

**STUDY ON ABO/RHD BLOOD GROUP AND RED BLOOD CELL
IMMUNIZATION AMONG CHRONIC LIVER DISEASE PATIENTS IN
HOSPITAL UNIVERSITI SAINS MALAYSIA**

BY

DR SITI ZALEHA S.ABDULLAH

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



UNIVERSITI SAINS MALAYSIA

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LIST OF ABBREVIATIONS

Anti	Antibody
AIH	Autoimmune hepatitis
ALD	Alcoholic liver disease
Anti-Le^a	Anti-Lewis a
Anti-Le^b	Anti-Lewis b
Anti-Fy^b	Anti-Duffy b
DM	Diabetes mellitus
CLD	Chronic liver disease
CKD	Chronic kidney disease
Hb	Hemoglobin
Hpt	Hypertension
HDFN	Hemolytic disease of fetus and newborn
HTR	Hemolytic transfusion reaction
USM	Universiti Sains Malaysia
IgG	Immunoglobulin G

IgM	Immunoglobulin M
LISS	Low ionic strength saline
MLD	Metabolic liver disease
Plt	Platelet
PRBC	Pack red blood cell
RBC	Red blood cell
Rh	Rhesus
RhD	Rhesus D
TLD	Toxic liver disease
WBC	White blood cell

ABSTRAK

KAJIAN MENGENAI KUMPULAN DARAH ABO/RHD DAN IMUNISASI SEL DARAH MERAH DALAM KALANGAN PESAKIT PENYAKIT HATI KRONIK DI HOSPITAL UNIVERSITI SAINS MALAYSIA

Latar belakang: Kumpulan darah ABO dan Rhesus (Rh) telah menunjukkan beberapa kaitan dengan pelbagai penyakit. Namun, hubungkait antara kumpulan darah ABO / RhD dengan penyakit hati kronik (PHK) masih tidak diketahui. Tambahan pula, pesakit PHK biasanya mengalami komplikasi anemia yang kadangkala memerlukan pemindahan darah dan meletakkan mereka berisiko untuk alloimunitisasi sel darah merah (SDM). Terdapat variasi dalam kekerapan alloimunitisasi SDM di kalangan populasi pesakit yang berbeza. Oleh itu, kajian ini bertujuan untuk menentukan hubungkait antara kumpulan darah ABO / RhD dengan PHK dan kadar imunitisasi SDM dengan faktor risiko yang berkaitan di kalangan pesakit PHK di pusat kami.

Bahan dan Kaedah: Ini adalah kajian kawalan kes yang melibatkan 441 pesakit PHK yang sedang dirawat di Hospital Universiti Sains Malaysia dan menjalani ujian pra-transfusi dari April 2012 hingga April 2022. Data klinikal dan makmal yang diambil daripada sistem maklumat bank darah dan rekod pesakit dianalisis secara statistik menggunakan regresi logistik berganda. $P < 0.05$ dianggap signifikan

Keputusan: Seramai 441 pesakit telah dimasukkan dalam kajian kami dengan majoriti adalah warga emas dengan purata umur pesakit 57.9 (SD±12.1) tahun, lelaki (65.1%) dan Melayu (92.1%). Penyebab PHK yang paling kerap ditemui di pusat kami ialah hepatitis virus (62.1%) dan penyakit hati metabolik (25.4%). Kekerapan kumpulan darah O dan B

diagihkan hampir sama di kalangan pesakit PHK, dengan 31.5% dan 31.3%, masing-masing. Kumpulan darah bukan-O didapati mempunyai kaitan yang signifikan dengan peningkatan risiko PHK (OR diselaraskan = 1.65, $P=0.027$). Dua puluh empat dan dua pesakit PHK dilaporkan mempunyai alloantibodi SDM dan autoantibodi dengan prevalens masing-masing 5.4% dan 0.5%. Kebanyakan pesakit mempunyai alloantibodi tunggal (83.3%) dan tergolong dalam kumpulan darah Rh iaitu anti-E (35.7%) dan anti-c (14.3%), diikuti oleh anti-Mi^a (17.9%) dalam kumpulan darah MNS. Tiada hubungkait faktor yang signifikan terhadap alloimunisasi SDM di kalangan pesakit PHK yang dikenal pasti.

Kesimpulan: Kumpulan darah bukan O didapati mempunyai kaitan yang signifikan dengan peningkatan risiko PHK. Kelaziman alloimunisasi SDM di kalangan pesakit PHK adalah rendah, tetapi majoriti daripada mereka mendapat alloantibodi SDM yang signifikan secara klinikal, kebanyakannya daripada kumpulan darah Rh. Oleh itu, semua pesakit PHK yang memerlukan pemindahan darah disyorkan untuk fenotaip Rh dan dibekalkan dengan fenotip Rh yang sepadan untuk mencegah alloimunisasi Rh.

