi-transfused patients with chronic kidney disease (p<0.001) and thalassaemia (p=0.032) were associated with a significantly higher risk for RBC alloantibody development.

CONCLUSION

Given the high prevalence of RBC alloantibody among multi-transfused patients, the provision of extended RBC antigen matched blood among these group of patients is essential to reduce the risk of transfusion induced RBC alloimmunisation and subsequently haemolytic transfusion reaction or delayed serologic transfusion reaction.

Keywords: Alloimmunisation, alloantibody, red cell antigen, multi-transfused, transfusion

CHAPTER ONE

INTRODUCTION

1.1 Overview

This chapter outlines the study on red blood cell (RBC) alloantibody development among multi-transfused patients in HRPB, Ipoh, Perak. This chapter also highlights the problem statements, research justifications and research questions.

1.2 Background of study

Blood transfusion therapy can offer benefits, improving quality and saving the lives of many patients with different diseases. To date, transfusion medicine is one of the five most performed practices in the world, with 10% of hospitalised patients receiving blood transfusion (1). However, allogeneic blood transfusion exposes the recipient to numerous foreign antigens and living cells that may persist for varying durations. As a result, administration of blood and blood products poses significant risks, including alloimmunisation, allergic transfusion reaction, febrile non-haemolytic transfusion reaction (FNHTR), incompatibility, and the transmission of infectious agents (2).

Alloimmunisation is defined as a humoral immune response following exposure to foreign human antigen(s). RBC alloimmunisation occurs when an alloantigen is introduced to an immunocompetent host and evokes an immune response, characterised by the development of RBC alloantibody. This response is stimulated via sensitisation through an antigen mismatch such as transfusion with antigen positive blood or when a pregnant mother lacks an antigen that is present on the foetal RBC (2). Therefore, RBC alloimmunisation resulted in the formation of RBC alloantibody.

The risk of developing RBC alloantibody after a transfusion depends on genetic disparities between donor and recipient, exposure to the foreign RBC antigen, and antigen immunogenicity (2). Additional factors include the frequency and number of transfusions, the recipient's age, gender, underlying clinical condition and immune status (3). The development of RBC alloantibodies remains an important hazard and pose a significant challenge in blood transfusion services (BTS). The resulting clinical consequences can differ significantly depending on the RBCs and specific antigens involved. This may also lead to laborious and costly investigations in the blood bank, which in turn limit and delay the provision of compatible blood products for transfusion.

This phenomenon pose a great challenge to patients, especially in regularly transfused patients as they are more likely to develop RBC alloantibodies in view of repeated exposure to a wide variety of different antigens with each transfusion. The prevalence of RBC alloantibody among the different patients' population varies due to the clinical heterogeneity, as well as transfusion policy and technical expertise of the transfusion medicine laboratory.

Hence, this study aimed to determine the prevalence, frequency, and specificity of RBC alloantibody among multi-transfused patients in a single tertiary centre. The findings of this study will contribute to a better understanding on the associated factors for RBC alloantibody development among these patients. Identifying the high risk group will be an important step towards improvement in blood transfusion safety.

1.3 Literature review

1.3.1 RBC alloimmunisation

RBC alloimmunisation leads to the formation of a specific RBC alloantibody against specific antigen present on the surface of RBC. This immune response is stimulated when an immunocompetent recipient lacking a particular antigen is exposed to the foreign antigen following blood transfusion, pregnancy or transplantation (4). For an alloantibody to develop, an individual must at least be exposed to a non-self RBC antigen, and have a human leucocytes antigen (HLA)-binding motif, capable of presenting a portion of the non-self antigen.

An immune response is elicited when these RBCs antigens are processed alongside HLA molecules on antigen-presenting cells and are presented to the T-cell receptor on T-lymphocytes. The recognition of RBC-derived peptide bound to the HLA class II molecule will activate $CD4^+$ T-cells, which subsequently induce specific B-cells to differentiate into plasma cells, producing the RBC alloantibodies towards the specific antigen (5).

