HAEMOLYTIC DISEASE OF FOETUS AND NEWBORN AND HAEMOLYTIC TRANSFUSION REACTION DUE TO KIDD ANTIBODY IN HOSPITAL UMUM SARAWAK

By,

DR IRNI BINTI MOHD YASIN

Dissertation Submitted in

Partial Fulfilment of the Requirement for the Degree of

Master of Medicine (Transfusion Medicine)

UNIVERSITI SAINS MALAYSIA

ADVANCED MEDICAL AND DENTAL INSTITUTE (AMDI)

2017

DECLARATION

I hereby declare that this dissertation has been sent to Universiti Sains Malaysia as a requirement for the degree; Master of Medicine in Transfusion Medicine. It has not been sent to other university. With this any part of this dissertation may be used for reference.

_____

Dr Irni binti Mohd Yasin

P-IPM0006/13

ACKNOWLEDGEMENTS

In the name of Allah, Most Gracious, Most Compassionate. Thanks to Allah with His blessing, mercy and guidance that I managed to complete this monumental task.

First, I would like to express my deepest sense of gratitude to my supervisor Prof Dr Narazah, and my co-supervisors Dr. Afifah and Dr. Mohamad Masrin for their guidance. Without their help and encouragement, this dissertation would not have been possible.

I am also grateful to members of Cluster of Regenerative Medicine, Advanced Medical and Dental Institute (AMDI) University Sains Malaysia (USM). I also would like to thank Immunohaematology staffs in Hospital Umum Sarawak (HUS) for their support and guidance. I also would like to thank my colleague for the friendship and moral support.

Finally, my deeply gratitude goes to my family for their unwavering encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

HDFN	Haemolytic Disease of Foetus and Newborn
HTR	Haemolytic Transfusion Reaction
DHTR	Delayed Haemolytic Transfusion Reaction
HUS	Hospital Umum Sarawak
NBC	National Blood Centre
USM	Universiti Sains Malaysia
WHO	World Health Organization
RDR	Rare Blood Donor Registry
RBC	Red blood cells
KL	Kuala Lumpur
IRDP	International Rare Donor Panel
NaCl	Sodium Cloride
BCSH	British Committee for Standards in Haematology
TTI	Transfusion Transmitted Infection

ABSTRAK

PENYAKIT HEMOLISIS JANIN DAN BAYI BARU LAHIR (HDFN) DAN HEMOLITIK TRANSFUSI DARAH (HTR) DISEBABKAN ANTIBODI KIDD DI HOSPITAL UMUM SARAWAK

Penyakit Hemolisis Janin dan Bayi baru lahir (HDFN) dan penyakit Hemolitik Transfusi Darah (HTR) boleh disebabkan oleh antibodi terhadap antigen Kidd. Di Malaysia, kelaziman antibodi Kidd menyebabkan HDFN dan HTR telah dilaporkan; bagaimanapun masih kekurangan data dari Hospital Umum Sarawak (HUS). Oleh itu tujuan kajian ini dijalankan adalah untuk menentukan jika antibodi Kidd menyebabkan HDFN dan HTR di HUS. Kes-kes '*alloimmunisation'* dari tahun 2011 hingga 2014 telah dikumpulkan daripada rekod perubatan pesakit. Seterusnya, untuk menentukan prevalens fenotip Kidd, kajian prospektif telah dilakukan. Dua ratus lima puluh (250) penderma darah di HUS dari 1 hingga 10 September 2015 telah direkrut. Sampel darah telah diuji untuk kumpulan darah sistem Kidd menggunakan kad gel Diamed-ID. Keputusan menunjukkan terdapat 1109 kes '*alloimmunisation'*di HUS. Daripada jumlah ini 44 (4.0%) kes '*alloimmunisation'* adalah disebabkan oleh antibodi Kidd dan 1065 (96.0%) kes adalah disebabkan oleh antibodi jenis lain. Sepuluh (10) daripada 44 kes (22.7%) '*alloimmunisation'* adalah disebabkan oleh antibodi Kidd menyebabkan HDFN manakala 4 daripada 44 kes (9.1%) menyebabkan HTR dan keputusan ini menunjukkan prevalens yang rendah ($p > 0.05$). Sementara itu, keputusan fenotip Kidd menunjukkan kehadiran fenotip Jk(a+b+) ialah 110 daripada 250 (44.0%) dan fenotip Jk(a-b-) ialah 7 daripada 250 (2.8%) penderma darah. Fenotip Kidd lain yang dikesan ialah Jk(a+b-) iaitu 60 daripada 250 (24.0%) dan Jk(a-b+) iaitu 73 daripada 250 (29.2%) penderma

darah. Fenotip Kidd dikesan dalam empat (4) kumpulan etnik; Cina, 127 daripada 250 (50.8%), Melayu, 96 daripada 250 (38.4%), Bidayuh, 25 daripada 250 (10.0%) dan Iban, 2 daripada 250 (0.8%). Keputusan juga menunjukkan bahawa fenotip Jk(a-b-) hadir hanya dalam kaum Melayu iaitu 7 daripada 250 (2.8%) tetapi tidak dijumpai di dalam kumpulan etnik yang lain, dan penemuan ini adalah signifikan (p <0.05). Kajian ini menunjukkan bahawa '*alloimmunisation'* disebabkan antibodi Kidd adalah tidak biasa bagi HDFN dan HTR di HUS. Fenotip Kidd yang paling kerap di kalangan penderma darah adalah Jk(a+b+). Fenotip Jk(a-b-) dalam ethnik Melayu di Sarawak adalah tertinggi berbanding dengan kajian terdahulu di Malaysia dan Asia. Kesimpulannya, antibodi Kidd jarang menyebabkan HDFN dan HTR di HUS dan kumpulan darah Kidd telah dikategorikan di kalangan penderma darah di HUS.