ABSTRACT

STUDY ON ABO/RHD BLOOD GROUP AND RED BLOOD CELL IMMUNIZATION AMONG CHRONIC LIVER DISEASE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA.

Background: ABO and Rhesus (Rh) blood groups showed some association with various diseases. However, whether ABO and Rh blood group is associated with chronic liver disease (CLD) remains unknown. Furthermore, CLD patients commonly complicated with anemia require blood transfusion support which puts them at risk for red blood cell (RBC) alloimmunization. Variations in the frequency of RBC alloimmunization have been noted among different patient populations. Thus, this study aimed to determine the association of ABO/RhD blood grouping with CLD, the rate of RBC immunization, and their associated risk factors among CLD patients in our center.

Materials and Methods: This is a case-control study involving 441 patients with CLD treated at Hospital Universiti Sains Malaysia and subjected to pre-transfusion testing from April 2012 until April 2022. Clinical and laboratory data were retrieved from the blood bank information system and patient's record and were statistically analyzed using multiple logistic regression. $P < 0.05$ is considered significant.

Results: A total of 441 patients were included in our study. The majority of patients were elderly, with a mean age of 57.9 (SD 12.1) years old, male (65.1%), and Malay (92.1%). The most common causes of CLD in our center were viral hepatitis (62.1%) and metabolic liver disease (25.4%). The frequency of O and B blood groups was almost equally distributed among CLD patients, at 31.5% and 31.3%, respectively. The non-O blood

group was significantly associated with an increased risk of CLD (adjusted OR = 1.65, P=0.027). Twenty-four and two CLD patients were reported to have RBC alloantibodies and autoantibodies with a prevalence of 5.4% and 0.5%, respectively. Most patients developed a single alloantibody (83.3%) and belonged to the Rh blood group, which were anti-E (35.7%) and anti-c (14.3%), followed by anti-Mia (17.9%) in the MNS blood group. There was no significant factor associated with RBC alloimmunization among CLD patients identified.

Conclusion: The non-O blood group was significantly associated with an increased risk of CLD. Although the prevalence of RBC alloimmunization among CLD patients was low, the vast majority developed clinically significant RBC alloantibodies, mostly from the Rh blood group. Therefore, all CLD patients requiring blood transfusion are recommended for Rh phenotyping and supplied with phenotype-matched Rh to prevent Rh alloimmunization.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Globally, 1.5 billion people are diagnosed with chronic liver disease (CLD) with an estimated incidence of cirrhosis in Southeast Asia being 23.6/100,000 (James et al., 2018). CLD is a set of diseases that are characterized by reduced hepatic function due to chronic inflammation or liver insult (Hope & Morrison, 2012). Progressive deterioration of liver functions occurs for more than six months, involving the synthesis of coagulation factors and other proteins, detoxification of unhealthy metabolism products, and bile excretion (Sharma A, 2020). The spectrum of etiologies is broad, including chronic alcohol consumption, infection, autoimmune diseases, genetics, and metabolic disorders (Moon et al., 2020; Sharma A, 2020; Sharma & Nagalli, 2022).

Only a few reports were available on the epidemiology of CLD in Malaysia. One study suggested that the landscape of liver diseases in Malaysia had changed dramatically. The major causes of cirrhosis were chronic hepatitis B (46.1%), chronic hepatitis C (18.5%), cryptogenic (15.4%), alcoholic liver disease (12.6%), and autoimmune hepatitis (AIH) (2.0%). Alcohol was the main etiology among Indians (51.1%), and hepatitis B was the predominant etiology in Malay (47.9%) and Chinese (58.8%) (Qua & Goh, 2011).

ABO and Rhesus (Rh) blood groups had been of great importance in different disease studies. It had shown to have some association with various non-infectious (Abegaz, 2021; Naeini et al., 2010; Zhong et al., 2019) and infectious diseases (Abegaz, 2021; Naeini et al., 2010). Few studies observed an association between the ABO blood group and the risk of certain malignancies, thromboembolic and cardiovascular disease (Pandey et al., 2014). A previous study reported that the non-O blood group is an independent risk factor for the progression of liver fibrosis in Hepatitis C virus (HCV) infection (Poujol-

Robert et al., 2006). In another study, ABO blood groups were found to be a risk factor for Hepatitis B virus (HBV) infection (Wang et al., 2012).

One study on the ABO blood group and the risk of non-alcoholic fatty liver disease (NAFLD) in the Han Chinese population concluded that non-O blood groups were significantly associated with an increased risk of developing NAFLD (Zhong et al., 2019). Their findings provided some epidemiological evidence for a possible role of ABO glycosyltransferase in the pathogenesis of NAFLD.