The development of RBC alloantibody relies on several factors, such as the genetic differences between recipients and blood donors, the amount and frequency of administration, and the antigen immunogenicity. RBC antigens differ greatly in density, chemical composition, overall functions and immunogenicity (1). The immunogenicity varies among the various blood group antigens, listed in descending order of immunogenicity as such: D > K > c > E > Fy > Jk (2).

1.3.2 RBC alloimmunisation in multi-transfused patients

The risk of RBC alloimmunisation increases with exposure to foreign RBCs. Observational studies on random patients, including those who received a single packed red blood cell (PRBC) transfusion and pregnant women, have estimated that the prevalence of RBC alloimmunisation ranges from 1-6%. However, there are some group of patients who require regular PRBC transfusion to improve their oxygen carrying capacity such as transfusion dependent thalassaemia, aplastic anaemia, myelodysplastic syndromes and other congenital or acquired chronic anaemia.

The definition of multi-transfused patient varies among researchers. There is no single universal definition, however examples of multi-transfused patient definition include patient who had been transfused more than ten units PRBC (6–10), patient who received at least six units PRBC in three months (11), patients who received transfusion of more than one unit PRBC in one month or at least ten units PRBC within three months (12), patient who had been transfused with two or more PRBC units within 3-12 weeks (4,13–15), or patients who had history of PRBC transfusion at least once in a month and dependent on transfusion (16).

Multi-transfused patients were thought to have higher risk to develop RBC alloantibody, approximately 1% for each PRBC transfused. The prevalence of RBC alloantibody varies greatly among different countries, ranging from 8-76% (3). The prevalence were reported to varies between 4-50% among beta thalassaemia major patients (10,17), while 1.9-13% haemato-oncology patients were found to have RBC alloantibodies.

In a case-control study in Chile, the highest prevalence of RBC alloantibody was found among patients with malignancies (29.2%) and gastrointestinal bleeding (16.7%), while the lower prevalence was reported among chronic kidney disease (CKD) patients (9.4%) (18). Patients with end stage renal failure reported a higher rate approaching 13% (19).

Other than the geographical differences, this disparity also reflects the differences in the transfusion practices and protocols, the age of the population, the use of leucoreduced PRBCs, as well as the clinical status of the patients. The presence of RBC alloantibodies can significantly impede the effectiveness of transfusion therapy by reducing in vivo survival of transfused RBCs thus leading to haemolytic transfusion reaction (HTR). The degree of severity of HTR can range from mild to severe, leading to significant morbidity and mortality (5).

Furthermore, RBC alloantibodies also pose additional challenges in the blood provision. When alloantibodies against high frequency antigens develop, finding an antigen negative compatible PRBC in a timely manner can be very challenging and laborious. This will lead to delayed blood supply and may affect patient's management, especially in an emergency or life threatening situation (20).

In most cases, the BTS typically match the PRBC units solely based on ABO and RhD antigen. As a preventive measure to reduce the risk of RBC alloimmunisation, it has been recommended and advocated that multi-transfused patients such as patients with haemoglobinopathies and sickle cell disease should receive PRBC transfusion with extended RBC antigen matching; Rh (C, c, E and e), Kidd (Jk^a and Jk^b), K (K, Kp^a and Kp^b), Duffy (Fy^a and Fy^b), S and s (21,22).

This practice is a proven strategy in preventing RBC alloimmunisation and has been widely practiced globally (22–24). Some reports have shown a significant reduction in the RBC alloimmunisation rate as well as the incidence of delayed HTR when extended RBC antigen matched PRBCs are used for multi-transfused patients (4,25).

1.3.3 Antibody Screening and Identification

The pre-transfusion testing which includes ABO and RhD grouping, antibody screening, and compatibility test play an important role in detecting the presence of RBC alloantibody thus reducing the risk of acute and delayed HTR (22,24).

Prior to transfusion, the patient's serum is analysed using standard blood bank methods to detect any unexpected antibodies besides antibodies to ABO antigens. As per national transfusion policy, antibody screening is mandatory for all requests for PRBC transfusion. The test is done by an indirect antiglobulin test (IAT), or indirect Coombs' test using a commercialised two-cell or three-cell panel. If the antibody screening is found positive, antibody identification will be carried out to determine its specificity.