Kata kunci: Penyakit Hemolisis Janin dan Bayi Baru Lahir (HDFN), Tindak balas Hemolisis Transfusi (HTR), antibodi Kidd, fenotip Kidd, ethnik, Hospital Umum Sarawak.

ABSTRACT

HAEMOLYTIC DISEASE OF FOETUS AND NEWBORN (HDFN) AND HAEMOLYTIC TRANSFUSION REACTION DUE TO KIDD ANTIBODY IN HOSPITAL UMUM SARAWAK.

Haemolytic Disease of Foetus and Newborn (HDFN) and Haemolytic Transfusion Reaction (HTR) may occur due to antibodies against Kidd antigen. In Malaysia, the prevalence of RBC alloimmunization due to Kidd antibody for cases of HDFN and HTR have been reported however there is insufficient data in Hospital Umum Sarawak (HUS). Therefore, the aim of this study is to determine whether Kidd alloimmunization causes HDFN and HTR. Records of alloimmunisation cases from 2011 to 2014 were retrieved and traced to the patients' medical records to determine whether Kidd antibodies is the underlying cause of HDFN and HTR in HUS. Secondly, to determine the prevalence of Kidd phenotype, two hundred and fifty (250) regular blood donors in HUS from 1st to 10th September 2015 were recruited. Blood samples were phenotyped for Kidd blood group using Diamed-ID gel card system. The results showed there were 1109 cases of alloimmunisation recorded. Out of this 44 (4.0%) cases of alloimmunisation were due to Kidd antibody and 1065 (96.0%) cases were due to other antibodies. Ten (10) out of 44 (22.7%) cases of alloimmunisation were due to Kidd antibody resulting in HDFN whilst 4 out of 44 cases (9.1%) resulting in HTR. These results were not statistically significant (p> 0.05). Meanwhile, the results of Kidd phenotype showed the presence of Jk(a+b+) phenotype in 110 out of 250 (44.0%) and Jk(a-b-) phenotype in 7 out of 250 (2.8%) blood donors. The other Kidd phenotypes detected were Jk(a+b-) in 60 out of 250 (24.0%) and Jk(a-b+) in 73 out of 250 (29.2%)

blood donors. Kidd phenotype was detected in four (4) ethnic groups; Chinese, 127 out of 250 (50.8%), Malays, 96 out of 250 (38.4%), Bidayuh, 25 out of 250 (10.0%) and Iban, 2 out of 250 (0.8%). The results also showed that Jk(a-b-) phenotype is present only in the Malays 7 out of 250 (2.8%) but not found in the other ethnic groups, and this is statistically significant (p<0.05). This study shows that alloimmunisation by Kidd blood group system is uncommon for the underlying HDFN and HTR in HUS. The most common Kidd phenotype among regular blood donors is Jk(a+b+). The prevalence of Jk(a-b-) phenotype in Malays in Sarawak is highest compared to earlier studies in Malaysia and Asia. In conclusion, there is low prevalence of Kidd antibody causing HDFN and HTR and Kidd blood group system was successfully characterised in regular blood donors in HUS.

Keywords: Haemolytic Disease of Foetus and Newborn (HDFN), Haemolytic Transfusion Reaction (HTR), Kidd blood group system, ethnicity, Hospital Umum Sarawak (HUS).

CHAPTER 1

1.1 INTRODUCTION

Blood transfusion has many benefits but adverse effects may also develop. The adverse effects include haemolytic transfusion reaction (HTR), transfusion transmitted infection (TTI), allergic reaction and febrile transfusion reaction (Sahu *et al.*, 2014). Transfusion reaction is defined as unwanted events that can happen to any patient receiving blood transfusion therapy. It can be classified as acute or delayed transfusion reaction (Bolton-Maggs and Cohen, 2013).

Acute haemolytic transfusion reactions are rare, but it can be fatal. It occurs when incompatible blood is transfused. This leads to the development of alloantibody resulting in destruction of red blood cells (RBC) leading to haemolysis (Shackle)

As a standard procedure, before any transfusion is given, pre-transfusion testing will be done. The pre-transfusion testing consists of ABO and Rh D typing, antibody screening test and cross-matching. Once the patients' blood group are known, it is safe to provide ABO compatible blood without cross-matching procedure (White *et al.*, 2016). However, a small group of people may have unexpected antibody against donor's RBC. There is also a possibility that antibody could be undetected by antibody screening test due to low titre. Therefore, a cross-match test is an essential step that needs to be taken before blood is transfused (Sood *et al.*, 2013).

Rios (2011) reported that the incidence of acute HTR is one (1) in 76,000 RBC transfusions (Rios *et al.*, 2011). The main causes are ABO and Rh incompatible with one (1) in 40,000 transfusions followed by non-ABO RBC alloantibodies. Even very little amount of incompatible blood transfused could lead to multi-organ failure and

patients may require intensive care and resuscitation. Clerical error may be one of the causes of fatal acute HTR (Bolton-Maggs and Cohen, 2013).

Delayed HTR may occur after 24 hours of receiving incompatible blood transfusion. This is due to RBC antibodies reacting with antigens in donor's RBC. These antibodies are produced in the recipients' blood causing haemolysis (White *et al.*, 2016). Low antibody levels are one of the causes of delayed HTR and different antigens may give different characteristics. The Kidd and Rh antigens commonly cause delayed HTR where Kidd antigen may activate the complement system causing severe intravascular haemolysis. (Villa *et al.*, 2007) reported a case of fatal HTR due to anti-Jka antibody. Other antibodies which can cause intravascular haemolysis include anti-P, P1, Pk, H and M (Al-Riyami *et al.*, 2014).