CLD of any cause is frequently associated with hematological abnormalities including anemia which is commonly seen in patients with advanced CLD due to multiple causes; reduced red blood cell (RBC) lifespan, hemolytic anemia as well as bleeding due to varices, thrombocytopenia and coagulation factor deficiency (Gkamprela et al., 2017). Current guidelines recommend blood transfusions to a goal of 7–8 g/dL in patients with cirrhosis (Angeli et al., 2018; Kakkar et al., 2021). As the survival of CLD patients has greatly increased with the advancement of medical treatment, they may require repeated blood transfusions throughout the course of their illness and may risk RBC alloimmunization (Bajpai et al., 2016).

RBC alloimmunization is a common and undesirable outcome, particularly among patients with a regular blood transfusion that occurs as a response of the recipient's immune system to foreign RBC antigens, particularly in chronically transfused patients (Bajpai et al., 2016). These antibodies may be clinically significant, leading to hemolytic transfusion reaction (HTR) or hemolytic disease of the fetus and newborn (HDFN) (Hendrickson & Tormey, 2016). RBC alloantibody may also cause some technical issues that lead to difficulty and delay in providing compatible blood (Tormey & Hendrickson,

2019). Therefore, RBC alloantibody detection and identification are critical in transfusion practice to provide antigen-negative blood to patients.

Nevertheless, exposure to a foreign antigen does not always result in an immune response in the vast majority of blood transfusion recipients (Qayyum et al., 2017). Few recent studies suggested that RBC alloimmunization is a multifactorial immune response that necessitates the presence of significant contributing factors simultaneously (Qayyum et al., 2017). Additionally, the frequency of RBC alloimmunization differs among all patients. Significant variations had been noted in different patient populations worldwide (Debnath et al., 2021; Hendrickson & Tormey, 2016). Another study concluded that the risks of developing RBC alloantibodies were complex and involve at least 3 main contributing elements: The RBC antigenic difference between the blood donor and the recipient; the recipient's immune status; and the immunomodulatory effect of the allogeneic blood transfusions on the recipient's immune system (Yazdanbakhsh et al., 2012).

Other factors that may contribute to the development of RBC alloimmunization include the person's age, sex, frequency of transfusions, as well as immunogenicity of the RBC antigen (Bilwani et al., 2015; Philip et al., 2014; Yousuf et al., 2013). Bajpai et al. studied RBC alloimmunization in multi-transfused CLD patients and revealed that CLD patients had an overall statistically significant impact on the rate of RBC alloimmunization (Bajpai et al., 2016). Few studies on multiple packed red blood cells (PRBCs) transfusion and alloimmunization showed a significant association between the mean number of PRBCs transfusions and the number of alloantibodies formed because a higher number of transfusions will increase the possibility of encountering a foreign antigen (Philip et al., 2014; Yangdon, 2019).

A better understanding of the risk factor of RBC alloimmunization is needed to optimize prevention strategies and increase transfusion safety (Hendrickson & Tormey, 2016). Many previous studies have concluded that preventing RBC alloantibody formation may prevent HTR, increases life expectancy, and reduce the amount of blood transfusion requirement (Bilwani et al., 2015; Hendrickson & Tormey, 2016; Kim et al., 2019; Nickel et al., 2016; Verduin et al., 2012).

Besides the risk of RBC alloimmunization, CLD patients also had been reported to develop autoimmune hemolytic anemia (AIHA) due to the high frequency of various autoantibodies, especially among patients with chronic HCV infection and AIH (Okazaki et al., 2015). To date, the frequency and significance of RBC autoantibodies have not been explored. Therefore, many studies in this area are needed to determine the significance of this medically treatable anemia seen in CLD patients.

In Malaysia, multiple ethnicities have caused genetic heterogeneity among the population, leading to a wide variation of antibody specificity (Haslina et al., 2019; Yousuf et al., 2013). So far, there is still limited study on the association of the ABO/RhD blood group with CLD and RBC immunization in CLD patients. Thus, this study aimed to determine the association of the ABO/RhD blood group with CLD, the rate of RBC immunization, and their associated risk factors among CLD patients in our center. To the best of our knowledge, this is the first study conducted in Malaysia.

Overall, the result of this study will help in a better understanding of the association between the ABO/RhD blood group and the development of CLD, as well as the associated risk factors for the development of RBC immunization among CLD patients. As the RBC alloimmunization rates vary with disease status, the benefit of extended or selected antigen matching as preventive measures for RBC alloantibody formation in

CLD patients for transfusion management will then be determined. Hopefully, the results of this study will be beneficial in the blood transfusion management of CLD patients.