Antibody identification is performed using commercial 10 or 11-cell identification panel. The British Committee for the Standards in Haematology (BCSH) guideline suggested that the panel cells for antibody detection should include the following most common clinically significant RBCs antigens: C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, P1, Le^a and Le^b (22).

IAT uses antihuman globulin (AHG) to detect in vitro sensitisation of RBC, and this method is applied for antibody screening and identification. Various techniques are available, including the test tube method, solid phase assays and column agglutination technique (CAT). These methods have been proven reliable for antibody detection when performed as per manufacturer's instructions and are thoroughly validated (26). CAT method uses micro-column cards predispensed with a neutral gel, a gel containing AHG, or specific reagents. This technique can be done manually, semi-automated or automated. It has an objective reading phase, and the results are standardised and reproducible (24).

Previous studies had demonstrated that IAT performed using the CAT system was superior to the test tube method in detecting alloantibodies of potential clinical significance (27,28). However, the presence of an alloantibody can be unrecognised, unidentified or missed during testing and could be for the following reasons: (29)

1. The serum is examined at an inappropriate time following antigenic exposure.
2. The antibody strength or the antibody titre is below the detection threshold.
3. The target cells lack the corresponding antigen.
4. The serological tests technique inaccurate, insensitive or not optimum.
5. The presence of autoantibody may mask the existence of alloantibody.
6. The immunogenicity is cellular not humoral.

1.3.4 Clinical significance of RBC alloantibody

Alloantibody that reacts with a RBC antigen can be classified based on the reactivity at different temperatures, phases, and types of antigenic exposure or stimulus. Naturally occurring antibodies are generally IgM. Antibodies of the IgM class are usually cold-reacting antibodies, react optimally at room temperature or below (around 4°C) and can cause agglutination of saline suspended RBCs (30).

Generally, most of these IgM antibodies are considered clinically insignificant except for ABO antibodies. IgM antibodies do not cause haemolytic disease of foetus and newborn (HDFN) because they do not cross the placenta. Although rare, these antibodies can activate complement and cause haemolysis if the thermal activity is above 30°C. Examples of such antibodies are those present in the ABO, Hh, li, Lewis, MN and P system (23).

On the other hand, immune antibodies are often IgG and are acquired through exposure and sensitisation to non-self or foreign RBC antigens during pregnancy, transfusion or transplantation. IgG class antibodies are warm-reacting antibodies which react optimally at 37°C and at the AHG phase. These antibodies are clinically significant as they can cross the placenta, causing HDFN and HTR (23).

The term clinically significant RBC alloantibody refers to an antibody that carries a substantial risk of HTR; whether causing an obvious HTR or no overt clinical symptoms but associated with laboratory or serological findings of RBC haemolysis (31). Table 1.1 shows the clinical significance of some RBC alloantibodies and the recommendation for the selection of PRBC for transfusion (26).

Table 1.1 Clinical significance of RBC alloantibodies and recommendation for the selection of blood (26)

System	Specificity	Likely clinical significance in transfusion	Recommendation for selection of red cells for transfusion[a]
ABO	Anti-A_1	No	IAT crossmatch compatible at 37°C
Rh	Anti-D, Anti-C, Anti-c, Anti-E, Anti-e	Yes	Antigen negative
Rh	Anti-C^w	No	IAT crossmatch compatible
Kell	Anti-K, Anti-k	Yes	Antigen negative
Kell	Anti-Kp^a	No	IAT crossmatch compatible
Kidd	Anti-Jk^a, Anti-Jk^b	Yes	Antigen negative
MNS	Anti-M (active 37°C)	Yes	Antigen negative
MNS	Anti-M (not active 37°C)	No	IAT crossmatch compatible at 37°C
MNS	Anti-N	No	IAT crossmatch compatible at 37°C
MNS	Anti-S, Anti-s, Anti-U	Yes	Antigen negative
Duffy	Anti-Fy^a, Anti-Fy^b	Yes	Antigen negative
P	Anti-P_1	No	IAT crossmatch compatible at 37°C
Lewis	Anti-Le^a, Anti-Le^b, Anti-Le^{a+b}	No	IAT crossmatch compatible at 37°C
Lu	Anti-Lu^a	No	IAT crossmatch compatible at 37°C
Diego	Anti-Wr^a (anti-Di3)	Yes	IAT crossmatch compatible
H	Anti-HI (in A_1 and A_1B patients)	No	IAT crossmatch compatible at 37°C
All	Others active by IAT at 37°C	Yes	Seek advice from Blood Centre

