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A STUDY OF THALASSAEMIA AMONG BLOOD DONORS IN HUSM

Dissertation submitted in partial fulfillment for the Degree of Bachelor of Sciences(Health) in Biomedicine

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2004

CERTIFICATE

This is to certify that the dissertation entitled

"A Study of Thalassaemia Among Blood Donors in HUSM"

is the bonafide record of research work done by

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CONTENTS

LIST OF TABLE

LIST OF FIGURE

Abstract

Thalassaemia is a common autosomal recessive disorder and have a high incidence among people of Asian Indian origin, Southeast Asian and Northern Thailand. It is classified into α-thalassaemia and β-thalassaemia. Thalassaemia is due to quantitative reductions in globin chains synthesis. Thalassaemia haemoglobinopathies are structural abnormalities of haemoglobin synthesis, where the synthesis of these haemoglobins are reduced in amount.

To data, there is limited study on the effect of blood donation from a thalassaemia donor. So objectives of this study were to determine the prevalence and the type of thalassaemia among blood donors in Hospital Universiti Sains Malaysia (HUSM); and to use the data for future protocol in blood transfusion therapy. A total of 80 blood samples were obtained from the donors at the Transfusion Medical Unit, HUSM. 91.3% of donors were Malays, 1.3% was Chinese and 1.0% was Indian.

The donors were selected according to the standard criteria. Thalassaemia screening was carried out using haemoglobin electrophoresis method. Hb A_2 elution technique and quantitation of Hb F were performed. Most of the blood donors, 15% (n = 12) were diagnosed as thalassaemia / haemoglobinopathy. Out of that, 10 donors presented as microcytic hypochromic and 2 donors were normocytic normochromic.

The type of thalassaemia were Hb E/ α-thalassaemia in 5 donors, Hb E trait in 3 donors, β-thalassaemia in 3 donors and Hb E /β-thalassaemia in 1 donor. This findings were consistent to previous study or population study done by Vella (1962); Lie-injo & Duraisamy (1972); Ganesan et al (1976); showing that Hb E haemoglobinopathy is prevalence among Kelantan population.

Screening test for thalassaemia trait is suggested to be included as the standard procedure of blood test before blood donation especially for the apheresis procedures. Further research is required to investigate the hypothesis that RBC from a donor with the thalassaemia will interfere with the purity of the platelet apheresis product.

Chapter 1

Introduction

In Malaysia, there is a need to raise public awareness of thalassaemia. With public education, screening for thalassaemia becomes feasible. In addition every medical practitioner should look for evidence of thalassaemia from a full blood count. If the mean corpuscular volume (MCV) is low, it should be investigated for iron deficient erythropoiesis or thalassaemia (George et. al 1994).

1.1 Thalassaemias

These are a heterogeneous group of genetic disorders, which result from a reduced rate of synthesis of α or β chains. Clinically they are divided into hydrops foetalis, β-thalassaemia major, which is transfusion dependent, thalassaemia intermedia characterized by moderate anaemia usually with splenomegaly and iron overload, and thalassaemia minor, the usually symptomless carrier (A.V.Hoffbrand & J.E.Pettit 1993).

1.1.1 Thalassaemia: Epidemiology

Thalassaemia is an inherited disease of haemoglobin synthesis. It is a public health problem in Malaysia. About 4.5% of people in Malaysia are heterozygous carries for β- thalassaemia and couples that are carries are at risk of producing a child with β- thalassaemia major. In Malaysian-Chinese, 4.5% are carries of the α-thal-1 or α^{o} -thalassaemia gene. The Malaysian-Chinese couples that are carries of the α^{o} -thalassaemia gene are risk of having a fetus with Hb Bart's hydrops fetalis (Elizabeth George 1998).

The thalassemias are a diverse group of genetic blood diseases characterized by absent or decreased production of normal hemoglobin, resulting in a microcytic anaemia of varying degree. The α-thalassaemias are concentrated in Southeast Asia, Malaysia and Southern China (Fucharoen S, Winichagoon P. 1992).