In the Malaysian population, the most common Kidd phenotype is Jk(a+b+) while the rarer Kidd phenotype are Jk(a+b-), Jk(a-b+) and Jk(a-b-). Jk(a-b-) phenotype is rare in Malaysia (Musa *et al.*, 2012) and other countries (Marion *et al.*, 2004). Individuals with Jk(a-b-) Kidd phenotype is often detected during an antibody screening and antibody identification test where they may form anti-Jk3 after sensitisation following blood transfusion or pregnancy. Kidd antibody and antigen can be detected by urea lysis test or molecular technique (Remeikiene, 2014). The consequence of formation of anti-Jk3 in women is Haemolytic Disease of Foetus and Newborn (HDFN) in subsequent pregnancies (Qureshi *et al.*, 2014). Whilst the formation of anti-Jk3 antibody in an individual can cause severe HTR if he or she is transfused with either Jka or Jkb positive RBC (Yousuf *et al.*, 2014).

In all ethnic groups, Jk(a+b+) is the commonest Kidd phenotype where Nathalang O (2015) reported similar findings in Thai and Asian populations (Nathalang *et al.*,

2015). Musa *et al* (2012) reported the low prevalence of Jk(a-b-) phenotype in blood donors in National Blood Centre (NBC), Malaysia. Similar findings were also reported in the Polynesians and Japanese where they reported that Jk(a-b-) phenotype is rare (Marion *et al*., 2004, Marli *et al*., 2014).

Kidd antibodies may cause delayed HTR (BCSH Guideline, 2014). Fatal HDFN due to Kidd antibody was reported by Kim and Lee (2006) where an infant died on the fourth day of admission due to intracranial bleed and multiorgan failure despite optimum treatment, such as exchange transfusion and intensive phototherapy had been given. Anti-Jkb was found in maternal serum and eluate of infant's blood (Kim and Lee, 2006).

In Malaysia, severe HDFN due to anti-Jkb also has been reported by Yousof *et al*., 2012. Blood investigations revealed anti-Jkb in the maternal serum and eluate of infant's blood however the child survived despite severe manifestations (Yousuf *et al.*, 2012). Hassan *et al* (2014) studied the incidence of HDFN due to maternal RBC alloantibodies in the Malay population. It was found that 0.58% women were found to have clinically significant RBC alloantibodies. The highest alloantibody was Rh system with 56.7% (n=17) followed by MNS system 13.3% (n= 4) and Kidd system 10% (n=3) (Hassan *et al.*, 2014).

Al-Joudi *et al* (2011) suggested blood donors RBC profile should be matched with the patients RBC profile to prevent further alloimmunisation. Certain RBC phenotype are common to specific ethnic, race and geographic distributions (Al-Joudi *et al.*, 2011). It is best to supply match phenotype blood to prevent complication from blood transfusion therapy (Dogra *et al.*, 2015). For this reason, it is essential to increase blood

donors' RBC database from the same ethnic group as the patients', to meet the demand (Rogers *et al.*, 2015).

There are many requests for Kidd phenotype blood in Malaysia, especially for chronic anaemia, and transfusion dependent patients such as thalassaemia patients (Figures 1.1, 1.2 and Table 1.1). In Malaysia, based on records of National Blood Centre (NBC), there are very limited amount of frozen blood stock available especially from the Kidd blood group system.

Problem arises when regular RBC phenotyping of blood donors are only practised in NBC and not in all other blood collection centers in Malaysia. Hence, phenotyped blood is requested only from the NBC when needed. The blood requested may take several days to be supplied and transfused to the patients. This may lead to delay in patient management and may have consequences on patient prognosis and outcome.

Table 1.1 Data of phenotype blood requests at National Blood Centre, Kuala Lumpur for 2015 (Unpublished data report on phenotype blood request from Immunohaematology Department, National Blood Centre Kuala Lumpur, 2015).

Phenotype	**Total (n)**	**(%)**
Rh	4346	46.02
Kidd	1779	18.84
Duffy	596	6.31
MNS	1686	17.85
Lewis	512	5.42
Kell	137	1.45
P1	62	0.66
Bombay	5	0.05
Mut (Mia)	253	2.68
Others	67	0.71
	9443	**100**

