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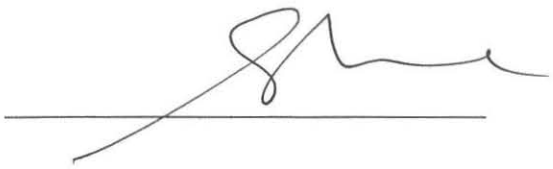
DISCLAIMER

I hereby declare that this thesis represents my own work and all the sources had been qouted and acknowledged by means of complete references. This thesis has been sent to Universiti Sains Malaysia for degree of Masters of Medicine in Transfusion Medicine and it is not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.



Dr Noor Sheereen Binti Adzaludin

P-IPM0070/15

ACKNOWLEDGEMENT

بسم الله الرحمن الرحيم

In the name of Allah S.W.T, the Most Compassionate, the Most Merciful. The salutation is upon His Messenger Muhammad S.A.W., His family and His companions. It is because of His blessing, that makes it posssible for me to finish this thesis. It is also an honour to me, to thank the following individuals who have provided their guidance in completing this thesis.

1. Dr. Siti Mardhiana Binti Mohamad, Supervisor, Medical Lecturer from Advanced Medical and Dental Institute, Universiti Sains Malaysia for her endless encouragement and support throughout the project. This thesis would not be possible without her guidance.
2. My Co-supervisor Dr. Afifah Binti Hassan, Dr. Nor Hafizah Binti Ahmad, and Dr Zulaiha Binti Muda. They have supported me, and provided valuable comments and suggestions in completing this thesis.
3. Dr Rohayu Binti Hami and Mr Nizuwan Bin Azman for their help in statistical analysis and data interpretation.
4. Staff of Pusat Darah Negara and Paediatric Institution Hospital Kuala Lumpur.
5. My husband, children and family for their prayer, understanding and encouragement to pursue my dream.
6. My fellow colleagues for their continuous support.

Last but not least, to those who continuously supported me throughout the process of writing this thesis.

Thank you.

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ABBREVIATIONS

RBC	Red blood cell
TTI	Transfusion transmitted infection
HKL	Hospital Kuala Lumpur
WHO	World Health Organisation
TDT	Transfusion-dependent thalassaemia
NTDT	Non-transfusion-dependent thalassaemia
Hb	Haemoglobin
Eβ	Hb E/β-thalassaemia
β major	β-thalassaemia major
SHOT	Serious Hazards of Transfusion
DHTR	Delayed haemolytic transfusion reaction
HDFN	Haemolytic disease of the fetus and newborn
Rh	Rhesus
Mi	Milterberger
BBIS	Blood bank information system
PDN	Pusat Darah Negara
SM	Standard matched
PM	Phenotype matched
F	Female
M	Male
Yo	Year
Mo	Month

ABSTRAK

Kadar dan Faktor Prediksi untuk Pembentukan Alloimunisasi Sel Darah Merah pada Kanak-Kanak Talasemia yang Bergantung Kepada Pemindahan Darah di Hospital Kuala Lumpur.

Pengenalan

Pesakit talasemia yang bergantung kepada permindahan darah sentiasa berhadapan dengan risiko alloimunisasi sel darah merah. Terdapat banyak faktor prediksi yang menyumbang kepada perkembangan alloantibodi pada pesakit-pesakit ini. Oleh itu, kajian ini dijalankan untuk menentukan kadar dan faktor prediksi untuk pembentukan alloimunisi sel darah merah dalam pesakit pediatrik talasemia di HKL. Tambahan pula, kajian ini dilakukan untuk membandingkan kadar alloimunisasi sebelum dan selepas pelaksanaan polisi transfusi darah yang padan dengan fenotip pesakit.

Kaedah

Kajian retrospektif secara kohort telah dijalankan. Sebanyak 44 pesakit direkrut dan kemudiannya dibahagikan kepada 2 kumpulan. Kumpulan *standard matched* (SM) bagi mereka yang didiagnosa sebelum Julai 2014, dan bagi mereka yang didiagnosa selepas bulan Julai 2014 dikelompokan ke dalam kumpulan *phenotype matched* (PM). Semua maklumat diperolehi dari fail pesakit dan status alloantibodi dari PDN. Data ini termasuk demografi pesakit, data berkaitan transfusi dan status alloimunisasi. Data-data ini kemudian dianalisis menggunakan perisian SPSS.

Keputusan

Seramai 5 (11.4%) orang pesakit dipastikan mempunyai alloimunisasi, 4 (18.2%) dari kumpulan SM dan 1 (4.5%) dari kumpulan PM . Alloantibodi yang banyak dikenalpasti adalah anti-E, dikesan dalam 3 dari 5 subjek yang mempunyai alloimunisasi. Dari kaji selidik ini, tidak ada perbezaan yang signifikan dalam kadar keseluruhan aloimunisasi. Tidak ada signifikan korelasi antara faktor prediksi yang dieksplorasi dengan kadar alloimunisasi pada pesakit-pesakit ini.

Kesimpulan

Kajian ini menyimpulkan bahawa penurunan angka yang besar pada kadar alloimunisasi selepas pelaksanaan dasar polisi transfusi mengikut fenotip walaupun pada hakikatnya tidak terdapat nilai statistik yang signifikan.

ABSTRACT

Rate and Predictors for Red Cell Alloimmunisation in Tranfusion Dependent Thalassaemic Children in Hospital Kuala Lumpur

Introduction

Transfusion dependent thalassaemia patients have an overall increased risk towards red cell alloimmunisation. Many predictors may be associated with alloantibody development. Thus, this study was conducted to determine the rate and predictive factors for red cell alloimmunisation development as well as to compare the rate of alloimmunisation before and after providing phenotype matched blood transfusion policy in transfusion dependent thalassaemic children in HKL.

