CARDIOPULMONARY ASSESSMENT
IN TRANSFUSION DEPENDENT
THALASSEMIA PATIENTS AT
HOSPITAL UNIVERSITI SAINS MALAYSIA

by

DR. MOHD RIZAL BIN MOHD ZAIN

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ABBREVIATIONS

1. Echocardiography parameters

    i.   LA – left atrium

    ii. IVS – interventricular septum

    iii. LVIDd – left ventricular internal diastolic diameter

    iv. LVIDs – left ventricular internal systolic diameter

    v.   LV free wall – left ventricular free wall

    vi. FS – fraction shortening

    *The detailed explanations of the parameters can be referred to Defination

    section (page 20)

2. Pulsed wave Doppler parameters

    i.   IVRT- isovolumic relaxation time

    ii. E - peak early diastolic flow velocity

    iii. A - peak late diastolic flow velocity

    iv. DT   - deceleration time of early velocity

    *The detailed explanations of the parameters can be referred to Defination

    section (page 22)

3. Pulmonary function parameter

    i.   VC – vital capacity

    ii. FEV_1 – forced expiratory volume at one second

    iii. FVC - Forced Vital Capacity

    iv. PEF – peak expiratory flow rate

    v. FEF_{25} – forced expiratory flow at 25%

    vi. FEF_{50} - forced expiratory flow at 50%

    vii. FEF_{75} - forced expiratory flow at 75%

viii.   MMEF (FEF $_{25-75}$) – max midexpiratory flow rate

*The detailed explanations of the parameters can be referred to Defination

section (page 23)

4. TDT   - Transfusion dependent thalassaemia

5. RBC   - Red blood cell

6. LV   - Left ventricle

7. Hb   - Haemoglobin

8. ATP   - Adenosine triphosphate

9. DNA   - Deoxyribonucleic acid

10. BSA   - Body surface area

11. IQR   - Interquartile range

12. AST   - Aspartate transaminase

13. ALT   - Alanine transaminase

14. ALP   - Alkaline phosphatase

15. β - Beta

16. α - Alfa

17. δ - Delta

18. γ - Gamma

ABSTRAK

Pengenalan

Thalasemia adalah sejenis penyakit yang disebabkan oleh mutasi gen yang bertanggungjawab menghasilkan rantaian globin α- dan β- hemoglobin. Ia boleh menyebabkan masalah kekurangan darah yang mempunyai pelbagai peringkat keterukkan. Kes β-Thalasemia terdapat di sepanjang kawasan Mediteranian, Afrika, Asia Barat, Benua India, Burma dan Asia Tenggara termasuklah China, Semenanjung Malaysia dan Indonesia.

Komplikasi penyakit jantung, endokrin dan penyakit hati adalah signifikan di kalangan pesakit thalasemia. Masalah kegagalan jantung masih merupakan penyebab utama kematian (60%), jauh mengatasi kematian yang disebabkan oleh masalah lain seperti jangkitan kuman (13%) dan penyakit hati (6%) di kalangan pesakit thalasemia.

Fungsi paru-paru yang abnormal telah diketahui di kalangan pesakit thalasemia yang memerlukan transfusi darah secara kerap. Walaubagaimanapun ianya masih tidak difahami secara terperinci dan keputusan kajian sebelum ini masih menunjukkan keputusan yang bercampur-campur. Keabnormalan boleh disebabkan oleh kekurangan darah (anemia), pemendapan zat besi di paru-paru dan juga faktor-faktor lain.

Tujuan Kajian

1. Untuk mengesan keabnormalan pada:

   1.1 Fungsi jantung (i.e fungsi sistolik dan distolik ventrikel kiri)

   1.2 Fungsi paru-paru (i.e kadar aliran dan isipadu paru-paru)

Di kalangan pesakit thalasemia yang bergantung kepada transfusi darah (TDT) yang menghadiri pusat rawatan harian kanak-kanak.

2. Untuk menentukan korelasi (hubungan) di antara keabnormalan pada fungsi jantung dan paru-paru dengan aras serum ferritin.

Metodologi

Kajian ini merupakan kajian keratan rentas, dijalankan di Pusat Rawatan Harian, Jabatan Pediatrik Hospital Universiti Sains Malaysia (HUSM) dari bulan Januari 2006 hingga bulan Disember 2006. Ianya melibatkan seramai 41 orang pesakit thalasemia yang bergantung kepada transfusi darah (TDT) yang menghadiri Pusat Rawatan Harian Pediatrik. Umur subjek ialah 11.4 ± 4.4 tahun yang telah didiagnosa sebagai pesakit thalasemia (melibatkan thalasemia alfa dan beta). Index sistolik dan distolik dopler ekokardiografi fungsi ventrikel kiri jantung dilakukan. Fungsi paru-paru dilakukan menggunakan Microloop Spirometer SPIDA 5 dan ianya hanya melibatkan 29 subjek.

Keputusan

Kajian penilaian fungsi jantung terhadap pesakit TDT menunjukkan bahawa apabila perbandingan dilakukan terhadap keputusan kajian dengan purata nilai normal (mean of normal values), fungsi sistolik ventrikal kiri (LV) tidak menunjukkan perbezaan yang signifikan. Walaubagaimanapun fungsi diastolic LV menunjukkan perbezaan yang signifikan dan setara dengan tanda-tanda masalah jantung ristriktif. Ujian fungsi paru-paru pula menunjukkan majoriti pesakit TDT iaitu seramai 29 orang (89.7%) mempunyai cirri paru-paru ristriktif, seorang pesakit mempuyai cirri-ciri obstruktif dan hanya 2 pesakit sahaja mempunyai fugsi paru-paru yang normal. Fungsi sistolik LV juga tidak menunjukkan sebarang korelasi dengan serum ferritin tetapi funsi diastolic

LV ada menunjukkan korelasi yang lemah. Walaubagaimanapun ujian fungsi paru-paru langsung tidak menunjukkan sebarang korelasi dengan paras serum ferritin.