The β-thalassaemias are seen primarily in the areas surrounding Mediterranean Sea, Africa and Southeast Asia. Due to global migration patterns, there has been an increase in the incidence of thalassaemia in North America in the last ten years; primarily due to immigration from Southeast Asia. The genetic defect of hemoglobin are the most common genetic disorders worldwide. They occur in tropical and sub tropical areas (Figure 1.0).

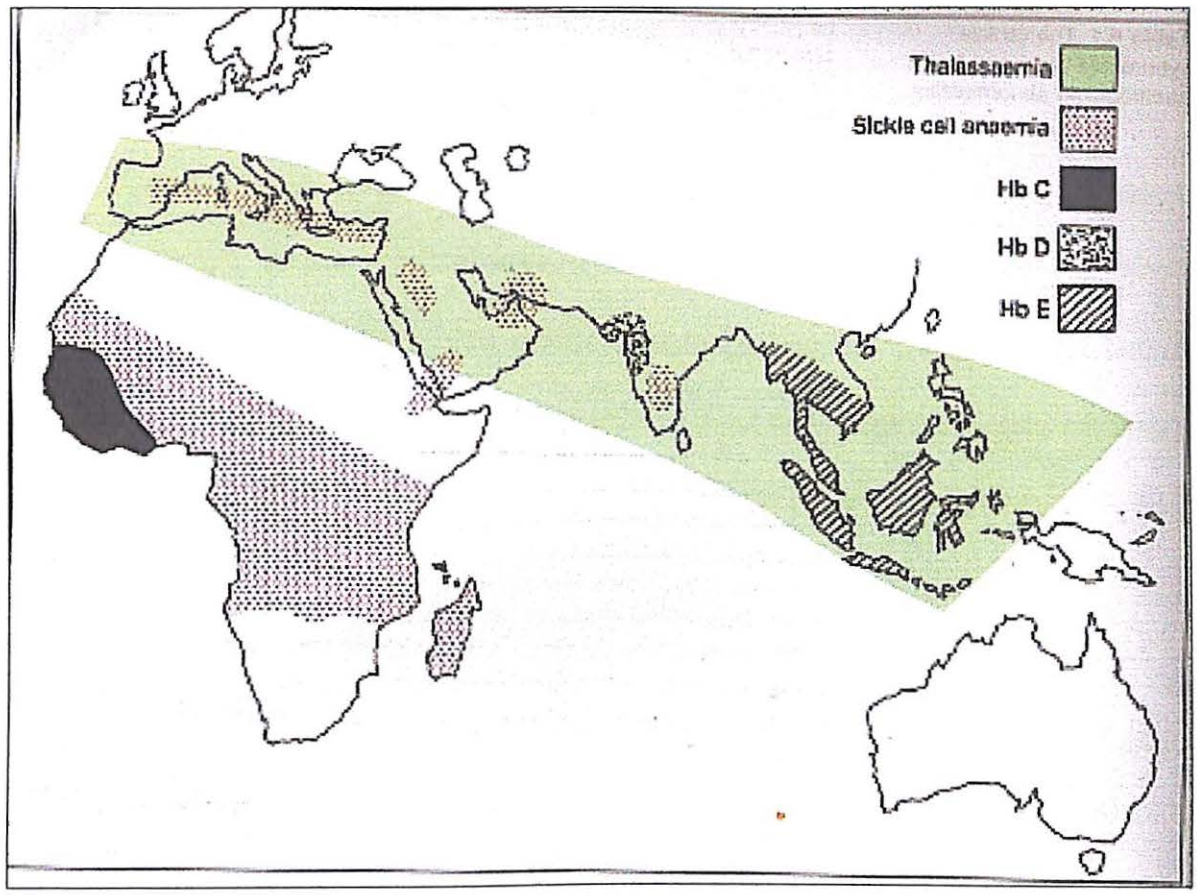

Figure 1.0: The geographical distribution of the thalassaemia and the more common, inherited structural haemoglobin abnormalities (A.V.Hoffbrand & J.E.Pettit 1993).