1.2 Objectives of the study

General Objective:

To study on ABO/RhD blood group and RBC immunization among CLD patients in Hospital USM.

Specific Objectives:

1. To determine association of ABO and RhD blood group with CLD in Hospital USM.
2. To determine association of demographic (age, gender, ethnic group), laboratory (Hb level, platelet count, number of transfusion) and clinical factors (patient's comorbid, types of CLD) with RBC immunization among CLD patient in Hospital USM.

CHAPTER 2

STUDY PROTOCOL

2.1 Full study protocol

Research title: Study on ABO/RhD Blood Group and Red Blood Cell Immunization among Chronic Liver Disease Patients in Hospital Universiti Sains Malaysia.

Researcher: Dr. Siti Zaleha S.Abdullah (MPM No: 61047)

Supervisor: Dr. Mohd Nazri Bin Hassan (MPM No: 39403)

Co-Supervisor: Dr. Marini Binti Ramli (MPM No: 37647)

2.1.1 Introduction and study background

Chronic liver diseases (CLDs) are a group of diseases that are characterized by reduced hepatic function due to chronic inflammation or liver insult. (Hope & Morrison, 2012). It is a progressive deterioration of liver functions for more than six months, involving the synthesis of coagulation factors, other proteins, detoxification of unhealthy metabolism products, and bile excretion. Recent estimates suggest that approximately 5.5 million persons suffer from CLD worldwide and as many as 40,000 persons will die of its natural course or complications. The majority of cases of CLD result from complications of alcohol and viral hepatitis, which together account for more than 75% of all cases (Hope & Morrison, 2012). The spectrum of etiologies is broad, and includes chronic alcohol abuse, infection, autoimmune diseases, as well as genetic and metabolic disorders.

Medically, the ABO and Rhesus (Rh) blood group system has been of great importance in different disease studies (Abegaz, 2021). Human ABO blood type antigens are genetically derived glycoconjugate structures that are located on the red blood cells (RBCs) surface which play an active role in the cells' physiology and pathology. Furthermore, oligosaccharide structures specific to the antigens defined the blood type of

a person. These carbohydrate components are perceived as foreign by the immune system of other individuals and produce antibodies to them (Abegaz, 2021). ABO and Rh blood groups have been shown to have some association with various infectious (Naeini et al., 2010; Wang et al., 2012) and non-infectious and infectious diseases (Abegaz, 2021). So far, there is limited study regarding the association of ABO/Rh D blood group with CLD. Previous study reported that the non-O blood group is an independent risk factor for the progression of liver fibrosis in HCV infection (Poujol-Robert et al, 2006). In another study, ABO blood groups were found to be a risk factor for HBV infection (Wang et al., 2012).

CLD of any cause is frequently associated with hematological abnormalities. Among these, anemia is a frequent occurrence, seen in 75% of patients with advanced CLD. The etiology of anemia, especially in cirrhotic patients, is complex and multifactorial. Various factors causing anemia in CLD include reduced RBC life due to increased lipid deposition in the membrane leading to macrocytosis and hemolytic anemia, splenic pooling of RBC in splenomegaly, bleeding varices, and acquired coagulation abnormalities (Gkamprela et al., 2017).

A major cause of anemia associated with CLD is hemorrhage, especially in the gastrointestinal tract. Currently, with the advances in medical treatment of CLD, the survival of individuals has greatly increased but the mainstay of treatment of anemia is blood transfusion. Blood transfusion support is crucial for the management of patients with CLD. These patients may require repeated blood transfusions during the course of the disease (Gkamprela et al., 2017).

Frequent blood transfusions may lead to many complications including RBC alloimmunization and transfusion-associated immunomodulation (Bajpai et al., 2016).

RBC alloimmunization causes induction of immunity in response to the foreign antigen on RBCs resulting in the production of clinically significant alloantibodies. Overall, these clinically significant alloantibodies may result in hemolytic transfusion reactions (HTR), hemolytic disease of fetus and newborn (HDFN), difficulty in cross-matching blood, decrease in RBC survival, and an increased transfusion requirement recipient. Moreover, the downstream effects of RBC clearance can lead to multiple organ failure, electrolyte disruption, coagulopathy, and death in some cases. Thus, humoral alloimmunization of RBC antigens is a major clinical concern (Pandey et al., 2014).

Alloimmunization make further blood transfusion troublesome as extensive matching of donor need to be done to provide antigen free blood component to the recipient for which alloantibodies are formed (Qayyum et al., 2017). Preventive methods vary between different medical centers. In the first approach, an extended RBC phenotype (ABO, Rh, Kell, Kidd, Duffy, Lewis, MNS) of the patient is performed before commencing blood transfusion for patients without prior alloantibody formation. However, the cost and the feasibility may not permit in some transfusion centers. The second approach involves transfusing matched blood only for patients who already developed clinically significant antibody, as this may reduce expenses and increase the availability of matched blood (Pandey et al., 2014).