[a]Where antigen negative red cells are recommended, these should also be compatible in an IAT crossmatch.

1.3.5 Blood Group and Immunogenicity

The ABO and Rh blood group systems are the most significant in transfusion practices. Nevertheless, the International Society of Blood Transfusion (ISBT) has recognised 354 RBC antigens from 44 blood group systems, genetically determined by 49 genes. Some antibodies against these RBC antigens potentially have clinical significance in causing the destruction of RBCs, which may result in severe clinical consequences. According to the ISBT, the 10 major blood group systems include ABO, Rh, Duffy, Kell, Kidd, MNS, Lewis, P1PK, Lutheran and I. However, the most clinically significant antibodies after ABO are those in the Rh, Kidd, Duffy and Kell blood group systems. Table 1.2 shows the 10 major blood group systems and their main antigens (23).

Table 1.2 Major blood group systems and their main antigens (23)

ISBT no.	Name of blood group system	Major antigens	Chromosome location no.
001	ABO	A, B, A_1	9
002	MNS	M, N, S, s, U	4
003	P1PK	P1, P^k	22
004	Rh	D, C, E, c, e	1
005	Lutheran	Lu^a, Lu^b	19
006	Kell	K, k, Kp^a, Kp^b, Js^a, Js^b	7
007	Lewis	Le^a, Le^b	19
008	Duffy	Fy^a, Fy^b, Fy3	1
009	Kidd	Jk^a, Jk^b, Jk3	18
027	I	I	6

1.3.5.1 Rhesus (Rh) blood group system

The Rh blood group is the most polymorphic and highly diverse blood group system, with 61 different antigenic specificities. The D antigen is highly immunogenic among the Rh antigens, followed by the c, E, C, and e antigens. Rh antibodies are mostly IgG antibodies and are the most clinically significant, second to ABO and H systems, as they are capable of causing severe HDFN and HTR (32).

It has been reported that following exposure to Rh-positive antigens in immunocompetent RhD-negative patients, about 80% will develop anti-D. Therefore, RhD-negative patients should only receive transfusion of RhD-negative PRBC.

1.3.5.2 Kell blood group system

There are around 39 antigens in the Kell blood group system. These antigens are present on the mature RBC, the erythroid progenitor cells, and many other tissues in lesser amounts. The K antigen is 8-10 times less immunogenic compared to the D antigen. Anti-K and anti-k are mostly IgG and react optimally at the AHG phase. They are known to have significant roles in HTR and HDFN. Their presence can induce in vivo RBC destruction through extravascular haemolysis (32). These antibodies develop following exposure through transfusion or pregnancy and may persist for many years. Providing antigen negative and crossmatch compatible PRBC is crucial once these antibodies are identified.

1.3.5.3 Kidd blood group system

The Kidd blood group system comprises of three important antigens: Jk^a, Jk^b, and Jk^3. However, the Jk^a and Jk^b antigens are the most commonly found antigens on the RBCs of most individuals. These antigens are fully developed on the foetal RBCs and can be identified as early as seven weeks gestation. Kidd antibodies are dangerous as they demonstrate significant serological variability in immunohaematology testing (32).

The reaction of these antibodies is often weakly reactive and the titre can decline rapidly in vivo. They demonstrate dosage effects, where the antibodies react more robustly towards homozygous RBCs expressing corresponding antigens than the heterozygous RBCs. Furthermore, their presence may be combined with other antibodies. Anti-Jk^a and anti-Jk^b are IgG subtypes and can cause complement activation leading to delayed HTR or acute HTR due to an anamnestic response (5). PRBC transfusion for patients with these antibodies must be antigen negative and crossmatch compatible.