1.2 Normal Haemoglobin Structure

In the normal adult, Haemoglobin A, which is composed of two alpha and two beta globins ($\alpha^2\beta^2$), is the most prevalent, comprising about 95% of all haemoglobin. Two minor haemoglobins also occur, Haemoglobin A_2, composed of two alpha and two delta globins ($\alpha^2\delta^2$) comprises 2-3.5% of haemoglobin, while haemoglobin F, composed of two alpha and two gamma globins ($\alpha^2\gamma^2$), comprises less than 2% of haemoglobin.

Haemoglobin F, or fetal haemoglobin, is produced by the fetus in uterus and until about 48 weeks after birth. Haemoglobin F has a high oxygen-affinity in order to attract oxygen from maternal blood and deliver it to the fetus. After birth, the production of adult haemoglobin rapidly increases and fetal haemoglobin production drops off.

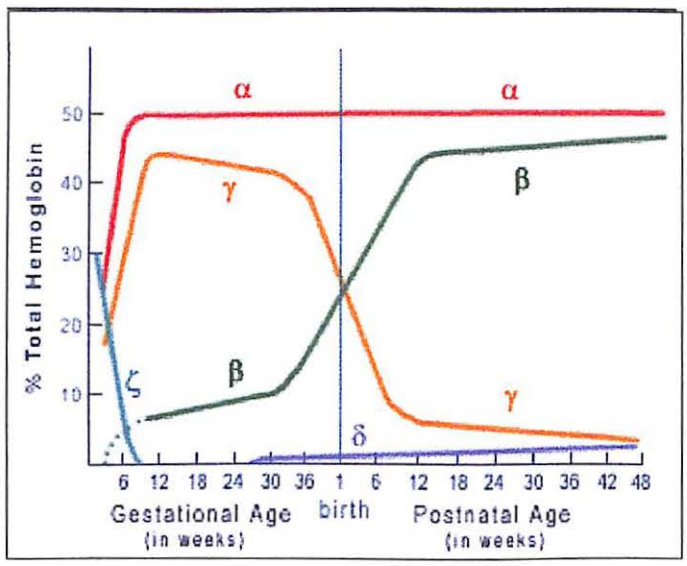

Figure 1.1: Globin Chain production and development.

The alpha globin, molecule concentration is rather stable in fetal and adult life, because it is needed for both fetal and adult haemoglobin production. The beta globin appears early in fetal life at low levels and begins to rapidly increase after 30 weeks gestational age, reaching a maximum about 30 weeks postnatal. The gamma globin molecule reaches a high level early in fetal life at about 6 weeks and begins to decline about 30 weeks gestational age, reaching a low level about 48 weeks post gestational age. The delta globin appears at a low level at about 30 weeks gestational age and maintains a low profile throughout life (Figure 1.1) (Robert S. Hillman, Kenneth A. Ault 1995).

1.3 Pathogenesis of Thalassaemia

In the thalassaemia patient, a mutation or deletion of the genes that control globin production will lead to a decrease production of the corresponding globin chains and an abnormal haemoglobin ratio (α: non-α). This abnormal ratio leads to decreased synthesis of haemoglobin and the expression of thalassaemia. The globin that is produced in normal amounts winds up in excess and forms red cell aggregates or inclusions. These aggregates become oxidized and damage the cell membrane, leading either to haemolysis, ineffective erythropoiesis or both. The quantity and properties of these globin chain aggregates determine the characteristics and severity of the thalassaemia.

1.4 Alpha Thalassaemia

The alpha thalassaemias are caused by a decrease in production of alpha globin chains due to a deletion or mutation of one or more of the four alpha globin genes located on chromosome 16. Alpha gene mapping can be obtained to determine the specific mutation. These are usually due to gene deletions. As there is duplication of the α-globin gene, deletion of four genes is needed to completely suppress α chain synthesis. (Figure.1.2).

The alpha thalassaemias can be generally categorized as:

i. Silent Carrier

ii. Alpha Thalassaemia Trait

iii. Haemoglobin H Disease

iv. Haemoglobin H-Constant Spring

v. Alpha Thalassaemia Major.

Frequently, the diagnosis of alpha thalassaemia trait in parents are discovered after the birth of an affected child (Elizabeth George 1998).

