A PILOT STUDY ON RED CELL IMMUNIZATION IN MULTIPLY TRANSFUSED THALASSAEMIC PATIENTS

By

DR NOOR HASLINA MOHD NOOR

M.D (USM)

Dissertation Submitted In Partial Fulfillment Of

The Requirements For The Degree Of

Master Of Pathology (Haematology)

UNIVERSITI SAINS MALAYSIA

MEI 2005

UNIVERSITI SAINS MALAYSIA
PUSAT PENGAJIAN SAINS PERUBATAN
SCHOOL OF MEDICAL SCIENCES

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No. Akaun : 304/PPSP/6131313

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Peruntukan 2004 (Tahun 1)	RM	9,000.00	Tajuk Projek:	A Pilot Study on the Determination of Type of Antibody and Factors Contributing to its Development Among Multiple Transfused Thalassemia Patients
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304	11000	PPSP	6131313		4,750.00	8,742.19	(3,992.19)	-	2,055.40	2,055.40	(6,047.59)
304	14000	PPSP	6131313		-	-	-	-	-	-	-
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304	21000	PPSP	6131313		500.00	-	500.00	-	-	-	500.00
304	22000	PPSP	6131313		-	-	-	-	-	-	-
304	23000	PPSP	6131313		300.00	-	300.00	-	-	-	300.00
304	24000	PPSP	6131313		-	-	-	-	-	-	-
304	25000	PPSP	6131313		-	-	-	-	-	-	-
304	26000	PPSP	6131313		12,444.00	-	12,444.00	-	-	-	12,444.00
304	27000	PPSP	6131313		-	200.21	(200.21)	-	4,434.60	3,984.00	(4,634.81)
304	28000	PPSP	6131313		-	-	-	-	-	-	-
304	29000	PPSP	6131313		2,000.00	2,333.55	(333.55)	-	1,150.00	890.05	(1,483.55)
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304	35000	PPSP	6131313		-	-	-	-	-	-	-
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Dr Ariffin Nasir

Dr Illuni Hayati Ibrahim

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3) Tajuk Projek:

A Pilot Study On Red Cell Immunization In Multiply Transfused Thalassaemic Patients.

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

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(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
Talasemia	Thalassaemia
Alloantibodi Rbc	Rbc alloantibody
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5) Output Dan Faedah Projek

(a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

Pembentangan poster:

1. Di 5 th Malaysian National Haematology Scientific Meeting, Putrajaya Marriott, Kuala Lumpur pada 9-11 April 2004
2. Di 9th National Conference on Medical Sciences pada 22-23 Mei 2004
3. Di 8th Internationnal Congress of the asean society of clinical pathology and laboratory Medicine Medan, Indonesia pada 29 Nov – 2 Dis 2004 (Memenangi " Best Free Paper Presentation")
4. Red cell immunization in multiply transfused Malay thalassaemia patients. (Submitted for review to Southeast Asian Journal of Public health And Tropical Medicine, April 2006)

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(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan)

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(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah: .....-.....
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ii) Pelajar Prasiswazah: .....-.....

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iii) Lain-Lain : .....-.....
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(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan)

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(c) Latihan Gunatenaga Manusia

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iii) Lain-Lain : .....-.....

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PROFESSOR ABDUL AZIZ BABA
Chairman of Research & Ethics Committee
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16150 Kubang Kerian, Kelantan

Red cell immunization in multiply transfused Malay thalassaemia patients.

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ABSTRACTS

BACKGROUND: The development of red blood cell (RBC) isoimmunization with alloantibodies and autoantibodies complicate transfusion therapy in multiply transfused thalassaemia patients. Thus, frequency, causes and prevention of these phenomena were studied among these patients.

STUDY DESIGN AND METHODS: Clinical and serological data of 58 Malay multiply transfused thalassaemia patients who seek their treatment in Hospital University Sains Malaysia were collected and analyzed prospectively. Blood samples were subjected to standard blood bank procedure for screening of antibodies and subsequent antibodies identification. All patients in our hospital received blood matched for only ABO and Rh (D) antigens.

RESULTS: There were 46 (79.3%) patients with Hb E/β thalassaemia, 8 (13.8%) β thalassaemia major, 3 (5.2%) Hb H Constant Spring and 1 (1.7%) Hb H disease. Overall, 8.6% of the patients had alloantibodies and 1.7% had autoantibodies. The alloantibodies identified were anti-E, anti-c, anti-K, anti-Jka, anti-N and anti-S.

CONCLUSION: The transfusion of matched blood is essential for chronically multi-transfused patients in order to avoid alloimmunisation. Considering high frequency of anti E in our hospital, it is advisable to genotype patients and match the red cell unit for E antigens in multiply transfused thalassaemia patients.

Keywords : Thalassaemia, alloimmunization, alloantibodies

INTRODUCTIONS

Thalassaemia is one of the major public health problems in Malaysia. It is a heterogeneous group of inherited autosomal recessive disorder of haemoglobin synthesis, which is characterized by the absence or reduced output of one or more globin chains of haemoglobin.[1]

The recommended treatment for thalassaemia major involves regular blood transfusions, usually administered every 2 to 5 weeks, to maintain the pretransfusion haemoglobin level above 9-10.5 g/dl. One of the complications of blood transfusion is the formation of alloantibodies and autoantibodies against RBC antigen. The results from a number of studies have demonstrated various frequencies and percentages of alloantibody and autoantibody formation in multi-transfused patients.[2, 3, 5] Some alloantibodies may cause haemolytic transfusion reactions and limit the availability of safe transfusion, while others are clinically insignificant. Red cell autoantibodies appear less frequent but can result in haemolysis and difficulty in blood cross-matching.[4]

The antibodies must be identified in the recipient's serum before every transfusion so that compatible blood can be provided. The causes of alloimmunisation in thalassaemia patients are not fully understood, however data suggest that the recipient's immune status, absence of spleen and difference in the red cell phenotype between donor and recipients are likely to further contributes to the phenomena.[6] This paper reports the result of a study carried out in this center to determine the prevalence of RBC alloantibodies and autoantibodies and the factors that might contribute to its development.

MATERIALS AND METHODS

This prospective study was conducted over a 1-year period from January 2004 until December 2004 at Hospital University Sains Malaysia. The study had been approved by the hospital ethical committees. Written consent was provided.

Patients

A total of 58 thalassaemia patients receiving multiple blood transfusions at interval of 2 to 4 weeks or had received at least 10 transfusions were included in this study. Diagnosis of thalassaemia was confirmed by standard haemoglobin electrophoresis and measurement of Hb A, A2 and F.