Kesimpulan

Fungsi sistolik LV kekal normal di kalangan pesakit TDT walaupun mempunyai paras serum feritin yang tinggi. Walaubagaimanapun fungsi diastolik LV terjejas lebih awal dari fungsi sistolik LV. Majoriti pesakit TDT mengalami masalah paru-paru ristriktif. Masalah lebihan zat besi dan pemendakan pada tisu-tisu jantung boleh membawa kepada masalah jantung ristriktif pengisisan ventrikal kiri. Hubungan di antara pemendakan zat besi dan perubahan dalam fungsi paru-paru masih lagi belum dapat dijelaskan.

ABSTRACT

Introduction

The thalassemias, a disease that result from mutations of genes encoding the synthesis of α- and β-globin chains of haemoglobin are responsible for anaemia of variable severity. The β-Thalassemia is widespread throughout the Mediterranean region, Africa, the Middle East, the Indian subcontinent, Burma and Southeast Asia including southern China, the Malay Peninsula and Indonesia.

Morbidity due to cardiovascular, endocrinological, and hepatic disease is considerable in β-thalassemia syndromes. Heart failure remains the major cause of death (60%), greatly exceeding deaths from the other causes such as infection (13%) and liver disease (6%).

Abnormal pulmonary function tests were described in thalassemic patients who need regular blood transfusion. However this is not well described and has mixed results from the previous studies. These abnormalities could be due to anaemia, iron deposition in the lungs, or other factors.

Objectives

1. To describe the abnormalities in:

   1.1 Cardiac function (i.e. left ventricular systolic and diastolic functions)

   1.2 Respiratory function (i.e. flow rates and lung volumes)

   in transfusion dependent thalassemia (TDT) patients attending paediatric day care centre.

2. To determine the correlation between the abnormalities of the cardiac and the respiratory functions with serum ferritin.

Methodology

This is a cross sectional study, conducted at the Day Care Centre Unit, Department of Paediatrics, Hospital Universiti Sains Malaysia (HUSM) from January 2006 till December 2006. It involved all the transfusion dependent thalassemia (TDT) patients who were attending the paediatric day care centre at HUSM. A total of 41 subjects were included in this study, aged 11.4 ± 4.4 years, with the diagnosis of thalassaemia (including both alfa and beta thalassaemia). Doppler echocardiographic indexes of systolic and diastolic ventricular function were assessed. Pulmonary function test was performed using Microloop Spirometer SPIDA and it only involved 29 subjects.

Results

This study revealed that cardiac assessment showed that when compared to the mean of normal values, there was no significant difference of LV systolic function but LV diastolic function showed significant differences and compatible with restrictive heart disease. Lung functions test showed that the majority of the patients, 26 (89.7%) of them predominantly had restrictive lung pattern, one patient had obstructive lung pattern and only 2 of them were normal. There were no correlation of serum ferritin level with LV systolic function and diastolic function only showed weak correlation. However lung function test had no correlation at all with serum ferritin level.

Conclusion

LV systolic performance is well preserved in TDT patients despite high serum ferritin level. However diastolic dysfunction affected earlier than systolic dysfunction. Majority of TDT patients had predominantly restrictive lung pattern. Severe iron overload and deposition in the myocardium may leads to the restrictive abnormalities of left ventricular filling. However relationship between altered lung function tests and iron deposition in the lung remains unclear.

CHAPTER ONE

INTRODUCTION
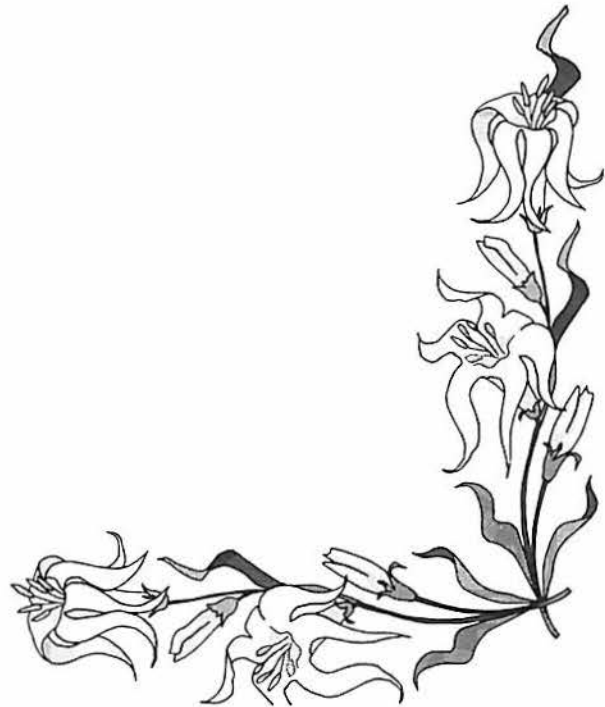
1. INTRODUCTION

1.1 Epidemiology

Thalassaemia, a recessive mendelian disorder is an increasingly serious public health problem throughout the Mediterranean region, the Middle East, the Indian subcontinent and South East Asia (Ismail *et al.*, 2006).

Dr. Thomas Cooley and Dr. Pearl Lee first described a form of severe anaemia in 1925, occurring in children of Italian origin and was associated with splenomegaly and characteristic bone changes. The name thalassaemia was first used in 1932. It originates from the Greek word, *thalassa* which mean *the sea* and anaemia, translating to *anaemia by the sea* (Olivieri, 1999).