1.3.5.4 Duffy blood group system

In transfusion practices, Fy^a and Fy^b antigens are among the most important antigens in the Duffy blood group system. The frequency of Duffy antigens across different populations varies significantly. Antibodies to Duffy antigens are IgG antibodies that react optimally at the AHG phase, and their development can be induced by transfusion or pregnancy. Identifying anti-Fy^a through serological testing can be challenging due to its frequent co-occurrence with other RBC alloantibodies, especially Rh antibodies.

Anti-Fy^a and anti-Fy^b are clinically significant antibodies and have been implicated in HDFN as well as acute and delayed HTR (32). Therefore, antigen negative and crossmatch compatible PRBC should be transfused once the antibody is identified.

1.3.5.5 MNS blood group

The MNS blood group system is second to the Rh blood group system in terms of complexity. There are currently 48 antigens identified; however, the M, N, S, s, and U are among the commonly found antigens on RBCs. Antibodies towards MN antigens are often cold-reacting IgM and naturally occurring. Generally, Anti-M and anti-N are clinically insignificant and rarely caused HTR or HDFN. Crossmatch compatible PRBC is supplied unless the antibodies react at 37°C.

On the other hand, anti-S, anti-s and anti-U are commonly IgG antibodies. They react optimally at 37°C with possible complement binding if IgM form is present. These clinically significant antibodies can potentially cause moderate to severe HTR. It is recommended to provide antigen negative and crossmatch compatible PRBC in the presence of these antibodies (33).

1.3.5.6 Lewis blood group

The Lewis blood group system is unique as these antigens are not intrinsic to the RBCs. Instead, they are passively absorbed from the plasma onto the RBCs membrane. The antigens of primary concern in this blood group are Le^a and Le^b. Lewis antigens are not fully formed on foetal RBCs. The antigenic strength varies and may change under different circumstances. Antibodies towards Lewis antigens are naturally occurring and often cold-reacting IgM. These antibodies are usually clinically insignificant and do not pose a risk of causing HTR or HDFN as they do not cross the placenta.

Crossmatch compatible PRBC is provided if these antibodies are present. However, in some rare occurrences, anti-Le^a may react strongly at 37°C, causing incompatibility during pre-transfusion testing (32). These antibodies should be considered clinically significant; therefore, antigen negative PRBC should be supplied.

1.3.6 Adverse Transfusion Reactions associated with RBC alloimmunisation

1.3.6.1 Haemolytic Transfusion Reaction

HTR is often caused by the transfusion of incompatible antibodies, resulting in accelerated destruction and clearance of transfused RBCs. This immune mediated reaction occurs when the recipient's preformed antibodies bind to the antigen on the transfused RBCs. The haemolysis can occur extravascular or intravascular, with or without complement activation. HTRs are classified based on the onset of reaction following transfusion, which is acute (within 24 hours) or delayed (after 24 hours) (30).

1.3.6.1.1 Acute haemolytic transfusion reaction (AHTR)

AHTR occurs during or within 24 hours following transfusion. It is characterised by a combination of signs and symptoms of haemolysis and laboratory evidence of RBCs haemolysis. The incidence of AHTR has been estimated at 1 in every 10,000 to 70,000 transfused blood components. AHTR usually occurs at the beginning of transfusion or a few hours upon completion (30).

This reaction is often caused by transfusion of incompatible RBCs, however may also be induced by the transfusion of incompatible plasma-containing products. RBC incompatibility may be detected through serologic testing. The offending antibodies commonly involved in AHTR are IgM antibodies; nevertheless, complement-fixing IgG antibodies may also cause similar reaction. The antigen-antibody complex activates the classical complement pathway, forming the membrane attack complex and leads to RBCs destruction. Examples of IgM antibodies are anti-A and anti-B (1).