Besides the risk of RBC alloimmunization, CLD patients also have been reported to develop autoimmune haemolytic anaemia (AIHA) because there is high frequency of various autoantibodies among patients with chronic HCV liver diseases and autoimmune hepatitis (AIH). However, the frequency and significance of anti-RBC autoantibodies have not been explored yet (Okazaki et al., 2015).

So far, there is still limited study on the association of ABO/RhD blood grouping with CLD and RBC immunization among CLD patients. Thus, the aim of this study is to determine the association of ABO/RhD blood grouping with CLD and also to determine the rate of RBC immunization and their associated risk factors among CLD patients. Hopefully, the results of this study will be helpful in the blood transfusion management of CLD patients.

2.1.2 Rationale/ Justification of the study

The rationale of this study is because of limited the data on the association of ABO/Rh blood group with CLD as well as RBC immunization in CLD patients. There is no previous study that had been done in Malaysia. There was one similar study among Chinese population, however it only involved small numbers of patient with non-alcoholic fatty liver disease (NAFLD). Compared to this study, it will include all patient with CLD. There was one study that had been done among Korean population involving risk factors of RBC alloimmunization in liver cirrhosis. As compared to this study, it involved an adult with chronic liver disease who had been admitted to Hospital USM. The sample for this study is bigger compared to previous study.

Furthermore, this study will not only focus on of RBC alloimmunization but also RBC autoantibodies which may also contribute to chronic anemia in CLD patients which may be left unrecognized in a number of patients. Therefore, many studies in this area are definitely needed to determine the significance of this medically treatable anemia seen in patients with CLD.

Overall, the findings of this study will contribute to a better understanding of the relationship between the ABO/Rh D blood group and the development of CLD, as well as the risk factors associated with the development of RBC immunization in CLD

patients. As the rate of RBC immunization varies between diseases, the benefit of extended or selected antigen matching for blood transfusions in CLD patients will then be determined.

2.1.3 Research questions

1. 1. Does patient with specific blood group have a significant risk of developing CLD?
2. 2. Is there any association between antibody development in chronic liver disease patient with other factors such as age, gender, race, Hb level, platelet count, type and stage of chronic liver disease and number of packed red blood cell or platelet transfusion?

2.1.4 Hypotheses

1. 1. There is a significant association between ABO and Rh D blood group with development of CLD in Hospital USM.
2. 2. There is a significant association between factors such as age, gender, race, Hb level, platelet count, type and stage of chronic liver disease and number of packed red blood cell or platelet transfusion in developing RBC immunization among liver disease patients in Hospital USM.

2.1.5 Objectives

General objective: To study on ABO/Rh D blood group and RBC immunization among CLD patient in Hospital USM.

Specific objective:

1. To determine association of ABO and Rh D blood group with CLD in Hospital USM.
2. To determine association of demographic (age, gender, race), laboratory (Hb, platelet, number of transfusion) and clinical factors (type, stage of CLD) with RBC immunization among CLD patient in Hospital USM.

2.1.6 Literature review

There have been only few reports on the epidemiology of CLD in Malaysia. None has reported regarding the incidence of CLD in Malaysia. There were ethnic and sex differences in the prevalence of the different aetiological types of chronic liver disease. The landscape of liver diseases in Malaysia has changed dramatically (Qua & Goh, 2011). Previously, alcoholic liver disease was the commonest, occurring in 36% (29 patients) followed by the idiopathic type (34%) and hepatitis B (33%). Alcoholic liver disease was commoner in Indians while the hepatitis B associated type was the commonest type in the Chinese (Khairullah & Merican, 2004).

From more recent study shown the etiology of cirrhosis has a peculiar pattern based on racial differences in alcohol intake and in the prevalence of hepatitis B. A total of 460 patients were included in the study: The major causes of cirrhosis were: chronic hepatitis B, 46.1%; chronic hepatitis C, 18.5%; cryptogenic, 15.4%; alcohol, 12.6% and autoimmune 2.0%. Alcohol was the main etiology in Indians (51.1%) compared to Malay (0%) and Chinese (4.4%) (both $P < 0.001$). Hepatitis B was the predominant etiology in Malay (47.9%) and Chinese (58.8%) compared to Indians (5.6%) (both $P < 0.001$) (Qua & Goh, 2011). Approximately 85% CLD cases seen in Malaysia are due to HBV

infection, 14% are due to hepatitis C virus (HCV) infection, and 10% are a result of mixed HBV and HCV infection, (Khairullah & Merican, 2004).