Method

This retrospective cohort study recruited a total of 44 patients. They were grouped into two groups. Standard matched (SM) group was for those diagnosed before July 2014, and those who were diagnosed after July 2014 were grouped into phenotype matched (PM) group. All clinical information were obtained from the patients' clinical case notes, whereas the transfusion information from PDN. The data includes patients' demographic information, transfusion exposure and alloimmunisation status. These data were then analysed using the Statistical Package for Social Sciences (SPSS) software.

Result

Out of 44 patients, 5 (11.4%) subjects were alloimmunised, 4 (18.2%) from SM and 1 (4.5%) from PM group. The commonest alloantibody was anti-E, 3 out of 5 alloimmunised subjects. There was no significant difference in the overall rate of alloimmunisation between the SM and PM group. There was also no significant association between the predictors explored with the rate of alloimmunisation in these patients.

Conclusion:

This study concludes that after the implementation of antigen matched phenotype transfusion policy, the rate of alloimmunisation showed a large numerical reduction despite the fact that there was no statistical significance.

CHAPTER 1

INTRODUCTION

1.1 Overview of Thalassaemia

Thalassaemia is a congenital genetic disorder caused by a partial or complete defect in a α– or β–globin chain synthesis (Matteocci and Pierelli, 2014). Defect in one of these globin lead to excess production of the other globin which responsible for the ineffective erythropoiesis and shortened red blood cell (RBC) survival (Wiener *et al.*, 1991).

Thalassaemia is widely distributed, which extended from the Mediterranean basin throughout the Middle East (Iran), and India to the Southeast Asia (Saied *et al.*, 2011). According to the Malaysian Thalassaemia Registry 2017, there is a total of 7509 registered patients of which 2623 consist of the transfusion dependent β-thalassaemia major and 2507 Hb E/β-thalassaemia patients (Chong Chia Chee, 2018). Based on previous study, it was estimated that 1 in 20 Malaysians are carriers of β-thalassaemia trait and further estimation showed that 120–350 infants are born each year with transfusion-dependent thalassaemia (Yatim *et al.*, 2014). The aim of treatment is to correct anaemia, suppress extramedullary haematopoiesis, prevent iron overload and essentially to maintain the quality of life (Obeidi *et al.*, 2011). The current target is to maintain the haemoglobin level to be between 10 to 11g/dL (Noor Haslina *et al.*, 2007).

Although stem cell transplantation has been established as the curative management for thalassaemia, there are still many issues such as donor availability, access and cost which makes this option to be less accessible to most thalassaemia patients. Regular transfusion and iron chelation therapy are still the mainstay treatment of thalassaemia major (Goss *et al.*, 2014). However, regular red cell transfusion is often associated with complications including iron overload, high risk of transfusion transmitted infections (TTI) and alloimmunisation against various blood group antigens (Ameen *et al.*, 2003). Red cell alloimmunisation and autoimmunisation will then result in difficulty in obtaining compatible blood, reactions to transfusion, haemolysis and occasionally life threatening adverse events (Ameen *et al.*, 2003; Singer *et al.*, 2000).

1.2 Overview of Alloimmunisation.

Alloimmunisation is defined as the development of antibodies in response to alloantigen after exposure to genetically different cells or tissues (Harmening, 2012). There are at least 3 main factors which contribute to the development of red cell alloimmunisation;

i. the disparity of RBC antigen between donor and patient
ii. patient's immune status
iii. the immunomodulatory effect of the allogeneic blood transfusions on the patient's immune system (Shamsian *et al.*, 2008).

Therefore, it is essential to ensure that the antigens of transfused RBC match the recipient RBC for safe blood transfusion. According to Blumberg *et al.* (1983), the rate of RBC alloimmunisation in chronic transfused patients was the highest in haemoglobinopathy (29%), followed by myelogenous leukaemia (16%), chronic kidney disease (14%) and lowest in both aplastic anaemia (11%) and upper gastrointestinal bleeding (11%) (Blumberg *et al.*, 1983). This goes to show that these population are at a greater risk for developing red cell alloantibody. The rate of alloimmunisation in chronically transfused thalassaemia patients alone greatly varied from 3.75% in Iran to 37% in Taiwan (Ansari and Moshtaghian, 2008; Wang *et al.*, 2006). The higher rate of alloimmunisation reported in Taiwan attributed to the heterogeneous population in Taiwan and also because they do not practice providing phenotype-matched red cells to thalassaemic patients (Wang *et al.*, 2006). On the contrary, in Iran, the rate of alloimmunisation reported was low due to the homogenous population (Ansari and Moshtaghian, 2008).

Alloimmunisation involves Rh, Kell, Duffy and Kidd system which are clinically significant that may result in haemolytic transfusion reactions (Bilwani *et al.*, 2005). The presence of alloantibodies will create significant problems in transfusion therapy. It will cause difficulty to provide crossmatched red cells especially in emergency situations and may contribute to morbidity and mortality. In Malaysia, it is crucial to perform red cell phenotyping for ABO, Rh, Kell, Kidd, Duffy and MNS in all newly diagnosed thalassaemia patients or before commencing the first transfusion (Karim *et al.*, 2016). However, there are still limited data on the impact of RBC

phenotype matching policy on alloimmunisation rates in thalassaemia patients. The rate of alloimmunisation was found to be significantly lower in patients receiving phenotype matched red cell compared to standard ABO-D matched blood (Singer *et al.*, 2000; Spanos *et al.*, 1990). Singer *et al.* (2000) reported the rate of alloimmunisation in the ABO-D matched blood was 33%, whereas the rate of alloimmunisation for phenotype matched was 2.8% ($p=0.0005$) (Singer *et al.*, 2000). Similar findings were also discovered earlier by Spanos *et al* in 1990, where the rate of alloimmunisation for patients receiving ABO, rhesus and kell antigen matched blood comparatively with those receiving only ABO and RH-D antigen matched blood were 3.7% and 15.7% respectively ($p<0.001$) (Spanos *et al.*, 1990).