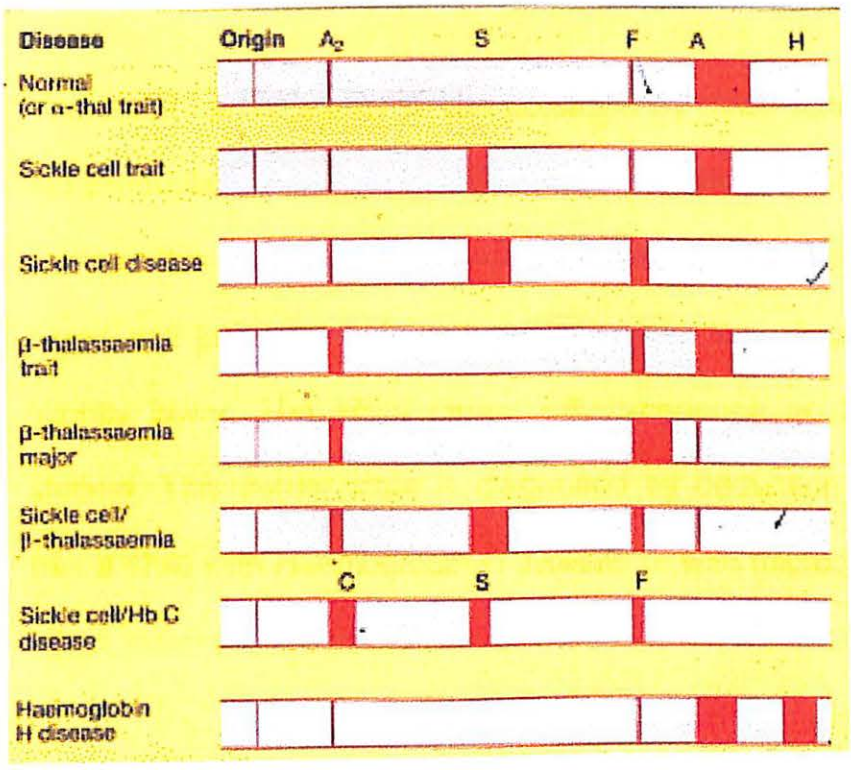

Figure 1.2: Haemoglobin electrophoretic pattern in normal adult human blood and in subject with sickle cell (Hb S) trait or disease, β-thalassaemia, β-thalassaemia major, Hb S / β-thalassaemia or Hb S/Hb C disease and Hb H disease (A.V.Hoffbrand & J.E.Pettit 1993).

1.4.1 Silent Carrier

The silent carrier status is characterized by three functional genes that code for the production of alpha globins (-α/αα). After the newborn period, it is not possible to make this diagnosis by conventional methods. There is overlap between the red blood cell indices of these individuals and normal, although the MCV may be slightly lower. The silent carrier will experience no health problems in his/her lifetime. This carrier state is diagnosed by deduction when a 'normal' individual has a child with Haemoglobin H disease or with microcytic anemia consistent with alpha thalassaemia trait.

1.4.2 Alpha Thalassaemia Trait

Alpha thalassaemia trait is characterized by two functional genes that code for the production of alpha globins [(-α/-α) or (--/αα)]. The two genes can either occur on the same chromosome (cis-type) or on each of the pair (trans-type). Cis-type α - thalassaemia trait tends to be found in individuals of Asian descent, while trans-type tends to run in individuals of African descent (Emmanuel C. et.al 1992).

Cis-type can be co-inherited with another cis-type or haemoglobin H disease to result in alpha thalassaemia major, or hydrops fetalis. Individuals who have alpha thalassaemia trait are identified by microcytosis, erythrocytosis, hypochromia, and mild anaemia. The diagnosis is made by a combination of family studies and the ruling out of both iron deficiency anemia and beta thalassaemia trait.

In the neonatal period, when Haemoglobin Bart's (γ_4) is present, the diagnosis can be strongly suspected. In children, there are no markers such as Haemoglobin A_2 and Haemoglobin F to make the diagnosis. (One exception is the case where both of the deletions occur on the same chromosome and zeta [ζ] globin is expressed in carriers. This is most common in Southeast Asians (Wasi P,et al 1985). The diagnosis is by exclusion. The clinician should be satisfied with the presumed diagnosis if the above criteria are met. During pregnancy, the microcytic anaemia can be mistaken for anemia of pregnancy. The individual with α thalassaemia trait will experience no significant health problems accept a possible slight anaemia, which cannot be treated with iron.