Clinical transfusion records of 58 thalassaemia patients who fulfilled the criterias were analyzed for the presence of allo- and autoimmunization, their antibody specificity and the time interval of RBC immunization from start of transfusion. Ethnic background, status of splenectomy, age at start of transfusion and the number of blood unit received were also recorded.

Laboratory investigations

Using standard blood bank methods, serum was analyzed prior to each transfusion for detection of new antibody to RBC antigen. All the pretransfusion sera were also tested to determine their phenotype for the following blood group systems namely ABO; Rhesus (D, C, E, c, and e); Kell (K, k), Kidd (Kpa, Kpb) and Duffy (Fya, Fyb).

An antigen panel was used for the antibody screening procedure where the serum was mixed with saline suspended red cells in LISS Coombs gel card incubated at 37 °C for 15 minutes. The antibody identification test was performed by a commercial RBC panel when the antibody-screening test was positive.

Polyspecific direct antiglobulin test was performed using 0.8% cell suspension of patient's RBC with anti human globulin. Elution and absorption methods were employed in patients with suspected autoantibody. Commercial RBC panel was used for the eluates and adsorbed sera to detect any specificity of the autoantibodies and alloantibodies respectively. The entire tests were done by using gel card method by Diamed ID (Switzerland).

Statistical Analysis.

Descriptive statistics and Fischer exact statistical test was performed and p value of less than 0.05 is considered significant. The results were analyzed using SPSS statistical software version 11.0

RESULTS

A total of 58 multiply transfused thalassaemia patients were included in this study. Demographic data is as shown in table 1. Twenty-two patients (37.9%) were blood group B, 16 (27.6%) were blood group O, 12 (20.7%) were blood group A and 8 (13.8%) were blood group AB. All the patients were rhesus positive. Twenty-six patients (44.8%) were genotyped as R1R1, 25 (43.1%) were R1R2, 6 (10.4%) were R1r and one (1.7%) was R2r.

Red cell alloantibodies were found in 5 of 58 patients (8.6%) and only one patient (1.7%) developed autoantibody.

Alloimmunised patients

Details of the patients with alloantibodies were shown in table 2. Three patients developed only 1 antibody, which were anti-E and anti-K respectively. One patient developed 2 antibodies, which was anti-E and anti-Jka and 1 patient developed 4 antibodies, namely anti-E, -c, -S and -N. Time at the development of antibody ranged between after 8 to 100 units of packed cell transfused.

There's no significant association between formation of alloantibody with gender (p=0.16), age at start of transfusion (p=0.58), number of packed cell transfused (p=1.00) and splenectomy (p=0.31).

DISCUSSIONS

To the best of our knowledge this is the first report on the incidence of RBC immunization among multiply transfused thalassaemic patients in Malay population. The frequency of alloimmunization ranged from about 5% to 30% in transfusion dependent thalassaemia patients.[5,6,7] However, the incidence of RBC alloimmunization and autoimmunization was low in this study, which was 8.6% and 1.7% respectively. This study was consistent with the study by Ho et al in Hong Kong.[4] Alloimmunization rate in study by Singer et al.[3] and R ameen et al.[2] were 22% and 30% respectively. The higher

alloimmunization rate in these two studies were probably due to the heterogeneity of the population living in Greece and Kuwait and mismatched RBC phenotype between donor and recipients compared to our study population which are more homogenous.

In the present study, anti E was seen most frequently followed by anti c (Rhesus system), anti S, anti N (MNSs system), anti Jka (Kidd system) and anti K (Kell system). All of our patients received compatible blood for ABO and Rh D antigen. In an Italian study, the alloantibody was almost entirely confined to the common antigen of Rhesus, Kell, Kidd and Duffy systems.[6] Several studies shown that anti E are the most prevalent alloantibodies among transfusion dependant thalassaemia patients. [2, 4, 6]

Autoantibodies was found in an 11 years old post-splenectomy Malay girl with HB E/β thalassaemia. She developed autoantibody without underlying alloantibody as determine by persistent positive direct Coombs test with no specific pattern in red cell elution test and panagglutination in absorption test. The monospecific direct Coombs test was positive for both Ig G and C3d in this patient. No secondary causes were identified in this patient. Study in Kuwait observed that 11% of their patients developed autoantibody which positive for both Ig G and C3d or Ig G alone[2]. However, majority of their RBC autoantibodies were associated with RBC alloantibodies. They reported that the presence of residual donor white blood cells (WBC) could have potential influenced on the rate of alloimmunization and autoimmunization seen among transfusion dependent thalassemic patients in Kuwait. RBC bound Ig G was found more abundant in splenectomised than nonsplenectomised subjects in thalassemia patients[8]. The antibodies also were found to have specificity for spectrin and band 3 proteins in thalassaemia patients.

Our results showed that there was no significant association between alloimmunization and gender however; we observed that all of the alloimmunized patients were female. Only 1 of our alloimmunized patient was adult, and the alloimmunization in her could be due to previous pregnancy or blood transfusions. Clinically significant alloantibodies have been reported to occur about twice as often in women compared to men.[9] Of the 5-alloimmunized patients in our study, 3 were adult and 2 were from paediatric age group. There's no association between alloimmunization and age demonstrated in this study. Few studies also reported that no significance relationship between age and alloimmunization in transfusion dependent thalassaemia patients [3,4,10]. Adult recipients, aged 16 to 88 years, apparently do not lose their ability to respond to red cell alloantigens as they aged.[9]

Our low alloimmunization rate in this study probably can be explained by the similarity in the ethnicity between patient and donor. All of our alloimmunized patients were Malays and most of our blood donors were also Malays, which comprised about 83%, of blood donors in our local population. In Hong Kong majority of immunized patients were Southern Chinese and all blood donors were predominantly of the same ethnic origin. Lower rate of alloimmunization in their study was explained by their access to phenotypically matched donors in Hong Kong.[4]

We observed that all 5 of our immunized patients were started on transfusion after the age of one year old. However, our results showed there was no statistically significant association between alloimmunization rate and the age at start of transfusion. R ameen et al.[2] found majority of alloimmunised patients formed first alloantibody between the aged of 2 and 10 years (58%). They observed that most of the alloimmunized patients involved in

their study developed alloantibody at a younger age. Michail V et al.[7] , Singer et al.[3] and T Spanos et al.[5] reported that low frequency of alloimmunization found in patients with thalassaemia major who started transfusion early, result also supported the view that there was some form of immune tolerance induce by an immature immune response mechanism to repeated blood transfusion. Immune response may also be affected by the patient's age at the start of transfusion and the number of blood units a patient receives. Despite exposure to many RBC and WBC antigen, infants do not produced alloantibody against blood cells antigen.[11]

In our study we found that the earliest development of antibody was after 8 units of packed cell transfused. However there was no significant relation between number of packed cell transfused and alloimmunization rate ($p > 0.05$). T Spanos et al.[5] in their study found that the earliest sensitization appear after 10 units transfusions. Blumberg et al.[12] conclude that most blood group antibody seen in multiply transfused patients were due to previous pregnancy and to the initial first ten transfusions. However, the number of antibody formation tends to increase with increasing number of transfusions.