1.3 Research Justification

Majority of patients with thalassaemia major are transfusion dependent patients which require life-long blood transfusion. Blood transfusion is currently the mainstay treatment for thalassaemia major. However, blood transfusion is often associated with complications such as adverse transfusion reaction and development of alloimmunisation, though many preventive measures were taken into account which includes providing antigen matched phenotype blood for most patients, fresh blood which are less than 14 days and leucodepleted red cell product. Many studies have been conducted to determine the prevalence as well as to identify the risk factors associated with the development of alloimmunisation in thalassaemia patients. Unfortunately,

despite of the efforts taken, there are still cases of alloimmunisation being reported.

Furthermore, there are still limited data on the prevalence and risk of development of alloimmunisation particularly on the impact of RBC phenotype matched policy on alloimmunisation rate among thalassaemic patients in other places. In Malaysia, the guideline for providing phenotype matched blood to thalassaemic patients was recommended in 2009 (Ibrahim *et al.*, 2009). Nonetheless, in order to improve the safety and efficiency of transfusion, the option of giving phenotype matched red blood cell was implemented to the newly diagnosed thalassaemia patients who were diagnosed after July 2014. No study was conducted to determine whether this practice is beneficial or not in preventing alloimmunisation in thalassaemia patients.

Hence, it is important to study the prevalence and predictive factors of RBC alloimmunisation in preventing further sensitisation especially in paediatric patients. The proposed study will provide evidence-based data that will help in determining whether the current policy of giving phenotype matched blood are beneficial or there is a need to explore new alternatives in thalassemia transfusion programme such as deoxyribonucleic acid (DNA) based or molecular extended antigen matching (Matteocci and Pierelli, 2014).

CHAPTER 2

OBJECTIVES

2.1 General:

To determine the rate and predictors of red cell alloimmunisation in transfusion dependent thalassaemic children in HKL.

2.1.1 Specific objectives:

i. To determine the rate of alloimmunisation and the alloantibody specificity in transfusion dependent thalassaemic children in HKL.
ii. To compare between the rate of alloimmunisation before and after the implementation of antigen matched phenotype transfusion policy in transfusion dependent thalassaemic children in HKL.
iii. To determine the association of gender, age of first blood transfusion and number of blood unit received with alloimmunisation status in transfusion dependent thalassaemic children in HKL

2.2 Hypothesis:

2.2.1 Null hypothesis:

i. There is no association between the rate of alloimmunisation before and after the implementation of antigen matched phenotype transfusion policy in transfusion dependent thalassaemic children in HKL.

ii. There is no association between gender, age of first blood transfusion and number of blood unit received with rate of alloimmunisation in transfusion dependent thalassaemic children in HKL.

2.2.2 Alternative hypothesis:

i. There is association between the rate of alloimmunisation before and after the implementation of antigen matched phenotype transfusion policy in transfusion dependent thalassaemic children in HKL.

ii. There is association between gender, age of first blood transfusion and number of blood unit received with rate of alloimmunisation in transfusion dependent thalassaemic children in HKL.

CHAPTER 3

LITERATURE REVIEW

3.1 Thalassaemia

3.1.1 Definition, Prevalence and Clinical Classification

Thalassaemia is a heterogeneous group of genetic disorders caused by a variant of one or more globin genes resulting in disruption of normal ratio of α to β–globin production chain synthesis (Edward J Benz, 2018). There are two major types of thalassaemia, α and β-thalassaemia, which resulted from the defective synthesis of the α- or β-haemoglobin chains respectively. The basic inheritance of these conditions followed the Mendelian-recessive manner (Weatherall, 2012).

The prevalence of thalassaemia varies in each country and are usually higher in historically Malaria-endemic areas such as sub-Saharan Africa, the Mediterranean, the Asian-Indian subcontinent, and Southeast Asia. About 5% of the world's population carries at least one thalassaemia variant allele and it is expected that during the early 21st century, a total of 900,000 individuals are estimated to have clinically significant disease, with the majority from Southern China, India, and Southeast Asia (Edward J Benz, 2018). The large number of cases are due to high prevalence of carriers in the population. About 3% to 40% of individuals living in endemic Malaria area carries one of these significant variants, and the prevalence of haemoglobin disorders ranges from 0.3 to 25 per 1000 live births. According to World Health

Organisation (WHO) (2018), it is estimated that there are 56 000 Thalassaemia major affected individuals, in which at least 30 000 are transfusion dependent, with β-thalassaemia major and Hb E/β-thalassaemia, and almost 5500 perinatal mortality due to α-thalassaemia major (Modell and Darlison, 2008). In Malaysia, there is a total of 7509 registered patients of which 2623 consist of the transfusion dependent β-thalassaemia major and 2507 Hb E/β-thalassaemia patients (Chong Chia Chee, 2018).

Pathophysiology of thalassaemia is due to the imbalance of α to β–globin unpaired globin chain that is produced in excess. This will precipitate the red blood cell (RBC) precursor which would trigger the destruction in the bone marrow (ineffective erythropoiesis) as well as in the circulation (Edward J Benz, 2018). This will in turn lead to RBC membrane damage and haemolysis that would result in anaemia, extramedullary haematopoiesis, hypermetabolic state and iron overload. This series of events would finally lead to life-threatening organ damage (Beth H. Shaz, 2013).