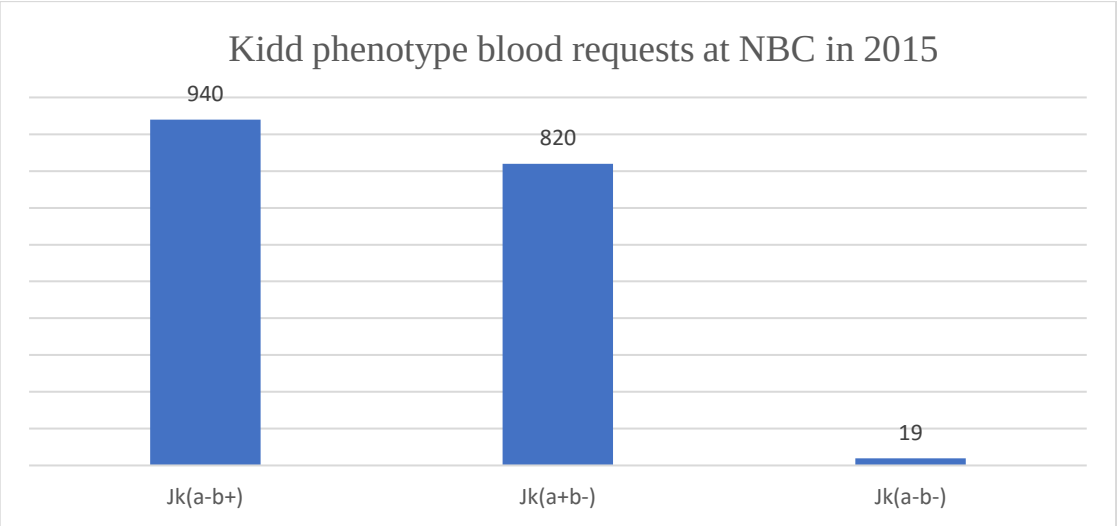

Figure 1.1. Kidd phenotyped blood requests at National Blood Centre, Kuala Lumpur in 2015, n=1779. (Unpublished data report on phenotype blood request from Immunohaematology Department, National Blood Centre, Kuala Lumpur, 2015).

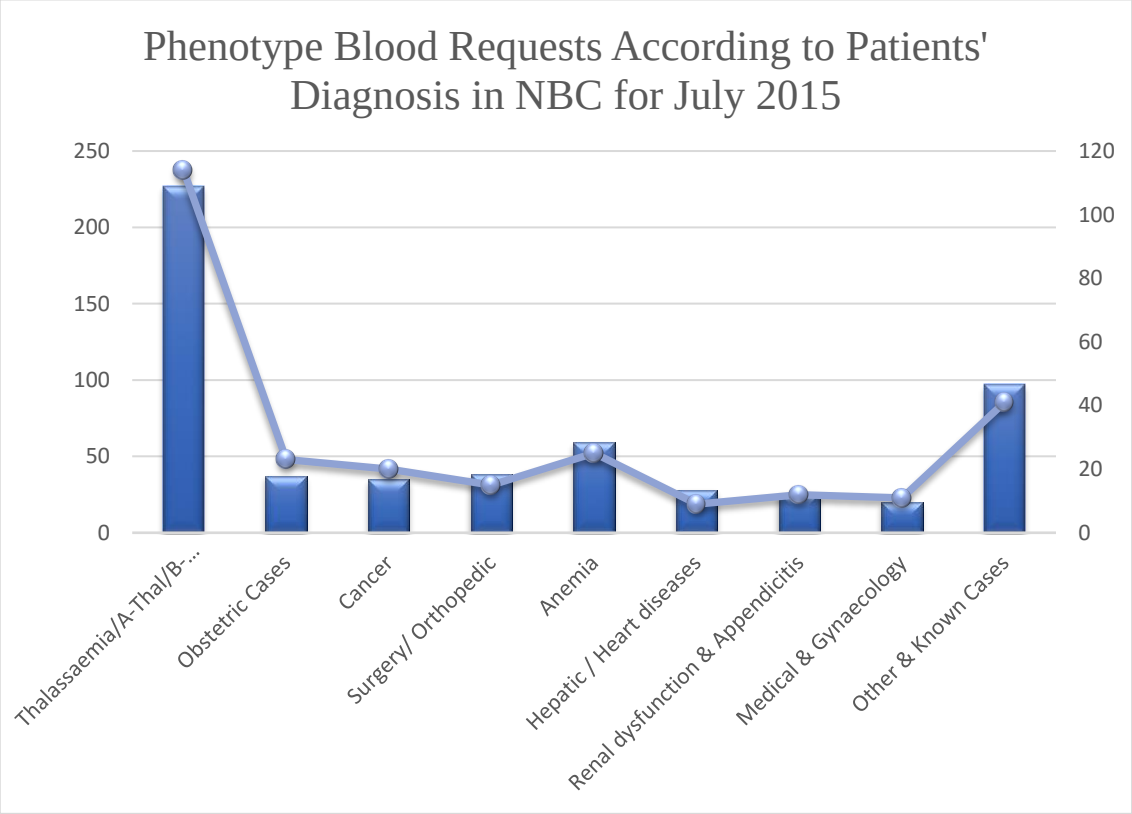

Figure 1.2 Phenotyped blood requests according to patients' diagnosis in National Blood Centre, Kuala Lumpur for July 2015, n=519 (Unpublished data report on phenotype blood request from Immunohaematology Departrment, National Blood Centre, Kuala Lumpur, 2015).

In Malaysia, the prevalence of Kidd alloimmunisation for cases of HDFN and HTR has been reported however there is insufficient data in Hospital Umum Sarawak (HUS). Therefore, the aim of this study is to identify Kidd alloimmunization that implicate HDFN and HTR. Additionally, the prevalence of Kidd phenotype in different ethnic groups of regular blood donors in HUS was investigated.

1.1 Rationale of Study

Sources of rare blood supply are from blood donors (fresh blood), patients' own siblings and frozen blood bank (Chaudhari, 2009). The target of this study is to increase rare blood donor pool in Malaysia registry and reduce the incidence of RBC alloimmunisation among transfusion dependent patients by giving matched phenotype blood.

There are many requests of Kidd phenotype blood in Malaysia, especially for chronic anaemia, haemato-oncology patients and transfusion dependent patients (thalassaemia patients), but in the NBC, there are very limited frozen blood (Kidd phenotype specific blood) to be supplied based on the statistic of phenotype blood request in NBC 2015. Since some types of Kidd phenotypes are rare among Malaysian population, the blood is kept in the freezer for many years.

Incompatible Kidd blood transfusion can cause severe acute or delayed HTR (Caamaño *et al.*, 2015). Blood with same phenotype is needed to prevent HTR (Al-Joudi *et al.*, 2011). In HUS Sarawak, random regular RBC phenotyping for blood donors is not practiced. Therefore, if patients are in need of Kidd phenotype blood, it has to be requested from the NBC.