In our study, despite a higher rate of patients with splenectomy, none of them had alloantibodies. This was in contrast to Singer et al.[3] observed that patient who had splenectomy had a higher alloimmunization rate. The absence of spleen may further enhance the immune response to the infused foreign antigens, which are not affectively filtered. .

In our study majority of the patients received packed cells aged between 2 to 7 days old. Frabetti et al.[13] noted that apoptotic was found to occur starting from first 48 to 72 hours of storage.

All of patients involved in this study had long-term exposure to non-leukodepleted packed cells due to limited budget. Study by Blumberg et al.[14] support the hypothesis that WBC reduction may be associated with a reduced frequency of RBC alloimmunization. However this result is in contrast with study by Uhlmann et al.[15] who observed that no significant difference in the transfusion reactions in patients receiving leucodepleted and non-leucodepleted RBC. Nuclear matrix protein that is released in the RBC unit from apoptotic white cells during cold storage, might induced antibody response in multiply transfused patients[16].

Our data show that the rates of RBC immunization to red cell antigens are low in transfusion dependent Malay thalassaemic patients despite the usage of non-leucodepleted blood. This probably can be explained by the homozygosity in ethnic between blood donors and thalassaemia patients and the usage of relatively fresh packed cells. We found that age at started of transfusion and splenectomy did not influence the formation of RBC antibodies.

Due to high incidence of anti E in our studied population, it is advisable to genotyped patients and matched red cell units for E antigen in addition to ABO and D antigen. Antigen matching transfusion will effectively prevent alloimmunisation for thalassaemia patients who have a life long transfusion dependent disease.

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Table 1 **Demographic data of thalassaemia patients who received regular blood transfusions**

Demographic data	Number of patients (n)	(%)
Total patients	58	
Diagnosis		
B Thalassaemia major	8	13.8%
HbE/B thalassaemia	46	79.3%
Hb H Constant Spring	3	5.2%
Hb H disease	1	1.7%
Gender		
Male	34	58%
Female	24	42%
Splenectomy		
Yes	15	25.8%
No	43	74.2%

Table 2 Data of patients with alloantibody

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5

ABBREVIATIONS: RBC = red blood cell; WBC = white blood cell; Ig = Immunoglobulin; Hb = haemoglobin; Rh = rhesus; LISS = low ionic saline solution

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project. My husband had taken good care of our children when I was busy writing the dissertation.

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Rbc	Red blood cell
Hb	Hemoglobin
α	Alpha
β	Beta
δ	Delta
γ	Gamma
Hb F	Hemoglobin fetal
Ig G	Immunoglobulin G
C3d	Complement 3d
Anti	Antibody
Wbc	White blood cell
IHTRs	Immediate hemolytic transfusion reactions
DHTRs	Delayed hemolytic transfusion reactions
DSTRs	Delayed serological transfusion reactions
NHFTRs	Non haemolytic febrile transfusion reactions
HPLC	High performance liquid chromatography
DCT	Direct Coombs Test
EDTA	Ethylenediamine tetraacetic acid
AHG	Antihuman globulin

ABSTRAK

Salah satu daripada risiko kemasukan darah adalah pembentukan antibodi terhadap satu atau lebih antigen sel darah merah. Pembentukan antibodi terhadap sel darah asing atau antibodi terhadap sel darah sendiri akan merumitkan lagi rawatan kemasukan darah kepada pesakit thalassaemia yang memerlukan kemasukan darah yang berulangkali. Maka, kekerapan, sebab-sebab dan cara mengatasi fenomena ini di kaji di kalangan pesakit ini.

Data serologikal dan klinikal daripada 63 orang pesakit thalassaemia yang memerlukan kemasukan darah yang berulangkali, yang mendapatkan rawatan di Hospital Universiti Sains Malaysia dan Hospital Kota Bharu di kumpul dan di analisa secara prospektif. Sampel darah di analisa berdasarkan prosedur tabung darah yang standard untuk ujian saringan dan penentuan antibodi.

Empat puluh sembilan (77.8%) pesakit adalah pasakit thalassaemia HbE/β, 10 (15.9%) adalah thalassaemia β major, 3 (4.7%) adalah Hb H constant Spring dan 1 (1.6%) adalah pesakit Hb H. Kesemua pesakit menerima darah yang dilakukan ujian penyesuaian darah hanya untuk kumpulan darah ABO dan Rh (D) antigen sahaja. Keseluruhannya, 7.9% daripada pesakit menghasilkan antibodi terhadap sel darah asing dan 1.6% pesakit menghasilkan antibodi terhadap sel darah sendiri. Tiga orang pesakit membentuk satu jenis antibody, seorang pesakit membentuk 2 jenis antibodi, dan seorang pesakit membentuk 4 jenis antibodi. Antibodi yang di hasilkan adalah anti E, anti c, anti K, anti Jka, anti N dan anti S. Didapati tiada perkaitan di antara bangsa, splenektomi, kekerapan kemasukan darah

dan umur semasa mula menerima kemasukan darah dengan pembentukan antibodi terhadap sel darah merah, mungkin disebabkan oleh saiz sampel yang kecil. Walaubagaimana pun didapati kesemua 5 pesakit yang menghasilkan antibodi terhadap sel darah asing menerima kemasukan darah yang pertama selepas berumur 1 tahun dan tiada pesakit yang telah menjalani splenektomi. Dua puluh tiga orang pesakit didapati positive untuk ujian antiglobulin langsung, dimana 2 orang pesakit menghasilkan Ig G dan C3d dan 21 orang pesakit menghasilkan hanya Ig G.

Kesimpulannya, kemasukan darah yang sesuai adalah perlu untuk pesakit yang menerima pemindahan darah secara kronik untuk mengelakkan penghasilan antibodi terhadap sel darah asing. Memandangkan tingginya kekerapan penghasilan anti E, adalah di nasihatkan sel darah merah di sesuaikan untuk antigen E untuk pesakit thalassaemia yang menerima darah berulangkali. Dengan itu juga, kesemua pesakit yang akan mulakan kemasukan darah seharusnya dilakukan ujian penentuan fenotip untuk antigen sel darah merah.