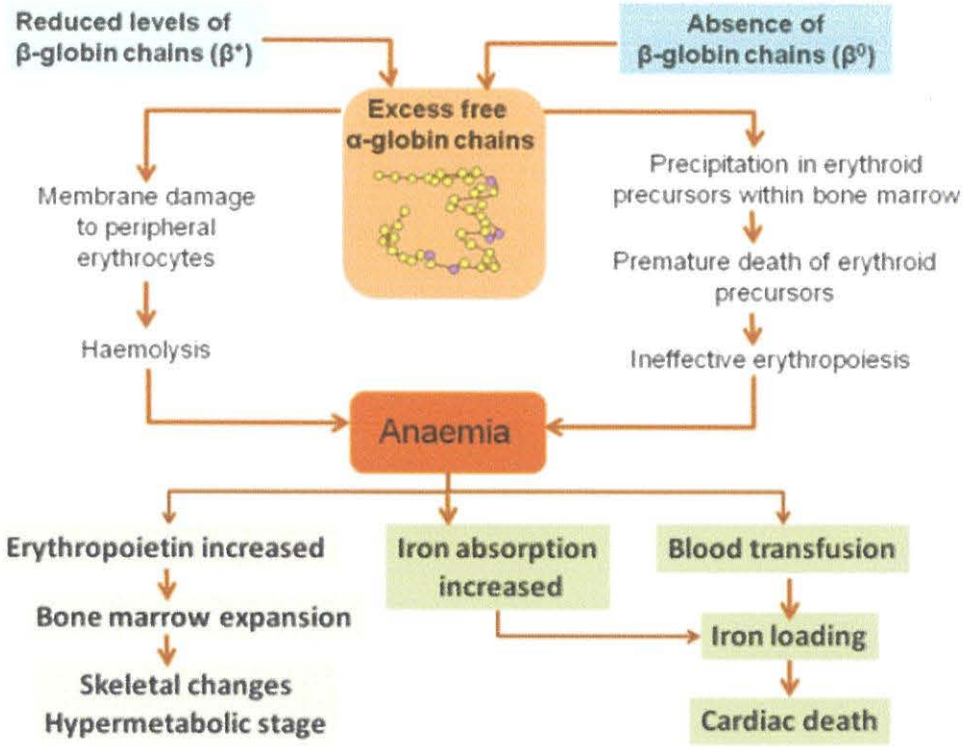

Figure 3.1: Effects of excess of free α-globin chains in β-thalassaemia. Figure adapted from (Cappellini MD, 2014)

Clinically, thalassaemia is categorised based on their severity of anaemia and transfusion requirement. There are three categories including; thalassaemia major, thalassemia intermedia and thalassemia minor (Cappellini MD, 2008). In 2012, the Thalassaemia International Federation adopted a new concept of clinical classification of thalassaemia namely; the transfusion-dependent thalassaemia (TDT) and non-transfusion-dependent thalassaemia (NTDT) (Cappellini MD, 2014). This classification requires a careful clinical evaluation using several clinical and haematological parameters in order to differentiate the new thalassaemia patients as either TDT or NTDT (Taher *et al.*, 2012).

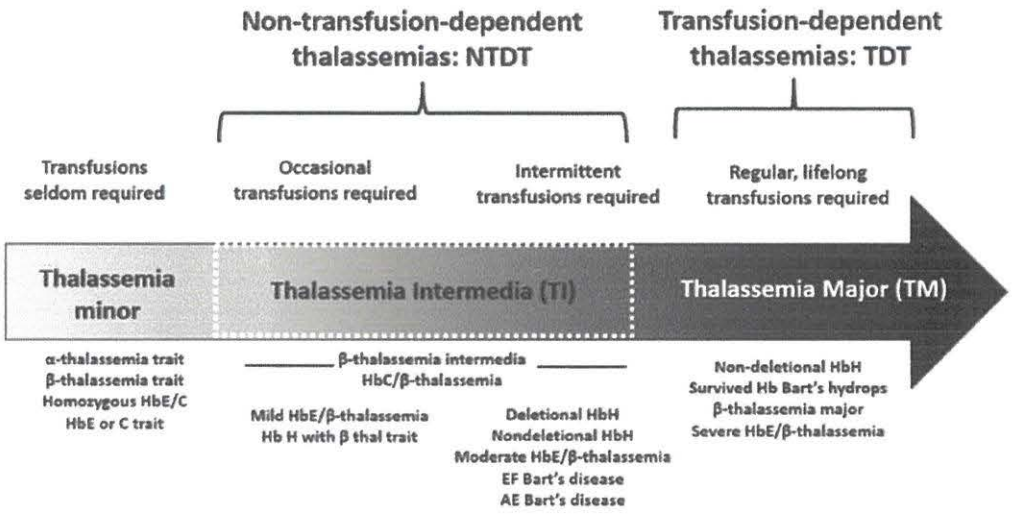

Figure 3.2: The clinical spectrum of thalassemia syndromes based on their requirement of regular blood transfusions into non–transfusion dependent thalassemia (NTDT) and transfusion dependent thalassemia (TDT). Figure adapted from (Vip Viprakasit, 2018)

3.1.2 Diagnosis and Management of Thalassaemia

The diagnosis of thalassaemia requires a comprehensive assessment including full history, physical examination and laboratory evaluation (Brancaleoni *et al.*, 2016). The diagnostic laboratory testing includes the full blood count, peripheral blood film, haemoglobin (Hb) analysis and molecular studies (Vip Viprakasit, 2018).