When a rare blood type is requested for a patient, the rare donor registry in NBC can be utilized to locate donors negative for a high-prevalence antigen or negative for multiple common antigens to ensure the patient receives compatible blood. Hence, we should track and organize rare donor information in blood collection centres in an effort to better meet the needs of patients with rare blood types in Malaysia and whole Borneo.

When blood is phenotypically matched, the patient has a lower risk of developing complications from transfusion therapy (Dogra *et al.*, 2015). Hence, it is extremely important to increase the number of available blood donors from all ethnic groups to meet the patients' demand. In this study, we chose Sarawakian as a study subject. Indirectly, this study will help HUS to set their own rare donor registry and to supply matched phenotype blood to Sarawakian without delay.

CHAPTER 2

LITERATURE REVIEW

Kidd blood group is one of the major blood group system. Kidd antigens are located on a red blood cell urea transporter. It contains alleles Jka and Jkb. It has 4 Kidd phenotypes: Jk(a+b+), Jk(a+b-), Jk(a-b+) and Jk(a-b-) (Agarwal *et al.*, 2013). Jk(a-b-) is the rarest in most populations, found in 0.9% Polynesians (Marion *et al*., 2004).

The gene encoding Kidd phenotype protein is found on chromosome 18. The Kidd blood group system is important in transfusion medicine (White *et al.*, 2016). This can lead to haemolytic transfusion reaction (HTR), in which the body destroys the transfused blood, leading to low red blood cell counts. Another disease associated with the Kidd antigen is Haemolytic Disease of Foetus and Newborn (HDFN), in which a pregnant woman's body creates antibodies against the blood of her foetus, leading to destruction of the foetal blood cells (Velasco Rodríguez *et al.*, 2014).

Prevalence of red cell phenotype including Kidd phenotype has been published in many countries including Malaysia (Musa *et al.*, 2012), India (Joshi and Vasantha, 2012), China (Zhong *et al*., 2012), New York (Marion *et al*., 2004), Nigeria (Osaro *et al.*, 2015), Thailand (Nathalang *et al.*, 2015) and Japan (Marli *et al*., 2014). These studies found that the prevalence of the typed antigens among Malaysian blood donors to be statistically different to those in the Caucasian and Black populations, but more similar to the Asian racial groups.

Kidd antibody is the commonest cause of delayed HTR (Bolton-Maggs and Cohen, 2013). Delayed HTR generally occurs after 24 hours post blood transfusion. Affected patients could possibly develop fever, leucocytosis, sign and symptom of

anaemia, elevation of serum bilirubin and positive direct coomb's test reported by de Montalembert (de Montalembert *et al.*, 2011).

Delayed HTR occur after RBC sensitization. Either from previous blood transfusion or pregnancy. Kidd antibody has amnestic response if re-expose with the same Kidd antigen. Many cases of DHTR are unnoticed due to slow destruction of RBC (Rios *et al.*, 2011). Plaut in 1953 discovered anti-Jkb can cause DHTR (Tantalo *et al.*, 1989).

Management of HTR due to antibody mediated is to discontinue the transfusion while maintaining venous access for emergency management. Anticipate hypotension, renal failure, and Disseminated Intravascular Coagulopathy (DIC) (Sachan *et al.*, 2015). Prophylactic measures to reduce the risk of renal failure may include low-dose dopamine (1-5 mcg/kg/min), vigorous hydration with crystalloid solutions (3000 mL/m ^{2}/24 h), and osmotic diuresis with 20% mannitol (100 mL/m ^{2}/bolus, followed by 30 mL/m ^{2}/h for 12 h). If DIC is documented and bleeding requires treatment, transfusions of fresh frozen plasma, pooled cryoprecipitates for fibrinogen, and platelet concentrates may be indicated (Al-Riyami *et al.*, 2014).

A clerical check of the information on the blood unit label and the patient's identification should be performed to ensure that the "right" blood unit was administered to the "right" patient. The residual contents of the blood component container should be returned to the blood bank, together with a freshly collected blood sample from the patient, and a transfusion reaction investigation should be initiated by Transfusion Medicine blood bank team (WHO Blood Safety., 2011).

Konstad and Halvonrsen in 1958 reported the first case of HDFN caused by anti-Jkb. Keir (2014) studied regarding HDFN due to Kidd antibody which is IgG in nature that can cross the placenta and caused HDFN. It can activate complement system and leads to intra and extravascular haemolysis in infant (Keir *et al.*, 2014).

A case study about severe HDFN due to anti-Jkb was reported in Malaysia and Korea. Bennardello (2015) proved that the prevalence of HDFN depends on RBC antigen negative that differs within ethnic and population. Antenatally, the first indication of HDFN caused by Kidd antibody is the presence of anti-Jka or anti-Jkb in maternal serum by indirect coomb's test (Bennardello *et al.*, 2015).

Clinical symptoms of HDFN infant varies from mild to severe. In mild cases, infant may develop mild neonatal jaundice. In moderate to severe HDFN cases, infant frequently presented with severe neonatal jaundice, pallor and hepatosplenomegaly. Kernicterus is a complication of severe neonatal jaundice and may leads to neurological impairment (Illanes Sá, 2013). Antenatally, hydrop fetalis, polyhydramnion, pericardial effusion, pleural effusion, ascites, hepatosplenomegaly and placenta may be thickened in severe cases of HDFN (Black and Maheshwari, 2009). The full blood picture shows anaemia, nucleated red blood cell and polychromasia reported by Gupte S. C (Gupte, 2015).