ABSTRACT

One of the risks of blood transfusion was formation of antibodies against one or more red cell antigens. The development of alloantibodies and autoantibodies complicates transfusion therapy in multiply transfused thalassaemia patients. Thus, frequency, causes and prevention of this phenomenon were studied among these patients.

Clinical and serological data of 63 multiply transfused thalassaemia patients who sought their treatment in Hospital Universiti Sains Malaysia and Hospital Kota Bharu were collected and analyzed prospectively. Blood samples were subjected to standard blood bank procedure for screening of antibody and subsequent antibody identification.

Of these patients, 49 (77.8%) were Hb E/β thalassaemia, 10 (15.9%) were β thalassaemia major, 3 (4.7%) were Hb H Constant Spring and one (1.6%) were Hb H disease. All patients received blood matched for only ABO and Rh(D) antigens. Overall, 7.9% of the patients had clinically significant alloantibodies and 1.6% had autoantibodies. Three patients develop one type of antibody, one patient developed 2 types of antibodies and one patient developed 4 types of antibodies. The specificities of the alloantibodies were anti E, anti c, anti K, anti Jka, anti N and anti S. There was no significance effects of ethnicity, splenectomy, frequency of transfusion and age at the start of transfusion on red cell immunization in this study due to small sample size. However we observed that all the 5 patients that developed alloantibodies had their first transfusion after the age of one year old and none of them had undergone splenectomy. Twenty-three patients were positive for

direct antiglobulin test, of which 2 were positive for Ig G and C3d and 21 were positive for Ig G only.

As a conclusion, transfusion of matched blood was essential for chronically multi-transfused patients in order to avoid alloimmunisation. Considering the high frequency of anti E, it is advisable to match red cell unit for E antigens in multiply transfused thalassaemia patients. Therefore, all thalassaemia patients who had beed started on transfusions should have RBC antigen phenotyping.

CHAPTER 1

GENERAL INTRODUCTION

1.0 GENERAL INTRODUCTION

Thalassaemia is one of the major public health problems in Malaysia. It is a heterogeneous group of inherited autosomal recessive disorder of haemoglobin syntheses, which are characterized by the absence or reduced output of one or more globin chains of haemoglobin (George, 1998).

Clinically thalassaemia are classified according to their severity into major, intermedia and minor forms. Thalassaemia major is a severe transfusion dependent disorder. Thalassaemia intermedia are characterized by anaemia and splenomegaly and require non-regular transfusion. Thalassaemia minor is the symptomless carrier states (George, 1998).
β thalassaemia are the most important types of thalassaemia because they are so common and produce severe anaemia in their homozygous and compound heterozygous state (Hoffbrand, 2001).

Present management consists of regular monthly blood transfusions to maintain the mean haemoglobin level of 10-11g/dl and this remains the main treatment for severe thalassaemia (George, 1998). One of the complications of blood transfusion is the formation by the recipients of alloantibodies and autoantibodies against red blood cell antigen. Results from a number of studies had demonstrated various frequencies and percentages of alloantibody and autoantibody formation in transfused patients. Some alloantibodies were hemolytic and might cause hemolytic transfusion reactions and limit the availability of safe transfusion, while others were clinically insignificant. Red cell autoantibodies appeared less frequent but

could result in clinical haemolysis and difficulty in cross-matching blood (Singer *et al*, 2000). Antibodies must systematically be identified in the recipient's serum before every transfusion so that compatible blood could be provided. Otherwise problems might occur which sometimes could even threaten the patient's life (Spanos *et al*.1990). Prevention of alloimmunisation ranged from provision of red blood cells matched for all major antigens associated with clinically significant antibodies to blood matched only for antibodies that had already been made. This was because the fact that many alloantibodies were not harmful and expensive prevention methods might therefore benefit only some patients (Singer *et al*, 2000).

Antigen matching transfusion would effectively prevent alloimmunisation. To do so, the patient's ABO, Rhesus, Kell, Kidd and Duffy systems should be typed at diagnosis or before institution of transfusion therapy. Blood to be transfused should always be matched at least with ABO, Rhesus and Kell system (Hoffbrand, 2001).

I hope that this study will contribute to a better comprehension of the problem and will help to find the ideal approach for future transfusion policy in our population.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 INTRODUCTION ON THALASSAEMIA

2.1.1 Definition

Thalassaemia : a heterogeneous group of genetic disorders of hemoglobin synthesis which result from a reduced rate of production of one or more of the globin chains of hemoglobin.

2.1.2 Background

Thalassaemia is a common condition, particularly in the Mediterranean region and South East Asia. The word 'thalassaemia' is a Greek term derived from thalassa, which means 'the sea' referring to the Mediterranean, and emia 'related to blood'. The first description of thalassaemia is given to American paediatrician, Thomas Cooley in 1925 and it was identified as a distinct entity at about the same time by several Italian clinicians. Undoubtedly, Cooley was responsible for the first description of severe, life threatening form of thalassaemia, while the Italian workers described a milder variety of the condition. In Europe, Fernando Riette described Italian children with unexplained hypochromic microcytic anaemia in almost the same year in which Cooley reported the severe form of anaemia. Wintrobe and coworkers in the United States reported a mild anaemia in both parents of a child with Cooley anaemia (Weatherall , 2001). Further cases were identified and the disorder was variously called splenic anaemia, erythroblastosis, Mediterranean

anaemia or Cooley's anaemia (Hoffbrand, 2001). The severe form then was labeled as thalassaemia major and the mild form as thalassaemia minor (Weatherall , 2001).

Thalassaemia are a heterogeneous group of genetic disorders of haemoglobin synthesis that resulted from a reduced rate of production of one or more globins chain (Hoffbrand, 2001). The term 'thalassaemia' used to describe a disorder with a significant decrease in the rate of synthesis of one or more globins chain (B.J Bain, 2000). The globin chain that is produced in excess is responsible for the ineffective erythropoiesis and shortened red cell survival (Weatherall , 2001). Thalassaemia are divided into α, β, δβ and γδβ according to which globin chain is produced but at a reduced rate (Hoffbrand, 2001).

Haemoglobinopathies might result from mutation of a globin gene (Barbara, 2000). Mutation of a β globin chain result in variant form of Hb A and mutation in a α globin chain resulted in variant form of Hb F, A and A2. Similarly mutations of γ and δ genes resulted in mutant forms of Hb F and A2 respectively. In some disorders there was both synthesis of structurally abnormal haemoglobin and a reduced rate of synthesis of the variant haemoglobin. This was the case with Hb H Constant Spring and Hb E, which could be referred as ' thalassaemic haemoglobinopathies' (Barbara, 2000).