1.4.3 Haemoglobin H Disease

Haemoglobin H disease is characterized by one functional gene that codes for the production of alpha globins (–/-α). Haemoglobin H disease should be considered in the case of a neonate in whom all of the red blood cells are very hypochromic. These neonates have a high percentage of Haemoglobin Bart's on the their newborn screening results. In older children, this haemoglobinopathy is characterized by moderate anaemia with haemoglobin between 8 to 10 gm/dl range, hypochromia, microcytosis, red cell fragmentation, and fast migrating haemoglobin (Haemoglobin H) on electrophoresis (Figure 1.3).

Haemoglobin H does not function as normal haemoglobin and has a high oxygen affinity (holds onto oxygen longer making it unavailable for use by the body), so the measured haemoglobin in these children is misleading. Individuals who have Haemoglobin H generally have a persistent stable state of anaemia, which may be accentuated by increased haemolysis during viral infections and by exposure to oxidant medications, chemicals and foods such as sulfa drugs, benzene and fava beans (similar to individuals who have G6PD deficiency). As the red cells mature they loose their ability to withstand oxidant stress and Haemoglobin H precipitates, leading to haemolysis.

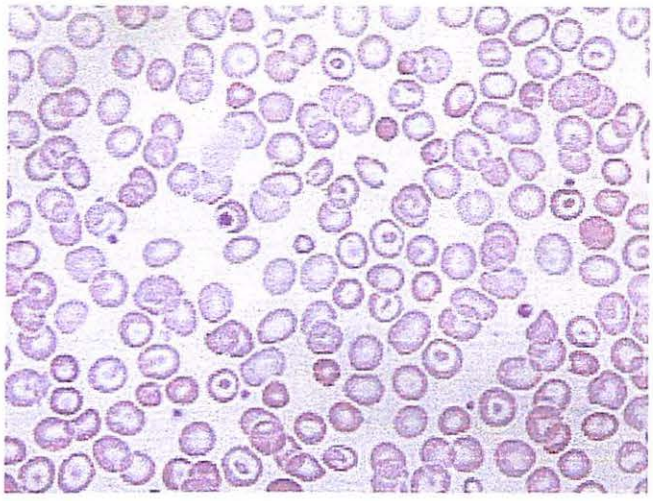

Figure 1.3: Alpha thalassaemia: Haemoglobin H disease (3 α- globin gene deletion). The blood film shows marked hypochromic, microcytic cells with target cells and poikilocytosis (A.V. Hoffbrand, J.E. Pettit. 1993) .

1.4.4 Haemoglobin Constant Spring

An unusual case of the silent carrier state is the individual who carries the Haemoglobin Constant Spring mutation. This is an elongated α–globin due to a termination codon mutation. Individuals who have this mutation have normal red blood cell indices, but can have children who have Haemoglobin H-constant spring disease if the other parent has alpha thalassaemia trait (–/aa).

Generally, children with Haemoglobin H-Constant Spring are more affected clinically than children who have classic Haemoglobin H disease. Two constant spring carriers can also pass on their genes to have a child with homozygous constant spring, a condition that has similar clinical implications as Haemoglobin H disease. Children with Haemoglobin Constant Spring (–/αα) have a more severe course than children who have Haemoglobin H. They have a more severe anaemia, with steady state haemoglobin ranging between 7 and 8 gm/dl.

1.4.5 Alpha Thalassaemia Major

The most severe form of alpha thalassaemia is α-thalassaemia major or hydrops fetalis, characterized by a deletion of all four genes that code for alpha globins (–/–). This diagnosis is frequently made in the last months of pregnancy when fetal ultrasound indicates a hydropic fetus. The mother frequently exhibits toxemia and can develop severe postpartum haemorrhage. These infants are usually stillborn.