In recent years, many studies have observed an association between the ABO blood group and risk of certain malignancies, thromboembolic and cardiovascular disease (Pandey et al., 2014). However, there are lack of studies on the association with chronic liver disease risk available.

There is one study among Han Chinese population (Zhong et al, 2019) regarding ABO blood group and risk of newly diagnosed non-alcoholic fatty liver disease (NAFLD). They concluded that non-O blood groups are significantly associated with an increased risk of NAFLD. Their findings provide some epidemiological evidence for a possible role of ABO glycosyltransferase in the pathogenesis of NAFLD. However, these findings need to be validated by future studies. In view of the above possible associations of the ABO blood group and different diseases, one of the objectives of this research is thus to look for an association between ABO and Rhesus blood groups with CLD patient.

CLD is known to be associated with hematological abnormalities especially anemia which seen in 75% of patients. Even though, survival rate of individuals with CLD has greatly increased but most of the patients need regular blood transfusion for the management of anemia (Gkamprela et al., 2017).

Hemolytic anemia had been reported as high as 54% of patients with cirrhosis regardless of etiology. There is high frequency of various autoantibodies among patients with chronic HCV liver diseases and autoimmune hepatitis (AIH). To date, the frequency and significance of anti-RBC antibodies have not been explored. Furthermore, the laboratory findings of hemolysis such as elevated indirect bilirubin and LDH levels, are also a

common laboratory finding observed in patients with chronic liver diseases (Okazaki et al., 2015).

It is highly possible that patients with mild to moderate hemolysis are left unrecognized in a considerable number of patients. Awareness of this complication and large studies are needed to demonstrate the contribution of this medically treatable anaemia seen in patients with chronic HCV liver diseases (Okazaki et al., 2015).

RBC alloimmunization is a common and potentially serious consequence of blood transfusions. The risk of alloimmunization is especially high in patients who receive multiple transfusions, such as patients with thalassemia and hematological malignancies (Philip et al., 2014). The frequency of alloimmunization is not uniform among all patients. Variations have been noted in different patient population. The risk of developing RBC alloantibodies depends on the age, sex, and genetic makeup of the patient, as well as the number and frequency of transfusions (Philip et al., 2014).

Few studies have investigated clinical risk factors for alloimmunization. In a case-control study, the characteristics of all patients in whom alloimmunization occurred for the first time after an RBC transfusion were examined. Female sex, diabetes mellitus and solid malignancy seem to be risk factors for RBC alloimmunization (Bauer et al, 2007).

In recent times, there has been a better understanding of the factors influencing RBC alloimmunization. These include recipient factors such as genetic disparity between donor and recipient, ability of recipient's HLA to present foreign antigens, genetic predisposition, recipient health status, prior exposures, and impact of the underlying disease, (Bajpai et al., 2016).

Bajpai et. al reported that the underlying disease may also be an important factor in determining the susceptibility of a patient to alloimmunization. CLD had an overall statistically significant impact on the rate of alloimmunization. High rates of alloimmunization were seen in patients with autoimmune hepatitis, cryptogenic liver disease, liver damage due to drugs/toxins, and liver cancer patients. Patients with alcoholic liver disease who comprise a major part of the patient population in the study had a low rate of alloimmunization. Evidence also suggests that recipient's inflammatory status affects alloimmunization rates in transfusion recipients. This study has also shown significant differences in the rates of alloimmunization in various liver diseases with highest rates in autoimmune hepatitis and cryptogenic cirrhosis.

Patients with alcoholic liver disease showed a much lower rate of RBC alloimmunization, this may be due to depressed immune response (decreased T cells, B cells, natural killer cells, and monocytes) in those patients (Bajpai et al., 2016). In another study suggested that viral-type inflammation leads to enhanced alloantigen specific immunity (Hendrickson et al., 2007). Previous study also found a high rate of alloimmunization to low incidence and low immunogenicity antigens in individuals with autoimmune disorders (Ryder et al., 2016).

The mean number of transfusions received was higher in alloimmunized patients as compared to non-alloimmunized patients although the difference was not significant indicate that RBC alloimmunization factor in this study probably contributed by the underlying liver disease (Bajpai et al., 2016).

Previous studies on multiple RBC transfusion and alloimmunization showed an association between mean number of red blood cell transfusions and number of alloantibodies (Debnath et al., 2021; Dogra et al., 2015; Patel et al., 2009; Philip et al.,

2014; Yusoff et al., 2020). Patients who received multiple red cell transfusions developed alloantibodies against one or many red cell antigens. A higher number of transfusions will increase the possibility of encountering a foreign antigen. RBC alloimmunization rate increases exponentially as numbers of transfused pints increase (Bajpai et al., 2016).