In Malaysia, thalassaemia is mostly seen among the Malays and Chinese and only in a small percentage of Indians. The Ministry of Health of Malaysia estimated that each year, between 150 and 350 babies were born with thalassaemia and there were about 5,600 patients who are blood transfusion dependent beta thalassaemia in Malaysia (Ismail *et al.*, 2006).

The thalassemias are a heterogeneous group of genetic disorders, which result from mutations, deletions or point mutation of the genes encoding the synthesis of the globin chains of haemoglobin. The production of normal haemoglobin is partly or completely suppressed because of defective synthesis of one or more globin chains and this lead to anaemia of variable severity. Several types of thalassemias have been described

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however the common types and of clinical importance are β-thalassemias, α-thalassemias and Hb Eβ-thalassemias.

1.2 Types of thalassemia

1.2.1 α-Thalassemia

α-Thalassemia results from the reduced or absent production of α-globin chains. These α-globin genes are duplicated and located in the telomeric end of the short arm of chromosome 16. α-Thalassemia is most commonly caused by deletions of large DNA fragments that involve one or both α-globin alleles.

Clinical disease is related to the number of α-globin genes affected and the degree of gene dysfunction leading to globin chain imbalance and ineffective erythropoiesis. The lack of α-globin protein synthesis results in excess γ-globin and β-globin accumulation that at birth is reflected by non-functional Hb Barts (γ4-globin) and lesser amounts of Hb H (β4-globin). Absence of production from all four α-globin genes produced Hb Bart's hydrop fetalis, the most severe form of α-thalassemia, results in foetal death in-utero from severe anaemia.

Hb Constant Spring is the other relevant structural variant, which is due to an elongation of α-globin chains, causes ineffective production. The mutation is found mainly in Asia. Co-inheritance of Hb Constant Spring and the deletion of two α genes results in a severe form of Hb H disease.

1.2.2 β-thalassemia

In β-thalassemia, a reduced production of structurally normal β-chains and the accumulation of unopposed α-chains lead to anaemia, largely as a consequence of ineffective haemopoiesis. In β-thalassemia major, the patients usually need initiation of blood transfusions during infancy due to severe anaemia. Patients with a less severe phenotype such as in β-thalassemia intermedia may become transfusion-dependent later in life (Hahalis *et al.*, 2005, Olivieri, 1999).

To date, more than 200 thalassaemic mutations have been reported. In β-thalassemia major, microcytosis and anaemia can be detected at birth. However, clinically apparent anaemia usually does not occur before 6 months of age (Palis and Segel, 1998).

1.2.3 Haemoglobin E (Hb E)

Haemoglobin E disorder is the most common structural variant with thalassaemic properties. Hb E is characterised by substitution of lysine for glutamic acid at position 26 of the β-globin chain. This mutation results in both qualitative and quantitative β-globin gene defect. It is the most common abnormal haemoglobin in South East Asia, reaching a carrier frequency of 50%. It is also prevalent in parts of the India subcontinent, including India, Pakistan, Bangladesh and Sri Lanka. Heterozygotes for Hb E are clinically normal and manifest only minimal changes in red blood cell indices, with 25-30% of Hb E on electrophoresis. Homozygotes for Hb E are clinically silent and may be only mildly anaemic.

Genetic compounds for Hb E and β-thalassemia, which are also common in South East Asia, have variable clinical manifestations, from thalassemia intermedia to severe

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transfusion dependent thalassemia major. The explanation for variable manifestation only partially been defined, and the subjects with identical genotypes may have very different severity in clinical manifestations.

1.3   Pathophysiology of Thalassaemia

The matured erythrocyte, or red blood cell (RBC), is a biconcave disc that has 40% more surface area than a spherical RBC of comparable volume. This excess surface area allows a normal RBC with a diameter of 7-8 micron to deform and elongate, thus enable to squeeze through 2-micron pores in the splenic cords and 3-micron capillaries where a full, plump sphere could not. The RBC carries haemoglobin containing of two α chains and two β chains, in which each of them carrying its own heme molecule. Each heme has an iron atom that allows the molecule to pick up oxygen in the lungs and carry it to the tissues, and offload it. Carrying haemoglobin in this form is the major function of the RBC.

Thalassemias were the most common RBC abnormality that evolved to mitigate the consequences of malaria. For reasons which are still obscure, this imbalance in the heterozygous state, protects against malaria infections. However, the consequences of this imbalance is quite significant. Because of reduced synthesis of either alpha or beta globin there was less haemoglobin per cell and this led to hypochromia, microcytosis, and variable anaemia. In addition to poor synthesis of haemoglobin there is accumulation of the excess unmatched globin chains, and these chains accumulate and cause haemolysis to the affected RBC. In the β-thalassemias, the accumulation of these unmatched globin chains damages the marrow erythroid precursors, leading to their intramedullary lysis or ineffective erythropoiesis. These processes can be trivial in

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some cases but very important in other cases by producing severe and even fatal anaemia. Patients with Cooley's anaemia (β-thalassemia major) are absolutely dependent on red blood cell transfusions. With each transfusion, iron accumulates in the liver and the heart eventually leading to death from haemochromatosis (Schrier, 1997).

Besides being poorly haemoglobinised, the thalassemic RBCs also have very abnormal shapes. The study using osmotic gradient ektacytometry to look at mechanical properties of RBCs found that α- and β-thalassemic RBCs are both very rigid. β-Thalassemic membranes failed quickly and therefore were mechanically unstable. However, as opposed to β-thalassemia, α-thalassemia (Hb H disease) the membranes were hyperstable. In α-thalassemia (Hb H) the RBCs are, as anticipated, uniformly overhydrated. In contrast β-thalassemic RBCs have some well hydrated forms, but there are large populations of very dehydrated, very rigid, very viscous RBCs. Therefore, α- and β- thalassemic RBC differ in at least two ways: overhydration in α-thalassemia and dehydration in β-thalassemia; membrane mechanical hyperstability in α-thalassemia, contrasted with instability in β-thalassemia (Schrier *et al.*, 1989). Besides the difference, there was also present of a large amount of oxidised globin present on the RBC membrane from both α- and β-thalassemia (Advani *et al.*, 1992).