There can be other congenital anomalies, though none are pathognomonic for α-thalassaemia major. Since α- globins are required for production of fetal and adult haemoglobin, the fetus suffers from significant in hypoxia uterus. The only haemoglobins found in these infants are: Haemoglobin Portland ($\delta^2\gamma^2$), Haemoglobin H (β^4), and Haemoglobin Bart's (γ^4), and no Haemoglobin A or A_2. These babies can have other complications associated with hydrops, such as heart failure and pulmonary edema.

1.5 Beta Thalassaemia

There are hundreds of mutations within the beta globin gene, but approximately 20 different alleles comprise 80% of the mutations found worldwide. Within each geographic population there are unique mutations. Individuals who have beta thalassaemia major are usually homozygous for one of the common mutations, or heterozygous for one of the common mutations and one of the geographically unique mutations. Both lead to absence of beta globin chain production.

The beta thalassaemia syndromes are much more diverse than the alpha thalasaemia syndromes due to the diversity of the mutations that produce the defects in the beta globin gene. Unlike the deletions that constitute most of the alpha thalassaemia syndromes, beta thalassaemias are caused by mutations on chromosome 11 that affect all aspects of beta globin production: transcription, translation and the stability of the beta globin product. Most haematologists feel there are three general categories beta thalassaemia :

i. Beta thalassaemia trait (β^{+}/β^{A},β^{o}/β^{A})

ii. Beta thalassaemia intermedia (β^{+}/β^{+},β^{+}/β^{o})

iii. Beta thalassaemia major (β^{o}/β^{o})

Splice site mutations also occur and are of clinical consequence, when combined with a thalassaemia mutation. Three splice site mutations occur in exon 1 of the beta globin gene. (Figure 1.4).

These mutations result in three different abnormal haemoglobins:

i. Hb Malay

ii. Hb E

iii. Hb Knossos

Haemoglobin E is very common abnormal haemoglobin in the Southeast Asian population, and when paired with a β^0 thalassaemia mutation, can produce severe transfusion-dependent ($E\beta^0$) thalassaemia (Elizabeth George.1998).

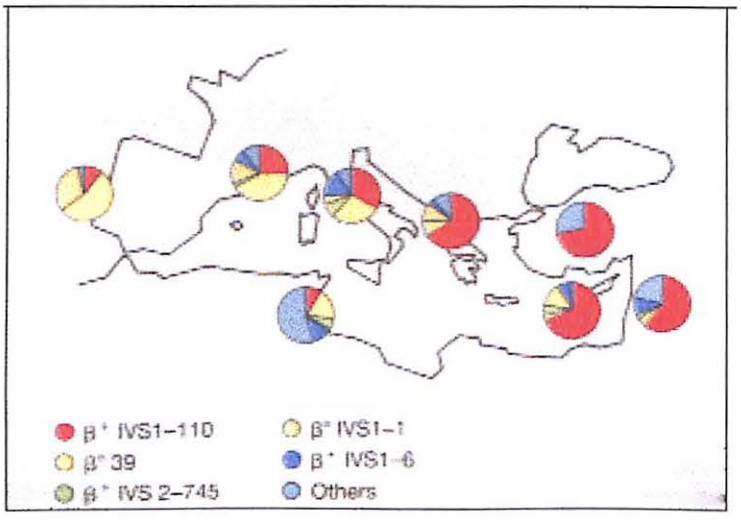

Figure 1.4: Distribution of different mutations of β-thalassaemia major round the Mediterranean (A.V. Hoffbrand, J.E. Pettit. 1993) .

1.5.1 Beta Thalassaemia Trait

Individuals who have beta thalassaemia trait have microcytosis and hypochromia ,target cell and elliptocytosis. Some individuals have an almost normal smear. Blood indices usually low MCV and MCH but red cell count high. The heterozygous state for β^+ or β^o-thalassaemia result in β- thalassaemia trait. Haemoglobin usually 10-15g/dl. Haemoglobins A_2 and F will be elevated on haemogram results.