2.1.3 Prevalence and Incidence

Thalassaemia and related disorders are the most common genetic disorder seen in Malaysia. As in other parts of the South East Asia, α thalassaemia, β thalassaemia, haemoglobin E and

haemoglobin Constant Spring are widely prevalent. A cross sectional study of a Malay community in Tanjung Karang showed that out of 111 individuals screened for full blood count, reticulocytes stain, haemoglobin electrophoresis, haemoglobin A2 and haemoglobin F estimation, 1 in 4 person carried one or more of these abnormal genes (Ainoon *et al*, 1994). About 4.5% of the people in Malaysia are heterozygous carriers for B thalassaemia. Couples who are carriers are at risk of producing a child with β thalassaemia major where affected births annually would be 2.1/1000 (George *et al*, 2001). A Study done by George *et al.*, 1990 on Chinese in West Malaysia, found that there are 3-5% of the Chinese population that carry β thalassaemia trait which cause severe transfusion dependent in homozygous state, and when combined with haemoglobin E trait, also cause severe anaemia requiring long term medical support and regular blood transfusions.

Thalassaemia major was seen in about 3% in the Singapore populations. Hb E trait was detected among Malays in Singapore and this accounted for 5% of the total number of Malays studied. Based on a survey on random samples submitted for haemoglobin electrophoresis, the incidence of β thalassaemia trait in Singapore was 3% (Chan *et al*, 2001). The overall incidence of B thalassaemia was 1% for Chinese and 1.4% for Malays in Singapore (Chong *et al*, 1984).

Study by Fucheron *et al.*, 2000 found that β thalassaemia, α thalassaemia, haemoglobin E and haemoglobin Constant Spring are prevalent in Thailand. The frequency are 20%-30% for α thalassaemia, 3%-9% for β thalassaemia, 10%-50% for haemoglobin E and 1%-8% for haemoglobin Constant Spring. There is high prevalence of haemoglobin E in Thailand and

Hb E/β is the most common β thalassaemia in this region. A similar finding among our Malay Kelantan population, from study by George *et al.*, 2001 showing the incidence of Hb E thalassaemia was 39%.

A study on 450 healthy adults was conducted in Surabaya, Indonesia and found 4% of them are β thalassaemia trait, 6.2% had haemoglobin E trait and 2.2% had α thalassaemia trait (Sandi, 1987). In Brunei a study was done on 1000 patient who was referred for low haemoglobin, and/or low MCH and MHC. It was found that 30% of these patients are proved to have thalassaemia or haemoglobinopathy. β thalassaemia was diagnosed in 22.7% and α thalassaemia was 4.3 % of this population (Jasdi, 1992). Distribution of Hb E and β thalassaemia in South East Asia was shown in figure 1.

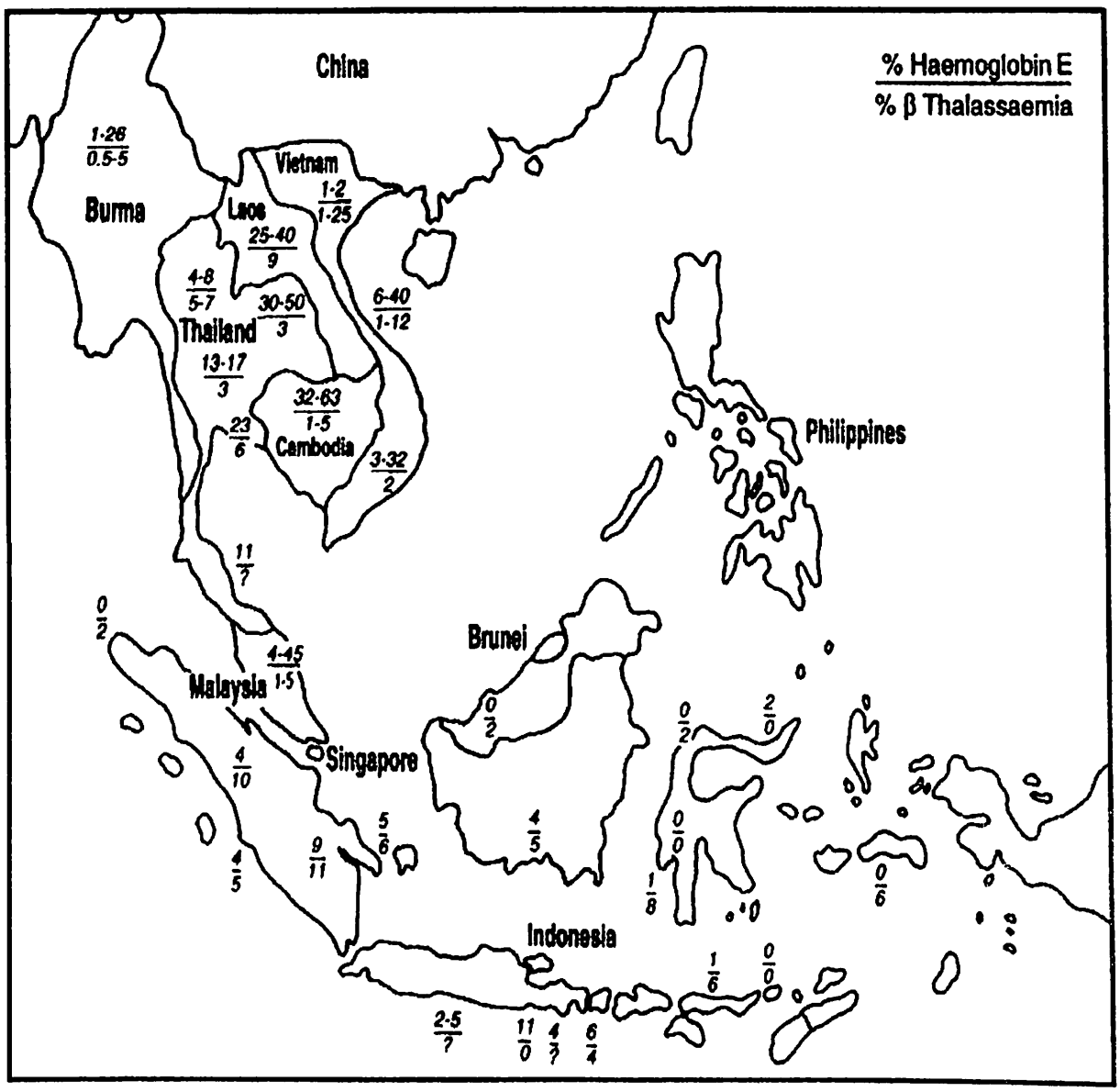

Figure 1. Distribution of B thalassaemia and Hb E in South East Asia.
(Reprinted from Weatheral DJ, 1998).