In general, the degree of severity of AHTR corresponds to the amount of incompatible blood transfused. The clinical manifestation vary widely from mild to severe. The most common symptom is fever which can be accompanied by chills or rigours. Patients may have pain at the infusion site, abdomen or flanks, nausea or vomiting, dyspnoea, hypotension, haemoglobinuria, and they may even develop multi-organ failure. Severe and life threatening features are associated with an increased mortality rate (30).

1.3.6.1.2 Delayed haemolytic transfusion reaction (DHTR)

DHTR is a delayed reaction that occurs within days to weeks (can be up to 28 days) following transfusion. Most cases appear 7-10 days post-transfusion. DHTR is usually caused by an anamnestic response of IgG alloantibody, which developed from previous exposure and was not detected during the initial pre-transfusion testing. DHTR is suspected in patient with an unexplained drop in haemoglobin level post transfusion.

The IgG alloantibody will accelerate the destruction of transfused RBCs via extravascular haemolysis through the FcR pathway (1). Consequently, a patient will have anaemia, unconjugated hyperbilirubinaemia and low serum haptoglobin level. Haemoglobinaemia and haemoglobinuria may present. Severe complication leading to coagulopathy or renal failure are rare, and the fatality rate is substantially lower than AHTR (21). Serologic workup may reveal a positive direct antiglobulin test (DAT) with IgG or complement. The most commonly implicated antibodies are towards Rh, Kidd, Kell and Duffy antigens. Patients with DHTR who require further PRBC transfusion should be supplied with antigen negative PRBC for the corresponding antibody (30).

1.3.6.2 Delayed serologic transfusion reaction (DSTR)

DSTR is characterised by serologic evidence of a new, clinically-significant RBC alloantibody or a positive DAT following a PRBC transfusion. However, compared to DHTR, the clinical signs and symptoms of haemolysis in DSTR are absent (30).

1.3.7 Factors affecting RBC alloimmunisation

The rates of RBC alloimmunisation are thought to be influenced by various factors such as antigenic differences, immunogenicity, immune status, also the amount and frequency of transfusion; however, the exact kinetics are still unknown (34). In a retrospective study conducted over three years involving 200 multi-transfused patients in a tertiary care centre in India, the prevalence of RBC alloantibody was 5.5%. There were 11 alloantibodies identified; the majority (72.7%) were against the Rh blood group system, followed by the Lewis, and MNS blood group systems (9.1% each) (6).

1.3.7.1 Age

Earlier research demonstrated that older age is a significant risk factor for developing RBC alloantibody. According to reports, patients with sickle cell disease older than 14 had a 2.8 times greater risk of alloimmunisation (35). However, another study found that RBC alloimmunisation was significantly higher in patients younger than 40 years (4).

Similarly, a study among thalassaemic patients reported that the rate of RBC alloimmunisation in patients younger than 10 years was lower than in other age groups. They found that early onset of transfusion (age of less than three years) was associated with a lower frequency of RBC alloantibody development by 20.9% compared to 47.5% of those whom it is started after that age (36). An immature immune system and possible an acquired immune tolerance to allogeneic RBC antigens are thought to be responsible for the reduced risk of RBC alloimmunisation.

1.3.7.2 Gender

Gender also has been debated to influence the RBC alloimmunisation rate. Few studies proposed that female gender as an independent risk factor for RBC alloantibody development following transfusion (4,37). The risk increases as women are more susceptible to alloantigen exposure during pregnancy, miscarriage, and childbirth.

However, some studies did not report this association to be significant (9,18,38). A meta-analysis concluded that RBC alloimmunisation was not associated in either male or female gender among transfused patients, where females patients showed no risk difference compared to males patients (39).

1.3.7.3 Frequency and amount of transfusion

Historically, it was believed that the amount of PRBC transfused influenced the rate of RBC alloimmunisation. The alloimmunisation risk among multi-transfused patients was reported to increase with the number of PRBCs transfused. An alloimmunised patient was also thought to have a higher risk of developing multiple RBC alloantibodies (2).

This was supported by a study across various patients' populations with different comorbidities, where patients with prior RBC alloantibody had a four to five fold increased risk of developing additional alloantibodies to low frequency antigens and autoantibodies upon subsequent exposure. These alloimmunised patients received a higher mean number of blood units than non-alloimmunised multi-transfused patients (3,11). Hence, extended RBC antigen matching is recommended in patients who have a higher risk of RBC alloimmunisation (4,22,23,40).