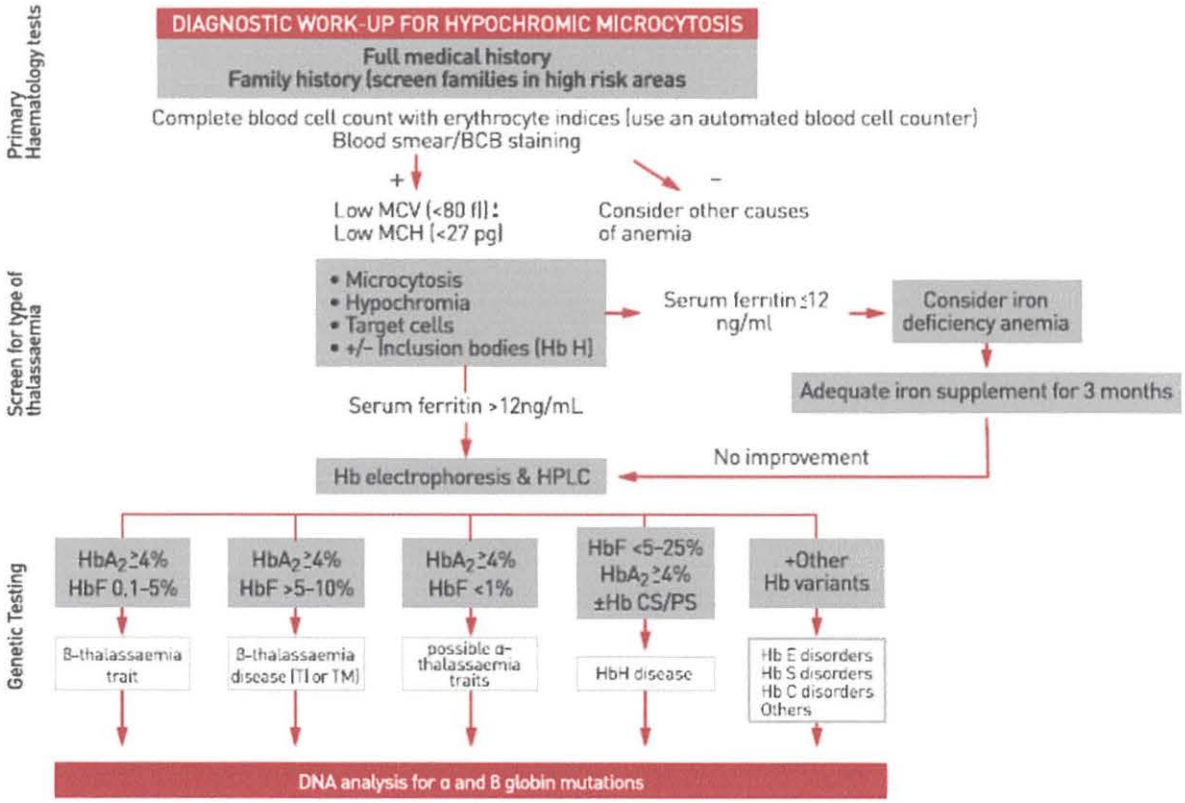

Figure 3.3: Diagnostic flowchart for thalassaemia. Figure adapted from (Cappellini MD, 2014)

The management of thalassaemia is decided based on the clinical classification and availability of the measures in the local setting. The definitive treatment of thalassaemia is allogeneic stem cell therapy but due to the multiple complications and limitations, it is mainly reserved for young children with an HLA-identical sibling donor (Lucarelli *et al.*, 2012). Hence, for transfusion-dependent thalassaemia, the current treatment are lifelong, which involves regular blood and iron chelation (Engert *et al.*, 2016). Ultimately, transfusion regime are intended to suppress the extramedullary erythropoiesis, promotes normal growths and normal physical activities, while minimising iron load (Cazzola *et al.*, 1997).

