CRANIOFACIAL AND DENTAL ARCHES CHARACTERISTICS IN TRANSFUSION DEPENDENT THALASSAEMIA PATIENTS

by

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بسم الله الرحمن الرحيم

(رب أوزعني أن أشكر نعمتك اللي أنعمت علي وعلى والدي وأن أعمل صالحا ترضاه وأدخلني برحمتك في عبادك الصالحين) سورة النمل-آية 19

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TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

TDT Transfusion dependent thalassaemia

CFD Craniofacial deformity

CVM Cervical vertebral maturation

CS Cervical vertebral maturation stage. There are six stages **CS1,CS2, CS3, CS4,CS5** and **CS6**.

FEA Finite element analysis

FEM Finite element morphometry

MFS Morpho studio software

Kg Kilogram

Cm centimeter

µg/L microgram per liter

g/dl gram per deciliter

SD standard deviation

IQR interquartile range

SPSS Statistical Package for the Social Science

DEFINITION OF TERMS

Transfusion dependent thalassaemia patients
Thalassaemia patients who receive regular blood transfusion (at least two blood transfusions per year).

Craniofacial deformity
Defined as having bony deformity in the form of frontal bossing and/or bulging of the cheeks and/or maxillary overgrowth

Haemoglobin level
Is a test that measures the level of free (outside red blood cells) hemoglobin in the blood. Normal values: 13.0 –18.0 g/dL in adult males, 12.0 - 16.0 g/dL in adult females.

Serum ferritin level
Ferritin is a protein found inside cells that stores iron so body can use it later. A ferritin test indirectly measures the amount of iron in blood. The amount of ferritin in blood (serum ferritin level) is directly related to the amount of iron stored in body.
Normal value: 20-300 µg/L in adult males and 18-300 µg/L in adult females.

Percentile
Rank position of an individual in a serial array of data, stated in terms of what percentage of the group the individual equals or exceeds.

Angle Class I malocclusion
A malocclusion in which the buccal groove of the mandibular first permanent molar occludes with the mesiobuccal cusp of the maxillary first permanent molar

Geometric morphometrics
Morphometrics is a field concerned with studying variation and change in the form (size and shape) of organisms or objects. This technique assesses the distribution of "landmarks": points described by a tightly defined set of rules, for example the suture between three named bones in a skull.

JLink analysis
A JLink is defined as the link between two landmarks. A JLink has a length that can be calculated, and during a transformation its length may change, so a Length factor can also be calculated, by dividing the final length by the initial one.

Finite element analysis
Finite-element analysis is a method of comparisons between forms. In this context, a specimen at an initial stage is compared with the same specimen at a final stage after a transformation.

Ciri-ciri Kraniofasial dan Lengkungan Gigi bagi Pesakit Talasemia-Transfusi- Dependen

ABSTRAK

Penyakit talasemia merupakan penyakit yang biasa di Asia Tenggara. Namun maklumat berkaitan dengan deformasi kraniofasial dan lengkungan gigi serta kerencatan dalam proses pematangan tulang, amatlah terhad.

Tujuan kajian ini adalah untuk menentukan prevalen kecacatan kraniofasial pesakit talasemia transfusi dependen (TDT) dan hubungan antara kecacatan ini dengan data klinikal. Kajian ini juga adalah untuk membandingkan ukuran sepalometrik, lengkungan gigi dan kematangan tulang vertebra servikal di kalangan pesakit talasemia–transfusi-dependen dengan kumpulan biasa kawalan.

Sebahagian dari penyelidikan ini merupakan kajian rentas lintang dimana prevalen kecacatan kraniofasial (CFD) di ukur daripada 43 pesakit TDT (purata umur 11.6 ± 4.75 tahun) dari Hospital Universiti Sains Malaysia (HUSM) menggunakan evaluasi klinikal. Data klinikal lain dibandingkan diantara pesakit TDT yang mengalami kecacatan kraniofasial (CFD+) dengan mereka yang tanpa kecacatan kraniofasial (CFD-). Sebahagian kajian ini pula menggunakan plan perbandingan, kes dan kawalan. Ukuran sepalometrik diukur dengan menggunakan perisian CASSOS 2001. Manakala morfologi lengkungan gigi dikaji menggunakan perisian Morpho Studio v3.01. Peringkat kematangan vertebra servikal (CVM) di nilai dengan menggunakan kaedah Baccetti *et al.,* (2005) dan purata umur setiap peringkat CMV dibandingkan. Ujian Independent *t,*

Mann-Whitney test and ujian chi-square dilayari untuk perbandingan hasil kajian. Analisis perangkaan telah dilakukan dengan menggunakan perisian Windows SPSS 12.0.1.