The prevalence of HDFN caused by unexpected alloantibodies have been published in many countries including Malaysia (Hassan *et al.*, 2014), Japan (Okutsu *et al.*, 2011) and India (Pahuja *et al.*, 2011); with differing prevalence among these countries. In Malaysia, Rh and Kidd were the commonest antibodies causing HDFN while in India, it was mostly due to Rh antibody. In Japan, HDFN was mostly due to M and N antibodies.

Majority of HDFN is caused by high maternal antibody titre. Mild cases require close monitoring of patients while moderate to severe HDFN cases usually requires resuscitation, intensive support, transfusion and correction of metabolic acidosis (Illanes Sá, 2013).

The spectrum of HDFN has changed over the last few decades. With the implementation of RhD immunoprophylaxis, HDFN due to ABO incompatibility and other alloantibodies has now emerged as major causes of this condition. Though in developing countries, anti D is still a common antibody in pregnant women, many Asian countries have identified alloantibodies other than anti D as a cause of moderate to severe HDFN (Bennardello *et al.*, 2015).

Many developed nations have national screening programs for pregnant women. This is necessary to ensure timely availability of antigen negative blood and reduce effects on the newborn. Although universal screening seems justified, the cost and infrastructure required would be immense. Developing countries and under resourced nations need to consider universal antenatal screening and frame guidelines accordingly (Gupte, 2015).

The transfusion medicine services Malaysia are currently studying the implications and feasibility to implement a more comprehensive screening programme of blood groups and antibodies in pregnant women to enable early foetal-neonatal intervention (Yousuf *et al.*, 2012). In addition, knowledge of the RBC phenotype frequency in a population with different ethnic origins can contribute in creating a donor data bank. The database on the distribution of blood groups are essential in providing compatible blood for patients with multiple alloantibodies to reduce the RBC alloimmunisation and complications (Al-Joudi *et al.*, 2011).

Furthermore, blood banks may also maintain a rare blood type database from amongst their regular voluntary donors and it may be practical to develop cryopreservation facilities for rare donor units (Musa *et al.*, 2012).

2.2 Conceptual Framework

Figure 2.1 and Figure 2.2 are conceptual framework developed for this study. Clinical manifestations due to Kidd antibody can be assessed by using indicators such as types of Kidd antibody, types of clinical manifestations (HDFN, HTR and others), and history of blood transfusion in affected patients. This study examined the demographic profile of regular blood donors in Hospital Umum Sarawak to determine associated factors with Kidd phenotypes. The demographic profiles are ABO blood group, Kidd phenotype and ethnicity of blood donors.