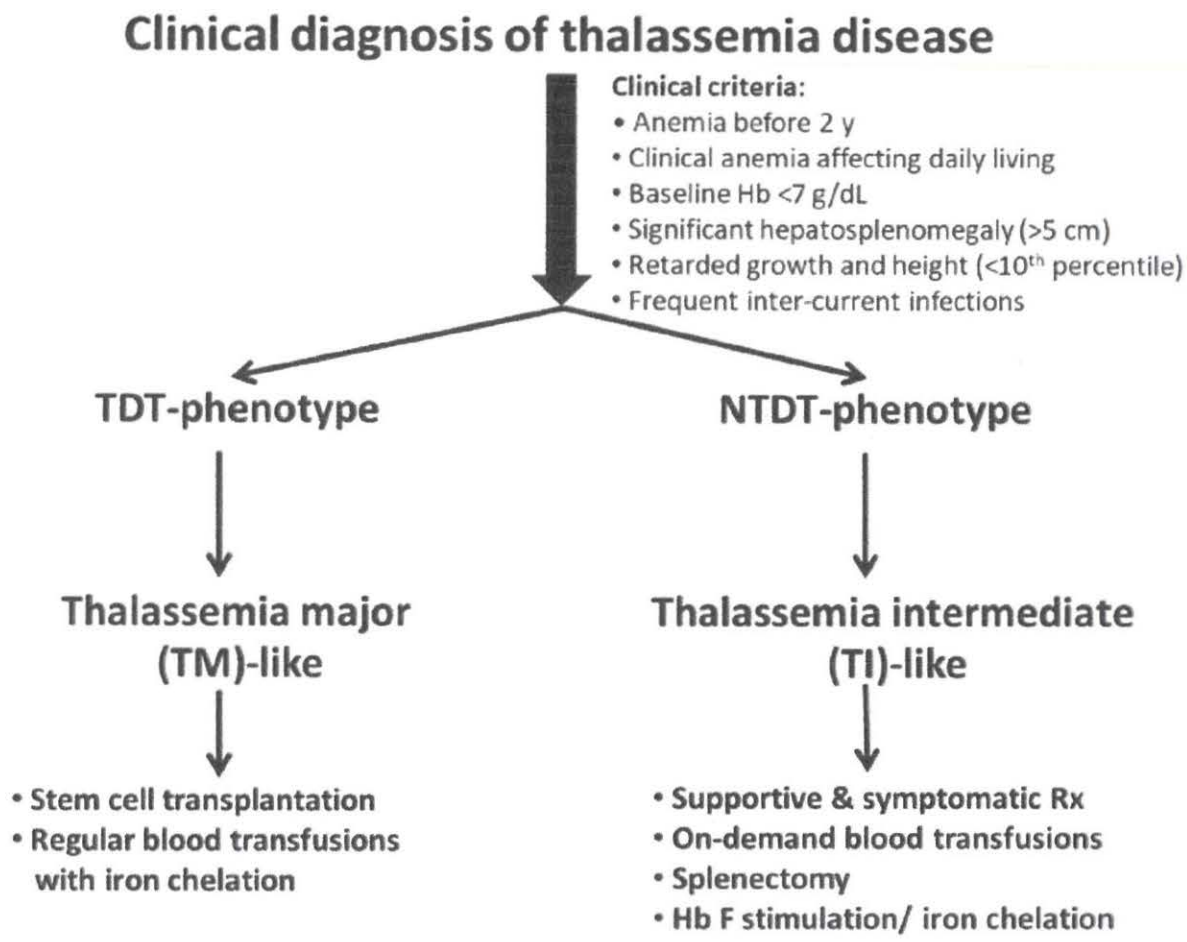

Figure 3.4: Differential characteristics for diagnosis thalassaemia to guide further clinical management. Figure adapted from (Vip Viprakasit, 2018)

3.2 Blood Transfusion

3.2.1 Blood Transfusion Practices in Thalassaemia

Effective red blood cell transfusion was introduced 50 years ago as one of the thalassaemia treatment modalities. It was a critical measure that successfully prevented early deaths in thalassaemia patients (Wolman, 1964). This is because, with effective transfusion regime, a severe, fatal anaemia can be alleviated to a chronic illness. Generally, the transfusion is administered between the duration of 2 to 5 weeks in order to maintain the pre-transfusion

haemoglobin above 9-10.5g/dL (Rebulla and Modell, 1991). In patients with cardiac complications or those failed to suppress the extramedullary erythropoiesis, the haemoglobin level is targeted higher at 11-12g/dL (Cazzola *et al.*, 1997).

The transfusion regime can be divided into 3 classifications, depending on the pre-transfusion haemoglobin target and disease complication. This classification consist of:

i. Super transfusion regime, which aims to maintain the pre-transfusion Hb of more than 12g/dL.
ii. Hyper transfusion regime, which aims to maintain the pre-transfusion Hb between 10-12g/dL.
iii. Moderate transfusion regime, which targets to maintain the pre-transfusion Hb between 9-10g/dL (Ibrahim *et al.*, 2009).

The decision of transfusion in thalassaemia is made when the diagnosis of thalassaemia is confirmed with Hb less than 7g/dL in 2 separate occasions, with more than 2 weeks apart excluding other causes such as infections or Hb less than 7g/dL with evidence of clinical criteria (Cappellini MD, 2014).

The clinical features of thalassaemia includes facial changes, poor growth, fractures and clinically significant extra-medullary haematopoiesis (Cappellini MD, 2014).

According to the Malaysian Transfusion Practice Guideline, prior to the first transfusion, all transfusion-dependent patients are required to have a transfusion baseline investigation such as;

i. RBC phenotype which includes Rhesus, Kell, Kidd, Duffy and MNSs.
ii. Transfusion transmitted infection (TTI) screening- should be repeated every 6 months.
iii. RBC antibody screening (Karim *et al.*, 2016).

Thalassaemia patients should be transfused with leucoreduced packed red blood cells with a minimum of 40g haemoglobin content and white blood cells count of less than 1×10^6 in each unit (Cappellini MD, 2014). All blood products that are intended for transfusion should be fully crossmatched, phenotype compatible if possible and filtered (Karim *et al.*, 2016).

The aim of the transfusion therapy and blood safety is for the optimisation of haemoglobin level in patients and avoidance of transfusion adverse reactions (Cappellini MD, 2014). By practicing safe transfusion and optimising iron overload management, patients with thalassaemia are able to achieve normal growth and life expectancy of more than 70 years (Borgna-Pignatti *et al.*, 2004).

3.2.2 Adverse Reaction of Blood Transfusion

Indicated blood transfusions are usually beneficial but it is not risk free and often associated with certain side effects (Marcucci *et al.*, 2004). Transfusion adverse reactions are defined as undesirable responses or effects in a patient that are temporally related to the administration of blood or blood components. Classification of transfusion adverse events is based on the pathophysiology and the onset of the adverse event. The importance to recognise the type of adverse transfusion reactions is that we can anticipate the management steps in order to minimise the reactions. For the non-infectious adverse transfusion reactions, they can be classified by the onset and then further sub categorised by the immune or non-immune aetiology (Sahu *et al.*, 2014).

Table 3.1: An overview of classification non-infectious adverse transfusion reactions. Adapted from (Sahu *et al.*, 2014).