In conclusion, in the thalassemias, large amounts of globin is attached to the membrane, mainly to the skeleton. It is the unmatched excess globin chain, α- in β-thalassemia, β in α-thalassemia which had undergone partial oxidation. When globin is attached to the membrane, it carries a heme with its iron, each of which can induce the generation of

reactive oxygen species. These in turn could oxidise adjacent membrane proteins and produce the instability or hyperstability and the associated changes in cellular hydration that were observed. The ferrokinetic observations made in the 1970s, indicate that the marrow is the site of major damage in β-thalassemia (Yuan *et al.*, 1993).

In normal people when the normal reticulocytes leave the marrow they are well hydrated. However, in β-thalassemia the reticulocytes are already variably dehydrated and in Hb H disease (α-thalassemia) the reticulocytes are already clearly overhydrated. Therefore the changes identified in α and β thalassemic RBC are substantially determined in the marrow (Schrier, 1997).

Besides, in β-thalassemia major it appear that the erythroid precursors undergoes accelerated apoptosis (Yuan *et al.*, 1993). Comparing erythroid precursors from normal, β-thalassemia trait, and beta thalassemia major, there is a significant increase in dead and apoptotic cells.

Thus, in conclusion the excess unmatched globin chains are the main role in the pathophysiology of the thalassemias.

1.4 Iron Overload and Complications

Iron is an essential mineral for normal cellular physiology, but excess of this mineral can result in cell injury. Iron in low-molecular-weight forms may play a catalytic role in the initiation of free radical reactions. These reactions have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrates resulting in wide-ranging

impairment in cellular function and integrity. There is substantial evidence that iron overload in experimental animals can result in oxidative damage to lipids in vivo, once the concentration of iron exceeds a threshold level. DNA has also been reported to be a target of iron-induced damage, and this may have consequences in regard to malignant transformation. Reduced cellular levels of ATP, lysosomal fragility, impaired cellular calcium homeostasis, and damage to DNA all may contribute to cellular injury in iron overload (Britton *et al.*, 2002).

Morbidity due to cardiovascular, endocrinological, and hepatic disease is considerable in β-thalassemia syndromes. Cardiomyopathy, diabetes mellitus, and hypothyroidism occurred in 15.1%, 8.6%, and 6.9% of patients with thalassaemia major, respectively. Delayed puberty was present in 38.4% of patients and short stature was common (Li *et al.*, 2002, Hahalis *et al.*, 2005).

In a recent study from the United Kingdom, it was found that 50% of the patients died before the age of 35. Heart disease was responsible for more than half of the deaths. Besides the other complications, osteoporosis and osteopaenia were common and affected virtually all patients. Blood transfusion related infections especially Hepatitis C virus was also common. Hepatitis C virus antibodies were present in 85% of multitransfused Italian patients, 23% of patients in the United Kingdom, 35% in the United States, 34% in France, and 21% in India. Hepatocellular carcinoma could complicate the course of hepatitis (Borgna-Pignatti *et al.*, 2005). In general Iron overload of the heart leading to cardiomyopathy remained the main cause of morbidity and mortality.

Thus, β-thalassemias not only affects a large number of people worldwide resulting in tremendous health care and social problem, but they also require a multidisciplinary medical team approach.

1.4.1 Iron deposition in the heart

Heart failure due to iron overload could develop either as a result of excess dietary absorption (hereditary haemochromatosis) or from repeated blood transfusions. Cardiac iron overload in thalassemia major is the most striking model, in which heart failure remains the major cause of death. Despite the introduction of the iron-chelating agent, desferrioxamine more than 30 years ago in the UK, 50% of patients still die before reaching the age of 35 years (Olivieri, 1999, Anderson *et al.*, 2002).

Even though the actual mechanism of iron-induced heart failure is still not well understood, the toxicity of iron in biological systems is attributed by its ability to catalyze the generation of oxygen-free radicals. The dose-dependent effects of chronic iron loading on heart tissue concentrations of iron, glutathione peroxidase (GPx) activity, free-radical production, and cardiac dysfunction were investigated in a murine model of iron-overload cardiomyopathy. It was shown that chronic iron-overload results in dose-dependent (a) increases in myocardial iron burden, (b) decreases in the protective antioxidant enzyme GPx activity, (c) increased free-radical production, and (d) increased mortality. These findings show that the mechanism of iron-induced heart dysfunction involves in part free radical mediated processes (Bartfay and Bartfay, 2000).

Iron has high affinity for transferrin. Transferrin and ferritin inhibit iron catalytic activity and formation of organ-impairing superoxides. Physiologically there is a feedback system, which inhibits iron absorption in the presence of high level of saturated transferrin and ferritin. However, this protective system can be overcome in pathological situations. Studies showed that iron could still be absorbed even when saturated transferrin levels were high. Similar observation was made in atransferrinaemia, a congenital condition in which, despite absent of transferring, iron absorption still occurred and led to an early organ damage. In these situations, iron was bound in the form of non-protein complexes of low molecular weight called NTBI (Non Transferrin Bound Iron). These complexes did not inhibit the catalytic activity of iron and thus they did not stop the formation of superoxides.