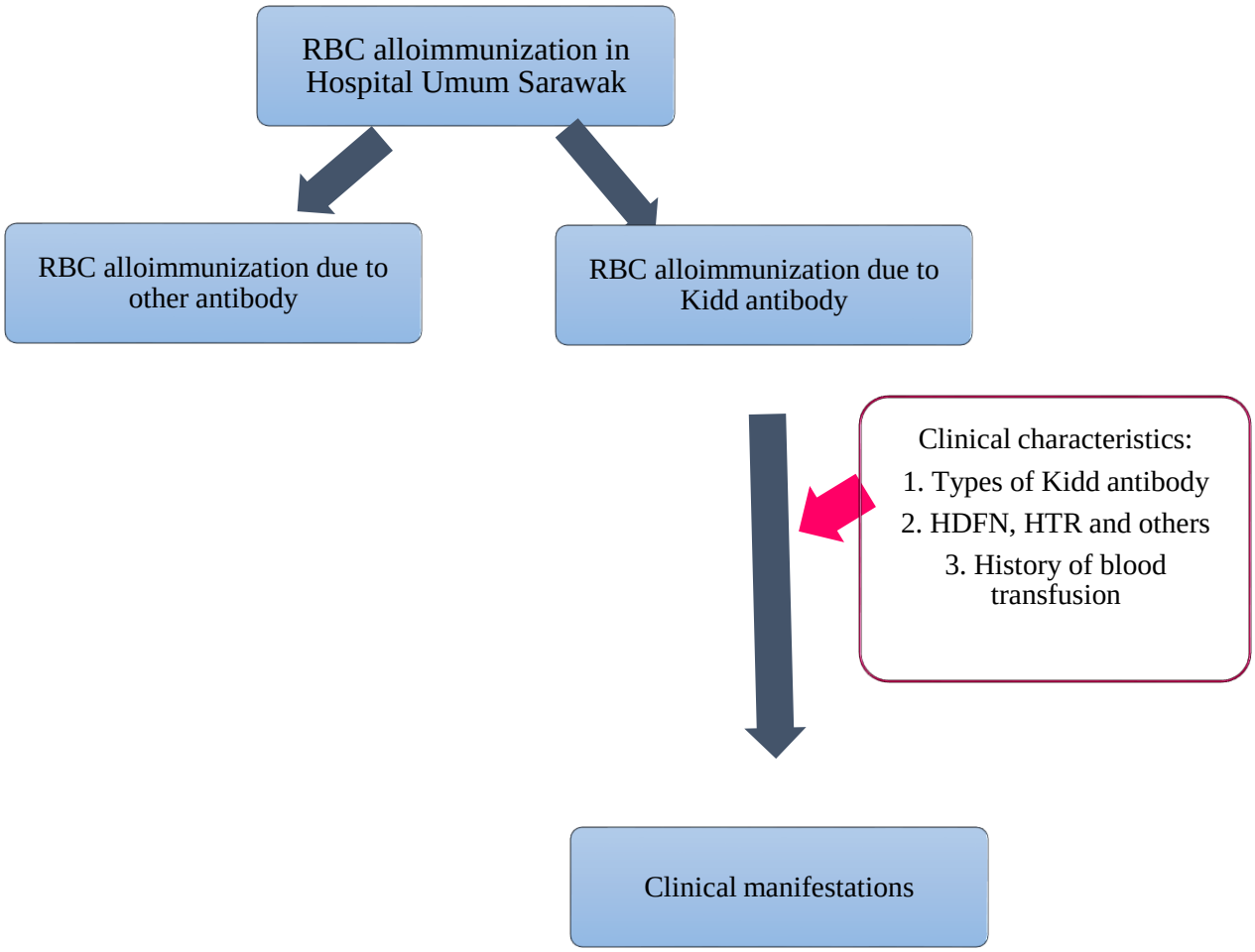

Figure 2.1: Conceptual framework of the study (Kidd antibody). Clinical manifestations due to Kidd antibody can be assessed by using indicators such as types of Kidd antibody, types of clinical manifestations (HDFN, HTR and others), and history of blood transfusion in affected patients.

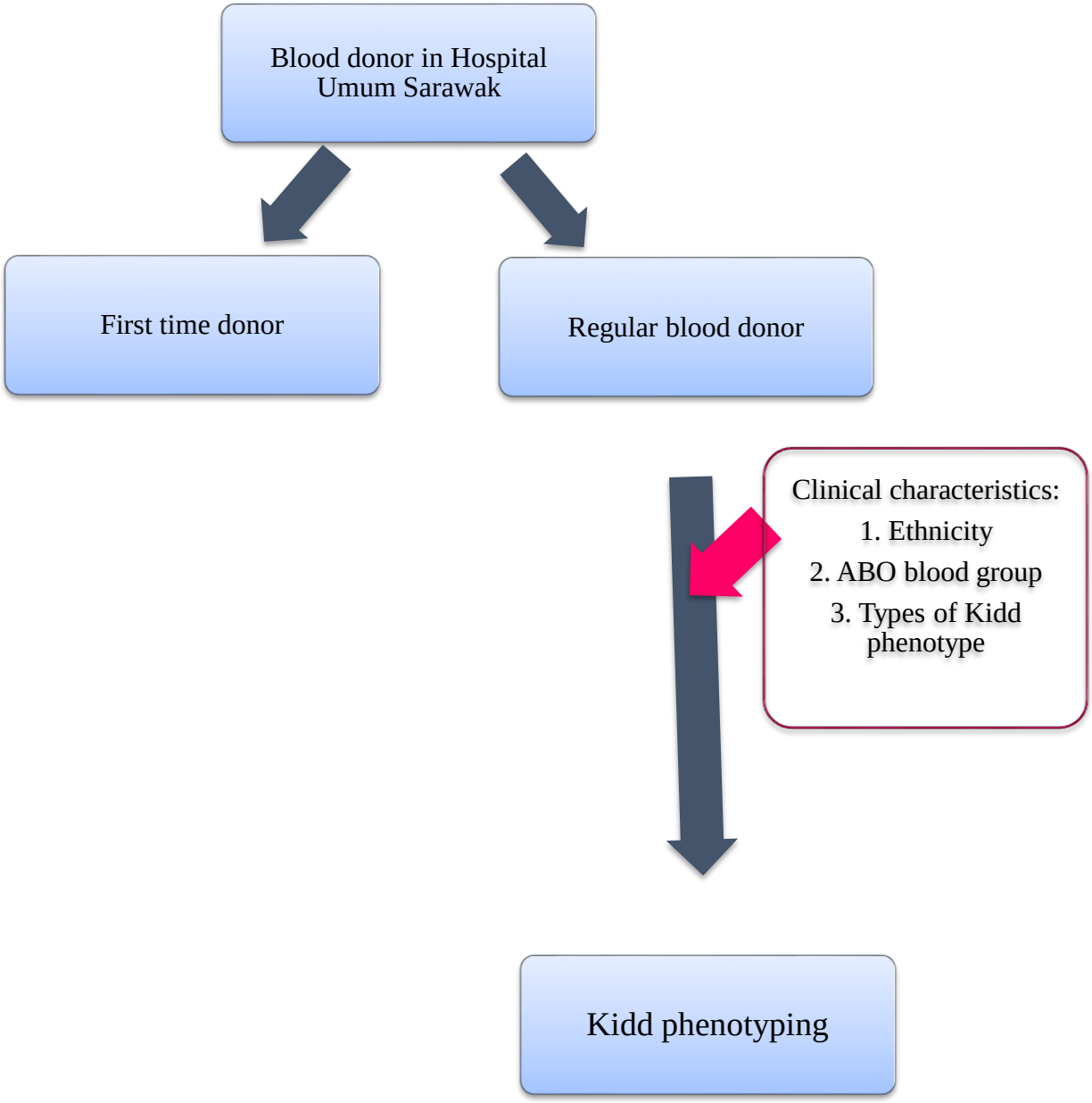

Figure 2.2: Conceptual framework of the study (Kidd phenotype). This study examined the demographic profile of regular blood donors in Hospital Umum Sarawak (HUS) and to determine the associated factors with Kidd phenotypes. The demographic profiles are ABO blood group, ethnicity and barcode of blood donation.

CHAPTER 3

RESEARCH QUESTIONS, OBJECTIVES AND HYPOTHESIS

3.1 Research Question

1. What is the prevalence of Kidd alloimmunisation for Haemolytic Disease of Foetus and Newborn (HDFN) and Haemolytic Transfusion Reaction (HTR) in Hospital Umum Sarawak (HUS)?
2. What are the characteristics of regular blood donors by ethnicity, ABO blood group and Kidd phenotypes in HUS?
3. What is the relationship between ABO blood group, Kidd phenotype and ethnicity among regular blood donors in HUS?