Currently, much attention focusing on recipient factors that influence RBC alloantibody responses. Other than exogenous factors or condition-associated inflammation, autoimmunity is also found as a risk factor for alloantibody formation.

These RBC antibodies may be clinically significant, leading to HTR or HDFN (Hendrickson & Tormey, 2016). A better understanding of alloimmunization triggers is needed for prevention strategies to be optimized. A common goal of such strategies is to increase transfusion safety (Hendrickson & Tormey, 2016). Therefore, it is important to detect these alloantibodies so that antigen-negative blood could be provided to patients.

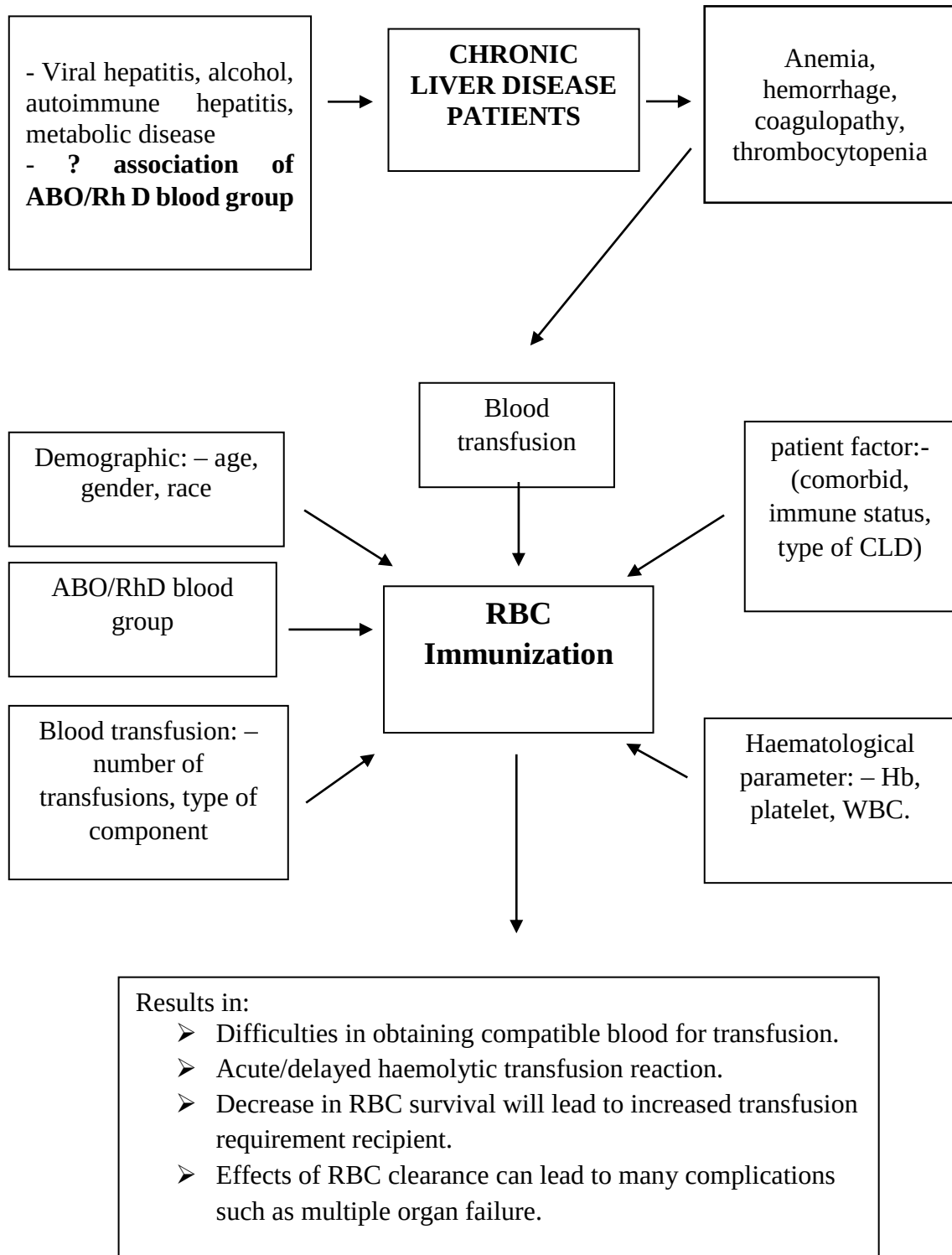
Philip et al, (2014) mentioned that transfusions given to patients who are likely to become transfusion dependent over a longer period of time should be matched for antigens other than ABO and Rh to prevent alloimmunization. Extended antigen matching intended to prevent the formation of RBC alloantibodies in patients who received multiple transfusions, has been advocated for selected patient populations (Philip et al., 2014).