It had been demonstrated that the level of NTBI was high in haemochromatosis and as well as in the thalassemia patients. This high levels on NTBI probably responsible for liver and myocardial damage. Moreover, animal studies had demonstrated that NTBI uptake by the myocardium is 200 times that of other parenchyma (Brissot et al., 1985, Mancuso, 2004)

1.4.2 Iron deposition in the lung tissue

Abnormal pulmonary function tests were described in thalassemia patients who were on regular blood transfusion. However it is still not well-described and showed mixed results among the previous studies. These abnormalities could be due to anaemia, iron deposition in the lungs, or other factors (Grisaru et al., 1990, Hoyt et al., 1986).

Although pulmonary dysfunction was not the most significant clinical manifestation of thalassemias, or indeed patient was asymptomatic, a certain reduction of pulmonary volumes had been reported to occur in most subjects with β-thalassemia (Piatti et al., 1999).

Among the abnormalities of respiratory function that are known to occur in patients with thalassaemia major include restrictive and or obstructive ventilatory defects, as well as impairment of transfer factor (diffusing capacity) for carbon monoxide. The actual aetiology of the restrictive abnormalities remains unknown. Factor et al in their study found that there was an inverse correlation between total lung capacity (TLC) and lifetime estimates of transfusional iron load and suggested that iron deposition in the lungs may play a key role in the pathogenesis of volume restriction in thalassemia patients (Factor et al., 1994). However, Tai et al., 1996 found that the calculated transfusional iron load did not correlate with TLC (Tai et al., 1996).

In the literature, there were contradictory results about the respiratory dysfunction. Most showed a restrictive spirometric pattern and a minority showed an obstructive pattern. However, none of the patients examined showed clinical symptoms due to lung dysfunction (Dimopoulou et al., 1999). Table 1.1 showed the summary of the results of pulmonary function in thalassaemia patients in the previous studies.

Pulmonary haemosiderosis, iron overload of connective tissue, alveolar septa and blood vessels, and interstitial fibrosis have only been observed in some patients affected by thalassaemia, but iron accumulation in these tissues has never been quantified.

Iron overload in the body produces damage to and dysfunction of these systems that is probably directly related to the defence mechanisms of the organs and tissues involved. The damage is related to the tissues' capacity to neutralize oxidative damage produced by free iron. This could explain why some organs, with the same degree of iron accumulation as others, are damaged more.

Table 1.1   Previous studies on pulmonary function in thalassaemia patients

Author	No of patients	Age	pO2	Pulmonary function	DL$_{CO}$
Cooper ,1980	17	6–18	hypoxemia in 15/17	restriction in 8/17	reduced in 13/15
Keens, 1980	12	18.4±2.6	hypoxemia in 10 /12	small airway obstruction in 11/12	normal
Hoyt, 1986	19	10–29	–	small airway obstruction	normal in 16/19
Grant, 1986	8	14–24	hypoxemia in 5/8	restriction	reduced but increased after transfusion
Fung, 1987	28		–	mild restriction	normal
Freedman, 1990	8		–	restriction	–
Grisaru, 1990	35	8–33	hypoxemia in 85 %	restriction in 24/35 obstruction in 2/35	reduced in 50 %
Lands, 1991	10	7– 23	–	normal	–
Bacalo, 1992	17	6–17	hypoxemia in 2/17	restriction in 7/17	reduced in 57 %
Luyt , 1993	15	5–18	hypoxemia in 6/13	restriction	reduced
Factor , 1994	29	6–40	hypoxemia in 1/29	restriction	reduced in 7/29
Santamaria , 1994	12	13.4±3.9	increases after transfusion	restriction	increased after transfusion

DL$_{CO}$ – single-breath carbon monoxidediffusing capacity

-Adapted from (Piatti *et al.*, 1999, Solymar *et al.*, 1980)-

Thus, the reasons for conducting this study in USM because despite of establish data regarding the cardiac and respiratory complications resulting from iron overload cause by recurrent transfusion. Unfortunately we still do not have our own local data of our

TDT patients. The effects of iron overload on the pulmonary functions, as mentioned above are still not well described in the literature and the results are also mixed. As a result we will combine the assessment of cardiac and pulmonary functions in this study among our TDT patients. We hope that it will help us to get a better understanding on this subject and this will help in managing patients in the future.

CHAPTER TWO

OBJECTIVES

2. OBJECTIVES OF THE STUDY

2.1 TITLE OF THE STUDY

Cardiopulmonary Assessment in Transfusion Dependent Thalassemia Patients at Hospital Universiti Sains Malaysia.

2.2 OBJECTIVES

2.2.1 To describe the abnormalities in:

i. Cardiac function (i.e. left ventricular systolic function and left ventricular diastolic function)

ii. Respiratory function (i.e. flow rates and lung volumes) in transfusion dependent thalassemia (TDT) patients attending paediatric day care centre.

2.2.2 To determine the correlation between the abnormalities of the cardiac and the respiratory functions with serum ferritin.

CHAPTER THREE

METHODOLOGY

3. METHODOLOGY

3.1 Study Design

This was a cross sectional study, conducted at the Day Care Centre Unit, Department of Paediatrics, Hospital Universiti Sains Malaysia (HUSM) from January 2006 till December 2006.

3.2 Study Population

All the thalassemia patients who were attending paediatric day care centre at HUSM.

Inclusion criteria

• All transfusion dependent thalassemia (TDT) patients (regardless of type) under paediatric HUSM follow-up who require regular blood transfusion (TDT patients).

Exclusion criteria

• Patient with underlying syndromes or congenital heart disease.

• Patient with HIV infection.

• Patient with underlying malignancy.

3.3 Calculation of Sample Size

The sample size was calculated using single mean calculation as shown below:

$$\text{No of sample, } n = \left[\frac{Z(S)}{\Delta} \right]^2$$

S =   standard deviation

Δ =   detectable difference of the measurement

towards the study population.

$$\text{Thus,} \quad n = \left[\frac{1.96(15.5)}{2.9} \right]^2$$

$$= 110$$

(Gharzuddine *et al.*, 2002)

However because of the limited number of the patients at our centre, the estimated number of patients was only 55.