2.2 COMPLICATIONS OF RED CELL TRANSFUSION

2.2.1 Definitions

Alloantibody: an antibody that is directed against an rbc antigen that is lacking on the patient's rbc.

Autoantibody: an antibody that is directed against an rbc antigen that is on the patient's rbc.

2.2.2 Haemolytic Transfusion Reactions

Haemolytic transfusion reactions are subdivided into 2 types, immediate and delayed. The most common cause of immediate haemolytic transfusion reactions (IHTRs) results from the transfusion of ABO-incompatible rbc because of a clerical error in the labeling of the patient's cross-match specimen (at the time of phlebotomy or in the blood bank) or the misidentification of the intended recipient at the time of transfusion. Delayed haemolytic transfusion reactions (DHTRs) is most often the result of an anamnestic response in a patients who was previously sensitized by transfusion, pregnancy or transplant in whom antibody was not detectable by standard pretransfusion methods (Patrice *et al*, 1994).

i. Immediate Haemolytic Transfusion Reactions (IHTRs)

IHTRs represent the destruction of transfused donor red blood cells by an immunologically mediated mechanism. Factors that influence the severity of IHTRs are class and subclass of immunoglobulin, its ability to activate complement, its plasma concentration, its avidity, its

specificity, red blood cell antigen density, activity of RES, sensitivity of rbc membrane to complement, the amount of incompatible red cell transfused and the thermal range of the antibody (Patrice *et al*, 1994).

The reported incidence of IHTRs reactions varies from 1 per 1,417 to 1 per 21,000 units transfused. The mortality rate of patients having IHTRs ranges from 7% to 40% (Patrice *et al*, 1994).

The IHTRs generally result from the formation of Ig M antibody-rbc antigen complexes that induce complement-mediated intravascular lysis. The membrane-associated immune complex provokes disseminated intravascular coagulation by activating both the intrinsic coagulation pathway (via Hageman factor and complement) as well as the extrinsic system (via platelet and leukocyte mediators). Concomitantly, Hageman factor activates a vasomotor response mediated by the production of bradykinin. This leads to vasodilatation and increased capillary permeability resulting in hypotension. Recent evidence strongly supports the role of proinflammatory cytokines (glycopeptides produced by a number of cells including monocytes, macrophages, lymphocytes, and endothelial cells) as mediators of many of the systemic effects of IHTR. Interleukin 1 β (IL-1 β), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) have been shown to be secreted by leukocytes that have been exposed to antibody-coated rbcs. These substances are known mediators of a shock-like state with fever, rigors, leukocyte and endothelial cell activation, and capillary leakage. Laboratory findings include haemoglobinuria, haemoglobinemia and a positive DCT result. An eluate made from the patient's rbcs will generally contain the pathogenic antibody. Management consists of immediate discontinuation of the transfusion, supportive measures

aimed at restoring or maintaining systemic perfusion and urine output, and treating complications of disseminated intravascular coagulation, if present (Patrice et *al*, 1994).

ii. Delayed Haemolytic Transfusion Reactions (DHTRs).

There is increased incidence over time due to the introduction of more a sensitive technique for antibody identification and greater awareness of the syndrome. Fatality caused by DHTRs is rare (Patrice *et al*, 1994).

DHTRs occur as a result of an anamnestic response from exposure to minor rbc antigens, typically those belonging to the Rh, Kell, Kidd, or Duffy blood groups. These reactions are not as severe as IHTRs and require a higher index of suspicion for diagnosis. The clinical picture is characterized by the onset of low-grade fever, jaundice, and a gradual decline in haemoglobin level 5 to 10 days following transfusion. In some cases haemolytic occurs within 3-21 day time frame (Patrice *et al*, 1994). Haemolysis is typically extravascular with elevated indirect bilirubin and LDH levels. The DAT result is positive. Identification of the antibody is performed to provide antigen-negative rbcs for all subsequent transfusions. The course is self-limited, as the transfused allogeneic cells become Ig G sensitized and subjected to gradual clearance by the RES. Serologically these findings are consistent with a positive DCT caused by drug or hypergammaglobulinemia. Medication history and quantitation of serum immunoglobulin levels is needed to exclude the cause of the positive DAT. Mechanism by which these patients developed a positive DAT remains unknown (Nancy *et al*, 1995).

iii. Delayed Serological Transfusion Reactions (DSTRs)

More often there is serological evidence of alloimmunisation (positive DAT and/or IAT) without clinical evidence of red cell destruction. This is called delayed serological transfusion reaction (DSTR). Nancy et al found that the rate of DSRT was most frequent. There was decrease in incidence of DHTRs and increase in DSTRs. These changes are most likely due to a combination factors, including a decrease in average length of stay for in patients where the patients were discharged before the antibodies reached the threshold of the antibody detection system and the adoption of polyethylene glycol antibody detection system, which has been reported to be more sensitive than the albumin and papain technique (Pineda *et al*, 1999).

2.2.3 Iron Overload

Hemosiderosis is a very real complication of repeated blood transfusions and commonly seen in long term blood transfusion therapy. It is most commonly seen in thalassaemia patients who commence transfusion early childhood. Each unit of blood has approximately 200 mg of iron, while daily excretion rate is about 1 mg. Iron chelating therapy does not completely overcome the iron load administered with blood, but has delayed the onset of problems due to haemosiderosis (Hoffbrand, 2001).

2.3 RBC IMMUNIZATION IN THALASSAEMIA

2.3.1 Introduction

One of the complications of blood transfusion is the production of alloantibodies against red cell by the recipients. The results from a number of studies have demonstrated various frequency and percentages of alloantibody formation in transfused patients. These risks apply mainly to those patients who received multiple transfusions including those suffering from thalassaemia. Such antibodies must be systematically identified in the recipient's serum before every transfusion so that compatible blood can be provided. Otherwise problems may occur which sometimes could even threaten the patient's life (Spanos *et al*, 1990).

Alloimmunisation may exist and not be recognized because 1) the serum is not examined at the appropriate time following antigenic challenge 2) the antibody strength is below the threshold for detection 3) the target cells do not possess the corresponding antigen 4) serologic test are not optimal or 5) the immunity is cellular not humoral (Richard *et al*, 1989).