1.3.7.4 Clinical Diagnosis

Clinical diagnosis of patients may lead to the alteration of immune status. Patients who are transfused in their baseline state of health are thought to be less likely to become alloimmunised than patients transfused in a state of inflammation.

Immunocompromised patients were shown to have a lower risk of developing RBC alloantibody. Patients with lymphoproliferative disorders and acute myeloid leukaemia had the lowest odds, especially those undergoing immunosuppressive treatments. This was attributed to lymphocyte dysfunction and concomitant chemotherapy, which suppressed the immune system (5).

1.4 Problem statements

Perak is one of the 13 states in Malaysia, the second largest state in Peninsular Malaysia and the fourth largest state in the country. According to the Department of Statistics Malaysia, in 2020, the estimated population of Perak is 2.51 million people. The population in Perak is composed of a majority of the Malay community (59.8%), Chinese (28.5%), Indian (11.3%) and others (0.4%) (41). Perak is divided into 13 administrative districts and each district is provided with government hospital facilities. Hospital Raja Permaisuri Bainun (HRPB) is the state hospital situated in the capital city, Ipoh. This hospital is the largest hospital in Perak and the third largest hospital in Malaysia.

Over the past 10 years, the total population in Perak has increased from 2.39 million people in 2010 to 2.51 million in 2020. Perak is the third oldest state in Malaysia with the percentage of senior citizens aged 60 years and above is at 10.7% (41). The increase in the elderly population reflects the increase in the life expectancy in the state of Perak. Therefore, the number of long term care hospitals is expected to increase as well. The physical and social changes associated with ageing are combined with the debilitating effects of multiple, acute and chronic diseases.

Among the chronic non-communicable diseases that were reported to increased significantly are ischaemic heart disease, cerebrovascular diseases, diabetes mellitus, CKD and malignant neoplasms such as lungs and colorectal carcinoma. As the survival of patients with chronic conditions have greatly improved with the advancement in medical treatment, these patients may require repeated blood transfusions throughout the course of their illness.

Hospitals in Perak state reported around 45,000 - 49,000 blood components transfused each year. Nationwide, Perak is currently the fifth for blood usage. Blood transfusion demands have significantly increased during the year 2018-2019 as compared to the year 2016-2017 where around 30,000 - 35,000 blood components were transfused each year (42). Thus, a major challenge faced by BTS in Perak is to maintain an adequate blood donor base and sufficient blood stock to meet the increasing demands.

The frequency of RBC alloantibody in Malaysia is not uniform and variations have been noted in different patients' populations. To date, data on the prevalence and factors that affect RBC alloimmunisation among patients, especially among multi-transfused patients is still scarce. Multi-transfused patients are associated with high PRBC transfusion burdens, thus have amongst the highest RBC alloimmunisation rates. For example, the prevalence of RBC alloantibodies was 0.76% and 1.28% among hospitalised patients in Selangor and Penang (43,44); higher prevalence was reported among thalassaemia (10.59%), haemato-oncology (3.2%) and CKD (12.4%) patients (8,45,46). However, such data and its related clinical indications are not available in Perak. Moreover, most studies did not take into account the different populations of multi-transfused patients or the frequency of the RBC transfusion.

In terms of blood supply and testing, HRPB is the referral centre for all the district hospitals in the state of Perak. The incidence of RBC alloantibodies detected in the Transfusion Medicine Department, HRPB have been in increasing trend over the preceding two years. Local unpublished data from hospital report found that there was 20-30% increase in the number of RBC alloantibody identified in this centre.

In addition, HRPB also is the referral hospital for thalassaemia patients in Perak and it is the fourth among the 10 centres in Malaysia with the highest number of thalassaemia patients (47). Besides, Perak itself is the third state in the nation with highest number of dialysis patients (48).