From the single mean formula above, the detectable difference of 2.9 was derived from the measurement of E wave of pulse wave Doppler after the discussion with the supervisor and the cardiologist.

3.4 Methodology

The names and registration numbers (RN) of the patients were obtained from the Paediatric Day Care Centre. All case notes of the patients were traced and reviewed. The patients were contacted and explanations of the participation in the study were provided. All of them were invited for an interview using a standardised questionnaire and a complete assessment of cardiopulmonary functions.

During the assessment day, which was held at Echocardiography Room, HUSM, written consent was obtained from the parents or guardians. If the patients were more than 18 years old, the consent was obtained from the patients. Subsequently, all the patients were interviewed individually in order to complete the questionnaire (appendix 1).

Physical examination was performed on each patient including anthropometrics measurements (weight and height), blood pressure measurement and oxygen saturation measurement. Electrocardiography (ECG) was performed on each patient.

Cardiac function assessment was evaluated via M-mode echocardiography and pulse-wave Doppler was recorded for analysis off line. The echocardiogram was performed by a specialist trained in echocardiography. He was the only person who was going to perform the echocardiography and pulse-wave Doppler. Parasternal long axis and short axis, and apical four chamber views was obtained. M-mode recording of left ventricle was obtained with the echo beam directed from the parasternal position to the tips of the mitral leaflets guided by real time 2D-echo. The measurements of echocardiography were listed below.

The results of echocardiography and pulse-wave Doppler was compared to the mean of normal values (Bu'Lock *et al.*, 1995, Myung and George, 2002, Snider *et al.*, 1997) based on similar body surface area (BSA) of the patients using paired t-test analysis.

Pulmonary function studies were carried out on a portable, computerised pulmonary function laboratory instrument. It was performed in all patients who were cooperative and capable of performing the test. The spirometry test adopted in this study used

Microloop Spirometer SPIDA 5. The measurements are listed below. Before recording commenced, the assessor would coach each child on the technique. The patient would perform the spirometry test while seated and a minimum of 5 FVC manoeuvres will be made. Recordings would be repeated until at least 3 technically satisfactory curves obtained with the measurements within 10 % of the largest value. Where more than 3 measurements were available, 3 with the largest value would be saved for subsequent analysis. The data then were transferred to Spida Spirometry Database System.

The result of lung function test than were compared with the mean of the normal values (Cogswell *et al.*, 1975, Solymar *et al.*, 1980) based on similar body surface area (BSA) of the patients using paired T-test analysis.

3.4.1. Echocardiography Measurements

i.   **Echocardiography**

- LA diameter

- LVIDd – left ventricular internal diastolic diameter

- LVIDs – left ventricular internal systolic diameter

- IVS – interventricular septum

- LV free wall – left ventricular free wall

- <u>LV systolic function</u> - fraction shortening (FS)

ii.   Pulse-wave Doppler of LV (LV diastolic function)

- E (cm/s)   - peak early diastolic flow velocity

- A (cm/s)   - peak late diastolic flow velocity

- E/A ratio

- DT (cm/s/s)   - deceleration time of early velocity

- IVRT (ms)   - isovolumic relaxation time

3.4.2. Pulmonary Function Test Measurement

i.   Lung Volumes

- VC - Vital Capacity

ii.   Flow rates

- FEV_1 - forced expiratory volume at one second

- FEV_1/VC

- PEF - peak expiratory flow rate

- $FEF_{25\%}$ - forced expiratory flow at 25%

- $FEF_{50\%}$ - forced expiratory flow at 50%

- $FEF_{75\%}$ - forced expiratory flow at 75%

- MMEF ($FEF_{25\%-75\%}$) - max midexpiratory flow rate

3.5   Statistical Analysis

SPSS version 12.0 for Windows was used to analyse the data. Comparisons to normal values based on similar BSA were made using paired t-test. The correlation of the results with the serum ferritin level was assessed using Pearson Correlation Coefficient (r). The correlations were considered significant if the corresponding p value was less than 0.05.

CHAPTER FOUR

DEFINITIONS

4. DEFINITIONS

1. Primary education

    - Attending school up to standard six.

2. Secondary education

    - Attending school from form 1 to form 6

3. Tertiary education

    - Having education at diploma level and above

4. Transfusion dependent thalassaemia (TDT)

    - All thalassaemia patients who require blood transfusion at least

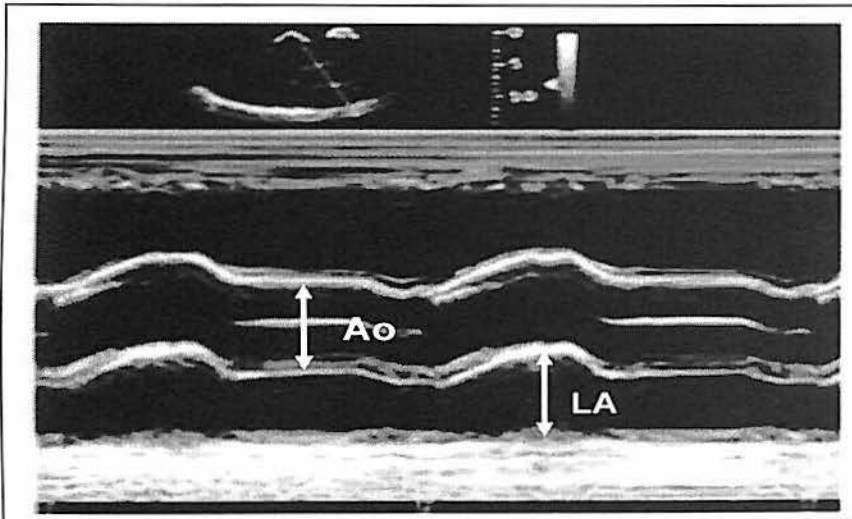   twice a year.