These haematologic features can be accentuated in women with trait who are pregnant and in individuals who are folate or iron deficient. In iron, B_{12} and folate replete individuals, the Haemoglobin A_2 can be as high as 3.5 to 8% and the Haemoglobin F as high as 1 to 5%. Generally, beta thalassaemia trait is milder in African-Americans (who frequently have a promoter gene mutation) but has a similar presentation in individuals of Chinese, Southeast Asian, Greek, Italian and Middle Eastern heritage. (D.J. Weatherall and J.B.Clegg. 2001).

1.5.2 Beta Thalassaemia Intermedia

Children who are diagnosed with thalassaemia intermedia have a homozygous or heterozygous β-globin mutation that causes a decrease in β-chain production, but not to the degree that chronic transfusion therapy is required. The phenotype can also occur in children who have a mutation that increases production of β-globin, in children who have co-inherited alpha thalassaemia and beta thalassaemia and in other rare mutations.

Children who have thalassaemia intermedia are able to maintain a haemoglobin of 7 gm/dl or slightly higher with a greatly expanded erythron and may manifest bony deformities, pathologic fractures and growth retardation. The homozygous state for β^{+}-thalassaemia (β^{+}/β^{+}) and the compound heterozygous state (β^{+} / β^{o}) result in thalassaemia intermedia.

Children who cannot maintain haemoglobin between 6 and 7 gm/dl should have an alternative diagnosis considered. If thalassaemia is the cause of the anaemia, transfusion and/or splenectomy should be considered (Elizabeth George.1998).

1.5.3 Beta Thalassaemia Major

Beta thalassaemia major is the most severe form of beta-thalassaemia. Present management consists of regular monthly blood transfusions designed to maintain the mean haemoglobin level of 10-11 gm/dl. The iron derived from the transfused red blood cells, and to a lesser extent from increased gut absorption of iron because of ineffective erythropoiesis accumulates in several parenchyma organs.

Beta thalassaemia major also occur in children who have a mutation that decreased production of β-globin, in children who have co-inherited α-thalassaemia and β-thalassaemia and in other rare mutations. The homozygous state for β° ($\beta^{\circ}/\beta^{\circ}$) causes a severe transfusion dependent anaemia termed thalassaemia major. There is a severe hypochromic microcytic anaemia with raised reticulocyte percentage with normoblasts, target cells and basophilic stippling in the blood (A.V. Hoffbrand, J.E. Pettit. 1983).

1.6 Thalassaemia Haemoglobinopathies

Thalassaemia haemoglobinopathies are structural abnormalities of haemoglobin synthesis, where the synthesis of these haemoglobin's are reduced in amount. The thalassaemia is caused by abnormal processing of mRNA or as a result of instability of the abnormal globin chain (Elizabeth George.1998).

Common thalassaemia haemoglobinopathies in Malaysia are:

i Hb E

ii Hb Malay

iii Hb New York

iv Hb Constant Spring

1.6.1 Variant Haemoglobin's

Haemoglobin E is very common among Southeast Asians, California Newborn Screening Program found that 1 in 12 Southeast Asians, and 1 in 4 Cambodian newborns had Hb E trait. Hb EE (homozygous) has not been shown to have serious medical implications. Haemoglobin E-Beta thalassaemia has a wide range of clinical manifestations, from mild anemia to significant anemia requiring chronic transfusions (Cao A, Rosatelli MC. 1993).

1.7 General Guideline for Donor

To give blood for transfusion to another person, a donor must be healthy, be at least 17 years old, weight at least 110 pounds and have not donated blood in the last 56 days. "Healthy" means that feeling well and can perform normal activities. Other aspects of each potential donor's health history are discussed as part of the donation process before any blood is collected.

Each donor receives a brief examination during which temperature, pulse, blood pressure and blood count (haemoglobin or haematocrit) are measured. Making donations for your own use during surgery (autologous blood donation) is considered a medical procedure and the rules for eligibility are less strict than for regular volunteer donations.(R.A.R., MD and M.A.P., RN,BSN the American red cross).

1.7.1 Who should not donate blood?

i Anyone who has ever used intravenous drugs (illegal IV drugs).

ii Men who have had sexual contact with other men since 1977.

iii Haemophiliacs.

iv Anyone with a positive antibody test for HIV (AIDS virus).

v Men and women who have engaged in sex for money or drugs since 1977.