Clinically significant rbc alloantibody in multiply transfused patients can pose major problems in long-term transfusion therapy. Traditionally the rate of alloimmunization was thought to increase with the total quantity of blood transfused (Brantley et *al*, 1988). Blumberg *et al*, (1984) found that rbc alloimmunization is most frequent during the first 15 transfusions. They also suggested thalassaemia patients become alloimmunised to a particular antigen because of underlying capacity for immune response in the initial

exposures. Study done by Spanos *et al.*, (1994) found that early onset of transfusion (age<3 years) is associated with lower frequency of alloantibody formation. When antibodies against high frequency antigens develop, it can be very difficult to find suitable blood donors. Most blood transfusion services match only red cell units for ABO and Rh antigens (Fluit at al, 1990). It has been advocated that patients with haemoglobinopathies and with aplastic anaemia should receive transfusion matched for minor blood group antigens such as those in the Rh, Kell, Duffy, Kidd and Lewis systems in an attempt to prevent alloimmunization (Blumberg *at al*, 1984).

2.3.2 Prevalence And Incidence

Study done by Singer *et al.*, (2000) on 64 transfusion dependent thalassaemia patients of predominantly Asians descent in Oakland found that 14 patients developed alloantibodies and 16 patients developed autoantibody.

Comparative study of usual vs. better match programmes by Michail *et al.*, (1987) in Greece found that a statistically significant difference does not exist in overall frequency of alloimmunization between unmatched (23.43%) and better match (14.28%).

There are overall 5.2% of thalassaemia major patients in Italy had clinically significant red cell alloantibody (Sirchia *et al*, 1985).

In Hong Kong, Ho et al found that rbc alloimmunisation was 7.4% among Southern Chinese transfusion dependent thalassaemia patients.

In Republic of China, 6% of Chinese patients who received multiple transfusions had rbc antibodies. Anti E, anti Mia and Anti c was the common alloantibody (Chow *et al*, 1994).

Study on patients received blood matched for ABO, Rh and Kell systems from their first transfusion; the immunization rate was very low (3.7%) (Spanos *et al*, 1990).

2.3.3 Alloimmunization

Alloimmunisation may result from prior exposure to donor blood components. As an adverse effect of blood component transfusion, alloimmunisation is a significant complication. Even very small amount of donor antigenic rbcs can elicit an alloimmune response.

With first exposure to foreign antigen, lymphocyte memory is invoked. This will result in a moderate production of Ig M and Ig G antibodies. Secondary exposure elicits rapid production of large amount of Ig G class antibody rising rapidly in the first two days after reexposure to the antigen. The antibody produced attaches to the antigenic surface and may interact with the complement system or RES.

The factors for alloimmunization are complex and involved at least 3 main contributing elements; the rbc antigenic difference between the blood donor and the recipient; the recipient immune status and immunomodulatory effect of the allogenic blood transfusion or the recipient's immune system (Singer *et al*, 2000).

A low rate of alloimmunization may be expected when there is homogeneity of rbc antigens between the blood providers and recipients (Ho *et al*, 2001). Ramsey et *al*, (1988) observed that, there is loss of red cell antibodies over time and patients under 20 years of agewere more likely to lose significant antibodies. Pretransfusion review of previous records is vital for the prevention of delayed haemolytic transfusion reactions, because of the high number of clinically significant antibodies that are undetected in subsequent routine screening. Anti Jka and anti C had particularly high frequency of loss while Kidd antibodies often disappear rapidly. Anti C alone without anti D was especially prone to subsequent loss (Ramsey *et al*, 1994).

2.3.4 Autoimmunization

Autoantibodies are directed against the individual own red cells. Most autoantibodies react with high incidence antigens; they agglutinate, sensitize the red blood cells of random donors as well as those of antibody producers. This circulating humoral antibody may shorten red cell survival (Richard *et al*, 1999).

Autoantibody appears less frequent, but they can result in clinical haemolysis and difficulty in cross matching blood. Patient with autoantibody may have higher transfusion rate and often require immunosuppressive drugs, splenectomy or alternative treatments. Study done by Singer et al found that 16 patients with autoantibody are associated with the presence of alloantibody. Of these autoantibodies, eleven autoantibody were typed as Ig G and 5 were typed as Ig M and 3 patients with this antibody developed clinically significant immune haemolytic anaemia.

The pathogenesis of erythrocyte autoantibody formation following transfusions is not well understood. However it was suggested that alloantibody binding to the rbc could lead to conformational changes of the antigenic epitope that ultimately stimulate production of autoantibody (Sharon M *et al*, 1999). Sharon M *et al* found that it is possible that certain persons are genetic 'responders' who have an increased tendency to develop rbc autoantibody and the tendency toward autoantibody formation could reflect an overall dysfunction of the immune system.

2.3.5 Contributing Factors

i. Age At Start Of Transfusion

Immune response may be affected by patient's age at the start of transfusion. Transfusion at early age (<3 years) may offer some immune tolerance and protection against alloimmunization in thalassaemia patients (Singer *et al*, 2000).

Early onset of transfusion (age <3 years) is associated with lower frequency of alloantibody formation. In a study done by Spanos *et al*, (1990) he observed that in whom transfusion began before the age of 3 the alloimmunization rate were lower than whom transfusion began after the age of 3. The resistance to alloimmunization perhaps due to immaturity of the immunological system.

Sirchia et al, (1984) found that the rate of red cell alloantibodies was significantly lower in those less than 6 years of age.

Red cell antibodies were particularly likely to occur in patients who started regular transfusion late, so excessive hesitation about starting transfusion should be avoided (Rebulla, 1991).

ii. Phenotypic Rbc Matched Blood

The highest rate of clinically significant alloimmunization occurred among patients who were transfused with non-phenotypically matched blood (Ho *et al*, 2001). Spanos *et al.*, (1994) found that there is statistically significant difference in the rate of alloimmunisation in patients who received compatible blood not only for ABO and Rh D but also for CcEe and K antigen. They suggest that patients who will need multiple blood transfusions should receive blood matched for ABO, Rh D, Cc, Ee and K antigen. Matching for Rh and Kell system is essential since the antigens of these systems trigger the greatest percentage of alloimmunisation. This policy and its application in multitransfused patients could overcharge the transfusion services with higher cost and perhaps little benefit. However extra expense involved in more complete blood matching is acceptable when weighed with the risk and problems entailed from transfusion of unmatched blood. In order to decrease the screening volume on long-term basis, the researchers proposed the possibility of using blood from known donors, matched for a single initial occasion for all future transfusion.