5. Echocardiography parameters

Figure 4.1   Parasternal long axis view with m-mode of aorta and left
              atrium

i.   Ao - Aorta diameter

      - Internal diameter of aorta measured at parasternal long axis

        view

ii.   LA – Left atrium diameter

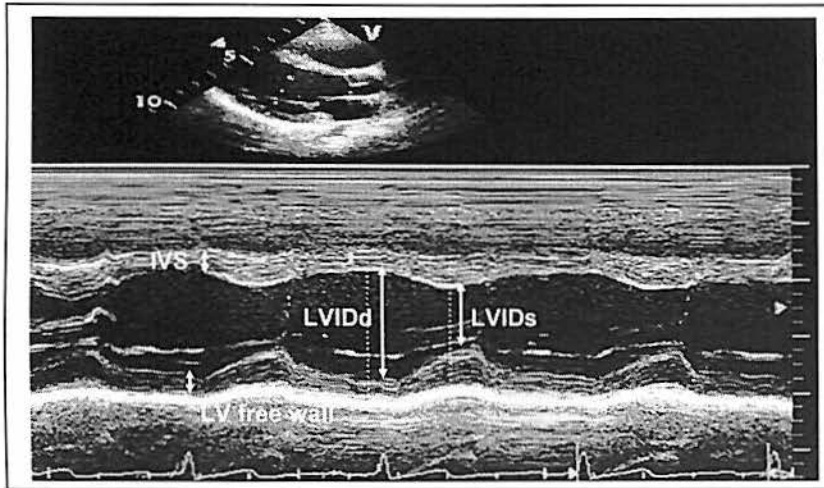- Internal diameter of left atrium measured at parasternal long axis view

Figure 4.2   Parasternal long axis view with m-mode of left ventricle

iii.   IVS – interventricular septum diameter/ thickness

iv.   LVIDd – left ventricular internal diastolic diameter

- internal diameter of left ventricle during diastolic phase (relaxation phase)

v.   LVIDs – left ventricular internal systolic diameter

- internal diameter of left ventricle during systolic phase (contraction phase)

vi.   LV free wall – left ventricular free wall

vii.   FS – fraction shortening

- M-mode indexes of left ventricular systolic function. It is the percent change in left ventricular diameter that occurs with systole

- It is calculated using the following equation:

$$FS = [(LVIDd - LVIDs) / LVIDd] \times 100$$
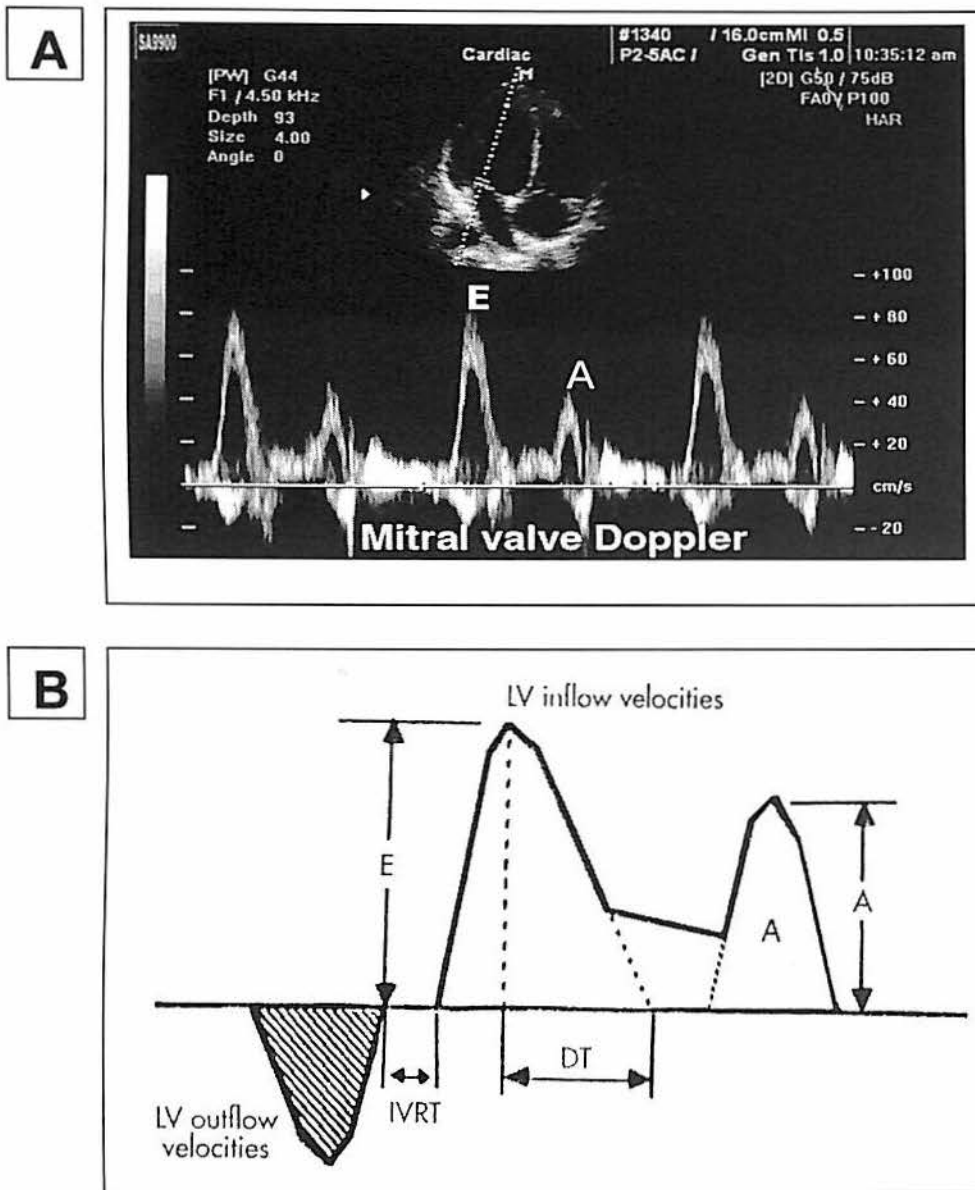
6. Pulse Wave Doppler parameters