Acute (occurring < 24 H after transfusion)	**Delayed (occurring > 24 H after transfusion)**

• Immune mediated
  ○ Acute hemolytic transfusion reactions
  ○ Febrile nonhemolytic transfusion reaction
  ○ Allergic/Urticarial/ Anaphylactic reaction
  ○ Transfusion-related acute lung injury (TRALI)
• Non immune mediated
  ○ Transfusion related sepsis
  ○ Non immune hemolysis
  ○ Transfusion-associated circulatory overload (TACO)
  ○ Air embolism
	• Immune mediated
	  ○ Delayed hemolytic transfusion reactions
	  ○ Alloimmunisation to RBC antigen, platelet and HLA-antigen
	  ○ Transfusion associated immunomodulation
	  ○ Transfusion-associated graft versus host disease
	  ○ Post-transfusion purpura (PTP)
	• Non immune mediated
	  ○ Iron overload.

According to the Serious Hazard of Transfusion (SHOT) data in 2017, the risk of transfusion related mortality was 1 in 114 273 whereas the risk of major morbidity was 1 in 21 426 blood components issued. In addition, the risk of transfusion transmitted infections (TTI) were reported to be much

lower in comparative to other transfusion complications (Poles *et al.*, 2018). The residual risk of TTI had greatly reduced after the implementation of nucleotide testing screening programme from approximately 1 in 500,000 to 750, 000 blood exposures (Perrotta and Snyder, 2001).

Presently, the common blood transfusion complications are the alloimmunisation to red cells, platelet and plasma protein which can lead to red cells destruction, febrile non-haemolytic and allergic reactions (Taylor *et al.*, 2008). Red cell alloimmunisation may be associated with the following clinical presentation.

i. Acute intravascular haemolytic transfusion reactions (more common in ABO antibodies).
ii. Delayed haemolytic transfusion reactions (DHTRs).
iii. Haemolytic disease of the foetus and newborns (HDFN) (Blackall, 2017).

As a result, patients with alloantibody would not only be at risk for serious clinical consequences but it will be timely and difficult to find a matched blood for him or her (Zalpuri *et al.*, 2012).

3.3 Red Cell Alloimmunisation

3.3.1 Overview of Red Cell Alloimmunisation

Exposure to RBC alloantigen through transfusion, pregnancy or transplantation will increase the risk of antibody development against the alloantigen that are expressed by the RBCs (Schonewille *et al.*, 2006). The risk of immunisation increases with the number of transfusion events and about 36% of patients developed the first antibody after or during the 10^{th} transfusion (Fluit *et al.*, 1990). Schonewille et al. (2006) have reported that the development of antibodies ranged from 1% to 6% in a single transfusion (Schonewille *et al.*, 2006). The rate of alloimmunisation was reported to be higher in chronically transfused patients such as those with myelogenous leukaemia (16%), chronic kidney disease (14%) and haemoglobinopathy (29%) (Blumberg *et al.*, 1983).

Red cell immunogenicity refers to the ability of an antigen which is present on the surface of the red blood cell to stimulate an immune response. This immune response is characterised by the development of antibody. Alloimmunisation to RBC includes RBC antigen recognition, processing, and presentation of antigen by HLA class II to TCR, activation of CD4 helper T cells, interaction of T and B cells, and finally B-cell differentiation into plasma cells (Yazdanbakhsh *et al.*, 2012).

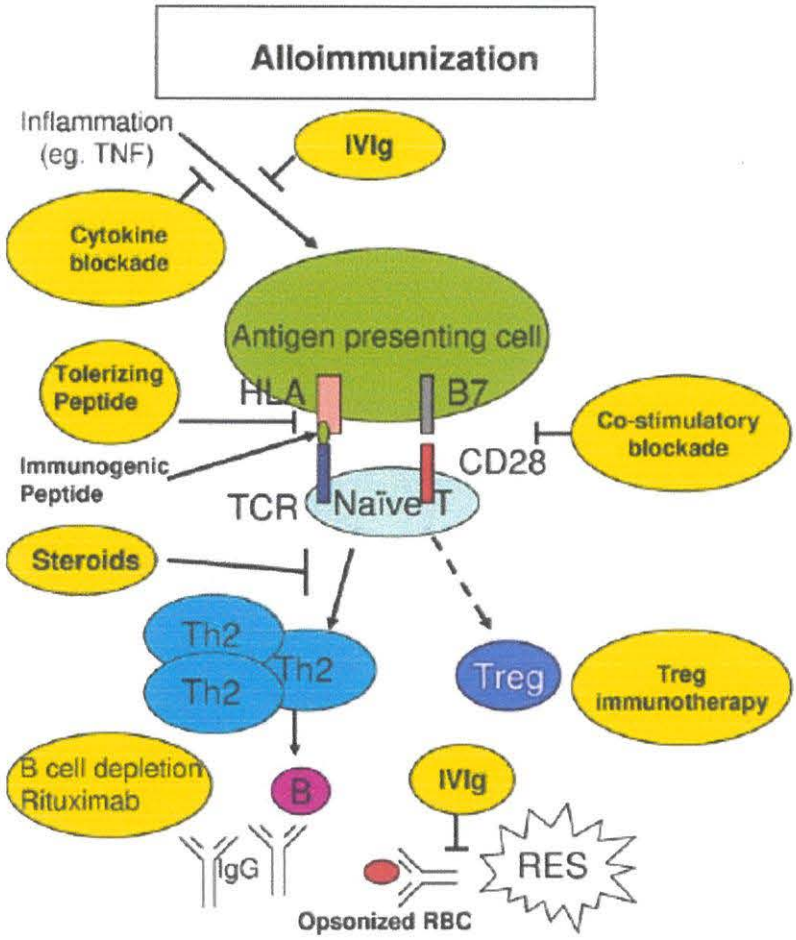

Figure 3.5: Hypothetical schema of immune response to RBC antigens in alloimmunised patients. Figures adapted from (Yazdanbakhsh *et al.*, 2012).