Theoretically, prophylactic matching for C, E, c, e, and K antigens able to reduce clinically significant antibody formation by 70% and by adding Fya, Jka, and S antigens will increase the rate to almost 90%. However, no randomized controlled studies have yet been performed to evaluate this strategy (Philip et al., 2014).

It was agreed by many previous kinds of research that prevention of RBC alloantibody formation in multi-transfused patients can prevent HTR, extend life expectancy, and

reduce the amount of blood transfusion required (Bajpai et al., 2016; Dogra et al., 2015; Patel et al., 2009). However, cost considerations and which patient populations will most likely benefit from this strategy are still unclear, making this an important issue for transfusion services (Debnath et al., 2021; Dogra et al., 2015; Philip et al., 2014).

2.1.7 Conceptual framework



2.1.8 Methodology

Study design

This is a case control study.

Study area

Transfusion medicine unit and Record office units in Hospital Universiti Sains Malaysia, Kubang Kerian.

Study period.

The study estimated from March 2021 and until March 2022

Study population

I. Reference population

Chronic liver disease patients in Kelantan.

II. Source population

Chronic liver disease patients treated in Hospital USM, Kubang Kerian.

III. Target population

Chronic liver disease patients under Gastro clinic follow up / admitted medical ward, Hospital USM.

IV. Sampling frame

All chronic liver disease patients under Gastro clinic follow up / admitted medical ward HUSM from Jun 2020 until May 2021.

Subject criteria

Objective 1: To determine association of ABO and RhD blood group with chronic liver disease in Hospital USM.

Case:

A) Inclusion criteria:

All patient more than 18 years old, diagnosed with CLD (Alcoholic liver disease, Viral hepatitis, Autoimmune hepatitis, Biliary cirrhosis, Metabolic liver disease, Cryptogenic liver disease).

B) Exclusion criteria:

- i. Comorbid (DM, HPT, CKD, Thalassemia, all malignancy other than liver cancer).
- ii. No data on ABO/RhD blood group.

Control:

A) Inclusion criteria:

- i. All non-CLD patient more than 18 years old.
- ii. Normal Liver function test (LFT).

B) Exclusion criteria:

- i. No data on ABO/RhD blood group.
- ii. Comorbid (DM, HPT, CKD, Thalassemia, all malignancy other than liver cancer).

Objective 2: To determine the sociodemographic and clinical factors associated with antibody development in chronic liver disease patient.

Case:

A) Inclusion criteria:

All CLD patient (Alcoholic liver disease, Viral hepatitis, Autoimmune hepatitis, Biliary cirrhosis, Metabolic liver disease, Cryptogenic liver disease), more than 18 years old, with positive antibody screening.

B) Exclusion criteria:

Comorbid (Thalassemia, sickle cell disease and hematological malignancy).

Control:

A) Inclusion criteria:

All CLD patient (Alcoholic liver disease, Viral hepatitis, Autoimmune hepatitis, Biliary cirrhosis, Metabolic liver disease, Cryptogenic liver disease), more than 18 years old, with negative antibody screening.

B) Exclusion criteria:

Comorbid (Thalassemia, sickle cell disease and hematological malignancy).

Sample size estimation

Objective 1: To determine association of ABO and Rh D blood group with chronic liver disease in Hospital USM.

Calculated by PS software using two proportion formula.

$\alpha = 0.05$ (the probability that will falsely reject the null hypothesis).

Power = the probability of correctly rejecting the null hypothesis.

P_0 = the probability of the outcome for a control patient.

P_1 = the probability of the outcome in an experimental subject.

Variables	P_0	P_1	Power	M	Sample size	Total Sample size x (depend on m)	+10% drop out
Non-O blood group	0.63 (proportion of Non-O blood among control group, Zhong et al., 2019)	0.71 (the probability of Non-O blood group among cases)	0.8	3	365	1460	1606

Sample size = 365

Total sample size = $365 \times 4 = 1460$

Total sample size + 10% drop out = 1606

Final sample size:

Sample size for case + 10% drop out = $365 + 36.5 = 401$ (Case).

Sample size for control + 10% drop out = $(365 \times 3) + 109.5 = 1205$ (Control).

Objective 2: To determine the sociodemographic and clinical factors associated with antibody development in chronic liver disease patient.

Calculated by PS software using two proportion formula

$\alpha = 0.05$ (the probability that will falsely reject the null hypothesis)

Power = the probability of correctly rejecting the null hypothesis

Po = the probability of the outcome for a control patient

P1 = the probability of the outcome in an experimental subject