Figure 4.3 (A) Pulse wave Doppler waveform, (B) Schematic diagram illustrating the pulse wave doppler

i. IVRT- isovolumic relaxation time

- the interval between the end of the LV outflow velocity and the onset of mitral inflow

ii. E - peak early diastolic flow velocity

- the velocity of an E wave occurring during early diastolic filling

iii. A - peak late diastolic flow velocity

- the velocity of an A wave occurring during atrial contraction

iv. DT - deceleration time of early velocity

- the interval from the early peak velocity to the zero intercept of the extrapolated deceleration slope
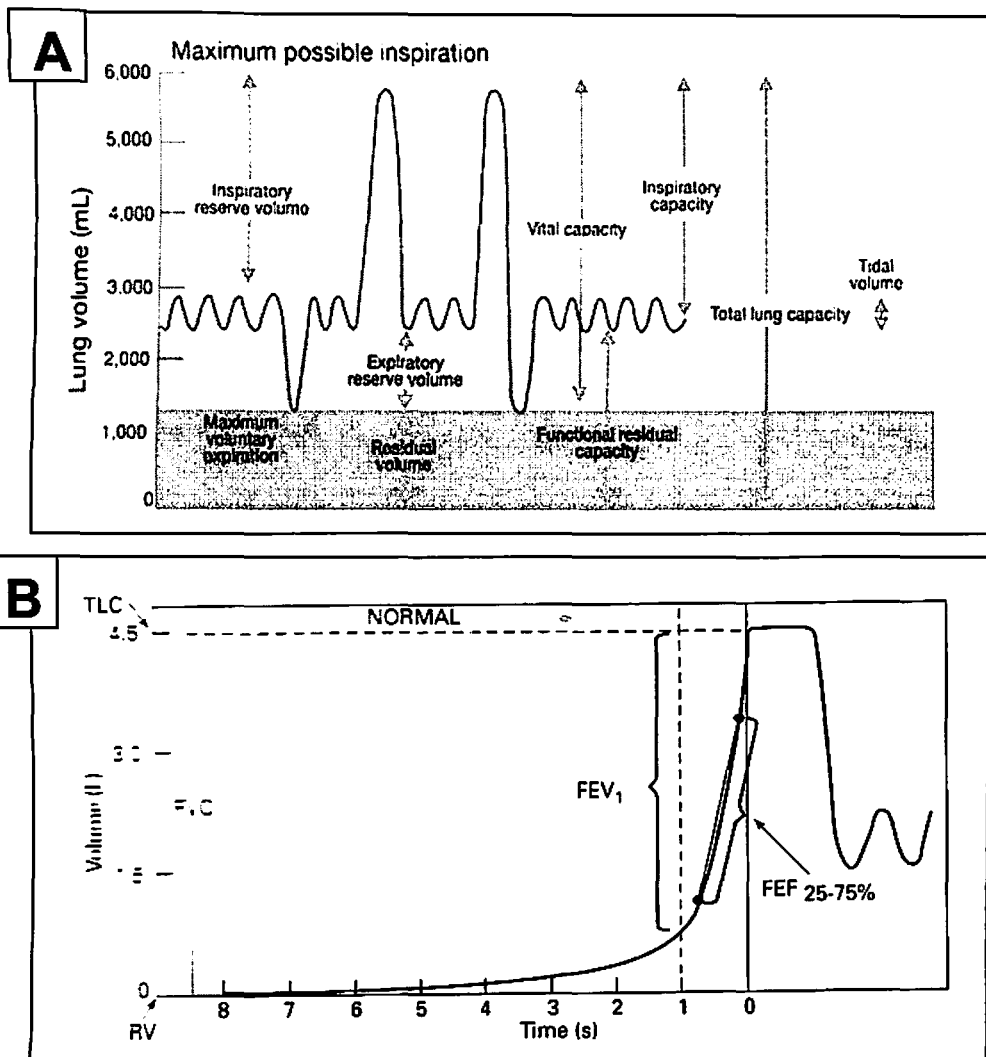
7. Pulmonary function parameters

Figure 4.4   (A) and (B) are the illustration of the lung function test parameters

i.   VC – vital capacity

  • volume of the gas measured from a slow, complete expiration after a
    maximal inspiration.

ii.   FVC - Forced Vital Capacity

  • the maximum gas can be expired when the subject exhales as
    forcefully and rapidly as possible.

iii. FEV_1 – forced expiratory volume at one second

  • the volume of gas expired over one second from the beginning of
    FVC.

iv.   PEF – peak expiratory flow rate

  • the greatest flow that can be obtained during a forced expiration
    starting from full inflation of the lung.

v. FEF_{25} – forced expiratory flow at 25%

  • FEF at 25% of the volume expired

vi. FEF_{50} - forced expiratory flow at 50%

  • FEF at 50% of the volume expired.

vii. FEF_{75} - forced expiratory flow at 75%

  • FEF at 75% of the volume expired.

viii. MMEF ($FEF_{25\%-75\%}$) – max midexpiratory flow rate

  • the average flow during the middle 50% of an FVC manoeuvre.