3.2 Objectives

3.2.1 General Objective

To determine the prevalence of Kidd alloimmunization for cases of HDFN and HTR and to investigate the frequency of ABO blood group, Kidd phenotype and its association with ethnicity in regular blood donors in HUS.

3.2.2 Specific Objectives

1. To determine the prevalence of HDFN cases among Kidd antibody in HUS.
2. To determine the prevalence of HTR cases among Kidd antibody in HUS.
3. To determine the frequency of ABO blood group, Kidd phenotype and its association with ethnicity in regular blood donors in HUS.

3.3 Study Hypotheses

Null hypothesis

1. There is no prevalence of HDFN among Kidd antibody cases in HUS.
2. There is no prevalence of HTR among Kidd antibody cases in HUS.
3. There is no significant difference between ABO blood group, Kidd phenotypes and ethnicity among regular blood donors in HUS.

Alternative hypothesis

1. There is prevalence of HDFN among Kidd antibody cases in HUS.
2. There is prevalence of HTR among Kidd antibody cases in HUS.
3. There is significant difference between ABO blood group, Kidd phenotypes and ethnicity among regular blood donors in HUS.

CHAPTER 4

MATERIALS AND METHODS

4.1 Study Design

This study design is a combination of retrospective and cross sectional study.

4.2 Study Duration

The study duration period was from 1st July 2015 until 30th June 2016. Retrospectively, data from 1st January 2011- 31th December 2014 (4 years) was collected and analysed. Cross sectional study for Kidd phenotyping, sample from 1stSeptember 2015 until 10th September 2015 was collected and analysed.

4.3 Study Location

This study was conducted in Blood Bank of Hospital Umum Sarawak (HUS). HUS is the largest hospital in the state of Sarawak, Malaysia. It is the main tertiary and referral hospital in Sarawak. HUS currently has more than 1000 beds and offers many specialisations services. HUS offers subspecialties in Medicine: nephrology, dermatology, neurology, rheumatology, chest medicine, cardiology, infectious diseases, gastroenterology and haematology.

4.4 Reference Population

All blood donors in HUS and all antibody positive in HUS.

4.4.1 Source Population

All regular blood donors in HUS and all Kidd antibody positive patients in HUS.

4.4.2 Sampling Frame

Two hundred fifty (250) healthy regular blood donors who donated in Blood Bank HUS was studied. All eligible blood donors who were fit to donate blood were approached for this study. They were explained regarding this study and informed consent were taken. Before undergoing blood donation all subjects were required to fill Donor History Questionnaire (Appendix J) and were examined by a qualified medical doctor. Only subjects who pass the medical examination were enrolled into the study to ensure the safety of subjects. Each subject was anonymised to ensure confidentiality.

Retrospectively, all Kidd alloimmunisation for cases of HTR, HDFN and others that was diagnosed by doctors in HUS since 2011 until 2014 was collected and analysed. The detailed medical history was extracted from patients' medical record and kept in data proforma.

4.5 Inclusion and Exclusion Criteria

Inclusion criteria for patients:

a) Alloimmunization positive cases due to Kidd Antibody.
b) Kidd antibody for HTR cases.
c) Kidd antibody for HDFN cases.

Inclusion criteria for blood donors:

a) Regular blood donor- defined as blood donor who has donated their blood yearly with negative result for all virology screening markers.

b) Eligible blood donor who fulfilled the criteria for blood donor as stated in National Guidelines for blood donation. (Transfusion Practice Guideline for Clinical and Laboratory Personnel 3rd edition 2008).

Exclusion criteria for patients:

a) Alloimmunisation positive cases caused by other antibody.
b) Other antibody except Kidd that cause HTR.
c) Other antibody except Kidd that cause HDFN.
d) Cases of Kidd autoantibody.

Exclusion criteria for blood donors:

a) First time blood donors
b) Foreigner
c) Non-eligible blood donor according to National Guidelines for blood donation. (Transfusion Practice Guideline for Clinical and Laboratory Personnel 3rd edition 2008).

4.6 Sample Size Calculation

Sample size for Kidd antibody: According to presence data of Kidd antibody positive in antibody identification in HUS for 4 years (from 2011 until 2014).

Sample size calculation for Kidd antibody using single proportion,

p=0.1 (10% of total RBC alloantibodies, cited by (Hassan *et al.*, 2014)

$N = (z/\Delta)^2 \times p(1-p)$ P=0.6 , z=1.96 Δ=20%

$= (1.96/0.2)^2 \times 0.1(1-0.1)$ + 20% drop out

= 96.04 x 0.09

= 8.64 + 20% (1.72)

= 10

Sample size calculation for Kidd antigen: Sample was calculated based on proportion from a study done by Williams *et. al* (Williams *et al.*, 2011).

a = 0.05

Power = 0.8 (80%)

Po= 0.1

P1= 0.2 (20% of total population; cited by Musa *et al*, 2012 (Musa *et al.*, 2012)

m= 1

n= 219

+-10% drop out= 219+22

Sample size =241

OR Sample size manually calculation, using Pocock's Formula for two proportions.

$$n=\frac{p_1(1-p_1)+p_0(1-p_0)}{(p_1-p_0)^2} \times (z_\alpha + z_\beta)^2$$

$$=\frac{[0.245(1-0.245)+0.1(1-0.1)]}{(0.245-0.1)^2} \times (1.96+0.84)^2$$