Antigenic differences, immunogenicity, dose and frequency of transfusion as well as the recipients' immune status are the factors that have been suggested to influence the rates of alloimmunisation (Zalpuri *et al.*, 2012).

3.3.2 Prevalence of Red Cell Alloimmunisation in Thalassaemia

Red cell alloimmunisation is a well-known complication and major challenge of transfusion dependent thalassaemia patients. As mentioned previously, the rate of alloimmunisation in transfusion dependent thalassaemia patients was reported to be between 3.75% in Iran and 37% in Taiwan (Ansari and Moshtaghian, 2008; Wang *et al.*, 2006). Lower rate of alloimmunisation was reported in Pakistan and Malaysia, with 8.7% and 8.6% respectively (Haslina *et al.*, 2006; Zaidi *et al.*, 2015). On the contrary, a higher rate of alloimmunisation was observed in a study done in Greece with 22% and 30% for Kuwait (Ameen *et al.*, 2003; Singer *et al.*, 2000). The differences in the rate of alloimmunisation between the studies were probably due to population diversity and homogeneity or heterogeneity of RBC phenotypes between the donor and the recipients (Michail-Merianou *et al.*, 1987). The rate of alloimmunisation was reported to be lower in some centers which provides extended antigen-matched products (Thompson *et al.*, 2011). According to Spanos *et al.* (1990), the rate of alloimmunisation was 3.7% in the group of patients receiving ABO, Rhesus (Rh) and Kell matched blood whereas the rate of alloimmunisation was 15.7% in patients receiving only ABO and Rh D antigen matching (Spanos *et al.*, 1990). However, there was no significant difference between the rate of alloimmunisation among the institution providing extended antigen-matched products with those practicing only ABO and Rh D antigen matching (Thompson *et al.*, 2011). Nonetheless, the data regarding these issues are still limited.

According to Ismail *et al.* (2016), their study which was conducted in Malaysia reported that the detected antibodies were anti-E 94.44%, anti-c 22.22%, anti-Jk b 11.1% and anti-e 11.1% and anti- Fy b 5.56% (Ismail *et al.*, 2016). Whereas, Zaidi *et al.* (2015) discovered that the most identified alloantibodies were against the Rh system and Kell antigens with anti-E, 2.5 %, anti-K 1.8%, anti-e 1.2% and anti-D 0.6% in Pakistan. More than 90% of the patients that were given an antigen matched RBC of Rh C, c, D, E, e and K were able to reduce the rate of alloimmunisation (Zaidi *et al.*, 2015). In addition, a study done by Cheng *et al.* (2012) demonstrated that the most common observed alloantibodies were anti-E, anti-Mia/Mur and anti-c in the Chinese population (Cheng *et al.*, 2012). There are greater likelihoods of developing anti-Mia/Mur in China rather than developing antibodies against Kell or Duffy antigen. This is because, the prevalence of Mia antigen among the Chinese and Southeast Asians; and Mur antigen among the Taiwanese and Chinese were 15% and 6% - 7% respectively. On the contrary, these antigens are rare in other population (Matteocci and Pierelli, 2014).

3.3.2 Predictive Factors of Red Cell Alloimmunisation in Thalassaemia

Patients are considered a significant factor and play a significant role in contributing to the development of red cell alloimmunisation. Age of patients when the transfusion initially started are associated with the rate of alloimmunisation. According to Gupta *et al.* (2011), rate of alloimmunisation was lower among patient who received the first transfusion before the age 2 year old compared to those who were transfused at a much later age (Gupta

et al., 2011). Furthermore, another study by Singer *et al.* (2000), concluded that patients who received their first transfusion when they were less than 3 years old were less likely to develop alloantibodies. This was thought to be due to the effect of immune tolerance which offer a protection against alloimmunisation (Singer *et al.*, 2000). Besides that, another risk for higher rate of alloimmunisation was associated with female gender (Bauer *et al.*, 2007). However, a study done by Hussein *et al.* (2014) showed that a higher rate of alloimmunisation were observed in the male gender (Hussein *et al.*, 2014).

Another factors that was also identified to be predisposed to the development of alloimmunisation was the total number of units transfused (Hassan *et al.*, 2004). The rate of alloimmunisation was found to be higher in patients with more units of blood transfused (Hussein *et al.*, 2014). However, the association was not observed in the study conducted in Penang, Malaysia in which they were not found to develop alloantibody even after receiving more than 100 units (Ismail *et al.*, 2016). A study done by Haslina *et al.* (2006) found that the development of antibodies was detected as early as after transfusing with 8 units (Haslina *et al.*, 2006). However, other study by Spanos *et al.*(1990) reported that alloimmunisation became prevalent after 10 units of transfusion (Spanos *et al.*, 1990).

Another factor that can contribute to the development of alloimmunisation is splenectomy. Most patients with splenectomy have a higher rate of alloimmunisation. This may be attributed by the fact that

splenectomy may enhance the immune response to the transfused foreign antigens that are not filtered efficiently (Singer *et al.*, 2000). No correlation was reported in a study conducted by Haslina *et al.* (2006) even though there was a higher rate of patients with splenectomy (Haslina *et al.*, 2006).

Hence, it is important to prevent the development of alloimmunisation especially in transfusion dependent patients. The consequences of multiple transfusion in transfusion dependent patients is that it will increase the risk of developing multiple antibodies in which may eventually cause the patients to develop adverse transfusion reactions such as haemolysis and occasionally life threatening events (Singer *et al.*, 2000). Furthermore, according to laboratory perspective, it will be a laborious and complex procedure to supply compatible blood for these patients.