Specificity of the alloantibody in thalassaemia patients were almost entirely confined to the common antigens of Rh, Kell, Kidd and Duffy systems. There was lower incidence of alloimmunisation in centres, which followed the 'better match 'policy than with those, which do not. Prevalence of red cell alloimmunisation was lower in thalassaemia major

than in patients with other disease. This indicates that it is not useful to look for compatibility for antigen other than ABO and D. Effort should be redirected to ensure availability of enough available blood for immunized patients, especially those who have mixtures of red cell alloantibodies in their serum. All new patients should be typed for the common antigen of the Rh, Kell, Kidd and Duffy to ensure that multi immunized patients received compatible transfusions (Sirchia *et al*, 1984).

Transfusion with blood matched for Rh and K antigen resulted in significant difference in the alloimmunisation rate. Singer *et al.*, (2000) observed that reduced new antibody formation in patients switched to or started in phenotypically matched blood.

In Hong Kong, anti K was not encountered in their patients. So they rarely need to transfuse K negative antigen matched blood. Only anti E has been reported to be clinically significant causing haemolytic transfusion reaction in their patients (Ho *et al*, 2001).

Matching of transfusion for additional antigens could increase cost dramatically. Blumberg recommends patients should generally receive red cells matched only for Rh(D) and ABO (Blumberg *et al*, 1984).

iii. Frequency Of Transfusion

Incidence of antibody formation increases with the number of transfusions. Small number of translusion needed to stimulate formation of anti K or anti E. It is advisable to match red cell units for the K and E antigens in patients at relatively high risk. Fluit *et al.*, (1990) found that mean number of transfusions before antibody formation was 25.

They observed that 36.4% of patients made their first antibody before or at 10^{th} transfusion and 91% made their first antibody before or at 35^{th} transfusion. They also found that once a person is immunized, the risk of making more than one antibody increases 2.7 fold. Neil Blumberg *et al.*, (1984) found that most blood group antibodies seen in multiply transfused patients are due to the initial 1 to 10 transfusions. The rates of antibody formation per transfusion actually decrease with increasing number of transfusions. They observed that many individuals make that antibody during the first few exposures to the appropriate antigen.

There is no significant difference by gender or age, indicating a similar pattern of antibody formation within the sub samples of men and women and the separate age categories (Fluit *et al*, 1990).

iv. Presence Of Spleen

Prevalence and specificity of alloantibody did not differ between patient who had undergone splenectomy and those who had not (Sirchia *et al*, 1984).

Singer at al found from their study that patients who had asplenectomy had a higher alloimmunisation rate. The absence of spleen may further enhance the immune response to the infused foreign antigens, which are not effectively filtered. Splenectomy may enhanced or promote an immune reaction as there is absence of an efficient filtering system for removal of damaged rbc (Singer *et al*, 2000).

Ho *et al.*, (2001) observed that there is no increase in alloimmunisation rate in patients underwent splenectomy.

Blood requirement in non-splenectomised patients is on average over 30% higher than that of splenectomised patients (Rebulla et al, 1991).

v. Donor Ethnicity

Singer et al., (2000) found that there is antigenic difference between the white donors and Asian patients. High alloimmunisation rate among Asian patients in Greece because the majority of the donor population is white and the majority of blood recipients were Asians. This will result in significant difference in the rbc antigen distribution.

Lower alloimmunisation rate in Hong Kong is explained by their access to phenotypically matched donors in Hong Kong (Ho *et al*, 2001).

Study done by Elliot *et al* on white patients with chronic anaemia and sickle cell anaemia patients who received long term transfusion demonstrated that alloimmunisation in the patients with sickle cell anaemia was due to antigen mismatching between a largely white donor population and blacks recipients.

It is important to study carefully all pertinent data, including phenotypes frequency of the donor population and of the patients, before initiating an antigen-matching program (Tahhan *et al*, 1994).

2.4 IMMUNOMODULATORY EFFECT OF BLOOD TRANSFUSIONS

Blood transfusion can induce immunomodulation. Allogeneic blood transfusion generally caused up regulation of humoral immunity and down regulation of macrophage and T cell immunity. Recent investigations into the mechanism of

allogeneic transfusion induced immunomodulation suggested that altered cytokine regulation might contribute to down regulation of macrophage and T cell function and up regulation of humoral immunity (KJ Kao, 2000).

Allogeneic transfusions predominately favors a Th2 type of immune response (Blumberg et al, 1990). Immunosuppressions of white cells are well recognized in blood transfusion. Marked absolute lymphocytosis were seen predominantly in splenectomised patients with the use of both non-leucoreduced and leucoreduced blood. Lymphocytosis is the result of immunomodulatory effect of blood elements, absence of spleen and recipients immune status. It was postulated that activated immune system increased the propensity to form antibodies (Singer *et al*, 2000).

Ho *et al.*, (2001) observed that significantly lower alloantibody rate had resulted from the introduction of leucodepletion.

Infants did not form alloantibody against rbc antigens following multiple transfusions given during the first 4 months of life. It was proposed that neonatal patients do not form antibodies to rbc due to functionally immature T and B lymphocytes (Floss *et al*, 1985).

Chronic transfusion therapy gives an effect on the distribution of lymphoid cell subsets in the peripheral blood of patients with thalassaemia major. T suppressor cells (CD 8) are markedly increased in thalassaemia patients who have received 300 or more transfusions. CD 4 cells were significantly lower than normal in splenectomised

patients. Transfusion of blood products may lead to decreased CD 4:CD 8 ratios (Robert *et al*, 1985).

2.5 RED CELL SEROLOGY TECHNIQUES

2.5.1 Introduction

Red cell antigen antibody reactions are usually detected by agglutination tests, in saline or macromolecular media, with or without unmodified or enzyme treated rbcs, and with or without the use of low ionic media, antiglobulin sera and potentiators. One of the most important factors affecting the accuracy of results is the reading of the reaction, especially when it is weak. To obtain good results, qualified person must examine the reaction within a short space of time, and, even then, it is some times difficult to interpret.

For maximum sensitivity in antibody detection, reagent rbc should have homozygous expression of the appropriate antigens (Rh D Cc Ee Jka Jkb Fya Fyb Ss) and cells should be used individually and not be pooled. Using such screening cells, antibody screening will detect 99.9% of atypical antibodies, those not detected having specificity for low frequency antigens.

2.5.2 The gel microtubes

The gel microtubes was presented in at Congress of the International Society of Blood Transfusion (ISBT) in1988. This gel system is marketed in the UK as DiaMed-ID system.