The national transfusion policy for thalassaemia patients has advocated BTS to provide RBC antigen matched PRBC for ABO, Rh and Kidd (and any other antigen negative blood for defined antibody) to minimise the likelihood of RBC alloimmunisation. Leucoreduced and fresh PRBC should be given whenever available. Thalassaemia patients also should have a baseline extended RBC phenotyping at their diagnosis and before the start of first PRBC transfusion (47,49). The policy was done in the year 2009 however was only implemented in the National Blood Centre since July 2014.

In practice, nevertheless, this current policy and guideline are not routinely performed and may be impractical in most states and district blood banks, mainly due to the unavailability of reagents, equipment and staff. Even with the availability of resources and technologies, some BTS are only able to provide partially matched PRBC units (ABO, Rh) instead of extended RBC antigen matching due to limited inventory of PRBC units. Furthermore, this transfusion strategies are only provided for transfusion dependent thalassaemia patients and does not include other multi-transfused patients with different clinical diagnoses.

1.5 Research Justifications

The genetic heterogeneity within Malaysians' population due to its diverse ethnicities have been observed through a unique distribution pattern of some blood groups which leads to a vast array of antibodies (50). Obtaining PRBC for transfusion in patients with RBC alloantibodies poses significant challenges to BTS. The process of locating crossmatch compatible PRBC for these patients can be extremely challenging and burdensome, especially with the limited time available in emergency situation.

Cost considerations and the question of whether routine extended RBC antigen matching would truly benefit the patient make this an important issue for most BTS. The patients' population that most likely to benefit from this strategy is still unclear and may not be cost effective to be applied for all patients. Therefore, the next best alternative is to provide preventive RBC antigen matching only for those transfusion recipients that are at high risk for RBC alloimmunisation. Hence this study aimed to determine the prevalence, frequency and specificity of RBC alloantibody and the characteristics of multi-transfused patients who developed the alloantibodies. Moreover, this study in Malaysia is the first to integrate multi-transfused patients with different diseases.

Knowing the diversity of RBC antigens that present among the multi-transfused patients' population, the prevalence of RBC alloantibody and the associated factors are crucial to improve the current transfusion practice. The findings of this study can help BTS to evaluate and identify patients at risk to develop RBC alloantibody and to consider the best prospective RBC antigen matching strategies if such measures are feasible. Altogether, prevention of RBC alloimmunisation is an important step towards preventing transfusion complications.

In addition, knowledge of the RBC antigen frequencies in a population with different ethnic origins can help BTS in creating a blood donor database and to formulate strategies for blood inventory management in providing antigen compatible PRBC for patients with multiple RBC alloantibodies. As a result, proactive and preventive measures can be strategised and implemented, including extended RBC phenotyping prior to transfusion therapy, blood donor genotyping, and establishing a more comprehensive registry of rare blood donors.

Furthermore, the findings from this study may also aid in establishing local as well as national transfusion policy for multi-transfused patients. As a result, enhancement in transfusion safety and improvement in transfusion practice for the these patients can be achieved.

1.6 Research questions

1. What is the prevalence, frequency and specificity of RBC alloantibody among multi-transfused patients?

2. What are the factors associated with the development of RBC alloantibody among multi-transfused patients?

CHAPTER TWO

OBJECTIVES

2.1 General Objective

To analyse RBC alloantibody development among multi-transfused patients in Hospital Raja Permaisuri Bainun, Ipoh, Perak.

2.2 Specific Objectives

i. To determine the prevalence, frequency and specificity of RBC alloantibody among multi-transfused patients.

ii. To determine the association between RBC alloantibody development with patients' demographic characteristics (age, gender, race, blood group), clinical diagnosis (thalassaemia, haematological disorders, chronic kidney disease or 'others'), PRBC transfusion frequency, and transplantation history.

2.3 Alternative Hypothesis

Patients' demographic characteristics, clinical diagnosis, PRBC transfusion frequency, and transplantation history are associated with RBC alloantibody development among multi-transfused patients.

2.4 Null Hypothesis

There is no association between patients' demographic characteristics, clinical diagnosis, PRBC transfusion frequency, and transplantation history with RBC alloantibody development among multi-transfused patients.