Hasil kajian mendapati prevalen kecacatan kraniofasial ialah 44.2 % dikalangan pesakit TDT (95 % CI= 30.2 %, 58.2 %). Tiada perbezaan statistik yang signifikan ($P>0.05$) ditemui antara CFD+ dan CFD- pesakit TDT pada semua data klinikal. Analisia sephalometrik menunjukkan perbezaan yang tidak signifikan pada hubungan ukuran anteroposterior ($P>0.05$) tetapi terdapat sedikit peningkatan sudut ANB pada kumpulan TDT, manakala, peningkatan ukuran angular mandibel adalah signifikan ($P<0.01$) pada kumpulan pesakit TDT. Kedua kumpulan menunjukkan perbezaan yang tidak signifikan ($P>0.05$) dalam ukuran panjang maksilari manakala ukuran panjang badan mandibel dan ramus adalah lebih pendek di kalangan pesakit TDT ($P\leq0.001$). Kedua-dua bibir atas dan bawah pesakit TDT menonjol ke depan ($P<0.001$) dan sudut nasolabialnya lebih kecil ($P<0.05$) berbanding kumpulan kawalan. Analisa lengkungan gigi menggunakan JLinks menunjukkan bahawa kumpulan pesakit TDT mempunyai dimensi maksila anterior dan mandibel posterior yang lebih kecil sebanyak 3-5%. Akhirnya, pesakit TDT menunjukkan ($P<0.05$) umur kronologikal yang lebih muda berbanding kumpulan kawalan pada peringkat servikal 1(CS1) dan kronologikal umur yang sama pada CS2 sementara mereka lebih tua daripada kumpulan kawalan pada CS3 ($P<0.05$) dan CS4 ($P>0.05$) dan tiada daripada mereka sampai ke CS5 atau CS6.

Kesimpulannya, pesakit TDT mempunyai prevalen yang tinggi dalam kecacatan kraniofasial tetapi tiada hubungan kait dengan data klinikal yang di kaji. Mereka mempunyai ciri-ciri rahang muka kelas II, penonjolan kedua-dua bibir atas dan bawah ke hadapan, pertumbuhan menegak tulang mandibel, lengkungan mandibel dan maksilari yang kecil dan kelewatan dalam kematangan vertebra servikal.

Craniofacial and Dental Arches Characteristics in Transfusion-Dependent Thalassaemia Patients

ABSTRACT

Thalassaemia diseases are common in Southeast Asia. However, information about craniofacial and dental arches deformity as well as retardation of skeletal maturation are deficient in this area.

The aims of this study were to determine the prevalence of craniofacial deformity (CFD) in transfusion-dependent thalassaemia (TDT) patients and the association between this deformity and clinical data. It was also to compare cephalometric measurements, dental arches features and cervical vertebral maturation of TDT with a normal control group.

This study was a cross sectional study, in part of it; where the craniofacial deformity of 43 TDT patients (mean age of 11.6 ± 4.75 years) from Hospital Universiti Sains Malaysia (HUSM) was evaluated clinically. Clinical data were compared between TDT patients with craniofacial deformity (CFD+) and those without it (CFD-). Other parts of this study followed a case control design. The cephalometric parameters were measured using CASSOS 2001 software. Morpho Studio v3.01 software was used for dental arches measurements while the stages of cervical vertebral maturation (CVM) were evaluated using Baccetti *et al.*, (2005) method. The mean chronological age of each stage of CVM was determined. Independent *t* test, Mann-Whitney test and chi-square test were utilized for previous comparisons. Statistical analyses were done using SPSS 12.0.1 for Windows.

Prevalence of craniofacial deformity was found to be 44.2% in TDT patients (95% CI= 30.2 %, 58.2 %). No statistically significant difference ($P>0.05$) was found between CFD+ and CFD- groups in all clinical data. Cephalometric analysis showed no significant difference in anteroposterior relationships ($P>0.05$) but slightly increased ANB angle in TDT group while, angular measurements of mandibular position were all significantly increased ($P<0.01$) in TDT group. Both groups showed insignificant difference in maxillary length while both mandibular body and ramus lengths were shorter in TDT group ($P\leq0.001$). Procumbancy ($P<0.001$) of upper and lower lips together with smaller nasolabial angle ($P<0.05$) were found in TDT group. Dental arches analyses with JLinks, showed that TDT group had narrower maxillary anterior and mandibular posterior dimensions by nearly 3-5%. Finally, TDT patients showed younger ($P<0.05$) chronological age in cervical stage 1 (CS1), the same chronological age in CS2 while older chronological age in CS3 ($P<0.05$) and CS4 ($P>0.05$) compared to control group. None of TDT patients has reached CS5 or CS6.

In conclusion, TDT patients had high prevalence of CFD that has no association with clinical data studied. TDT patients were characterized by mild class II skeletal pattern, protruded upper and lower lips, prominent vertical growth direction of the mandible, smaller maxillary and mandibular arches dimensions and retarded cervical vertebral maturation.

CHAPTER 1

INTRODUCTION

Thalassaemia and other haemoglobin disorders are the most common monogenic disorders among humans. Nearly 7% of the world's population carriers of potentially pathological haemoglobin genes (Weatherall and Clegg, 2001 b, WHO, 2002). Each year about 300, 000 infants worldwide are born with haemoglobin disorder; 30% of them with thalassaemia (WHO, 2006). β-thalassaemia is most common in the Mediterranean basin, Middle East and Asia. Severe alpha thalassaemia is common in Southeast Asia and sickle-cell anaemia predominates in Africa (WHO, 2006). Increasing global migration has introduced haemoglobin disorders into many areas where they were not originally endemic (Vichinsky, 2005).

Haemoglobin E is the most common structural haemoglobin variant globally (Vichinsky, 2007). It is found in the Eastern half of the Indian subcontinent and throughout Southeast Asia where carrier rates may exceed 60% of the population in some areas (Weatherall and Clegg, 2001 b). Although HbE trait and HbE disease are asymptomatic conditions, the interaction of HbE and β-thalassaemia can cause a severe clinical disorder that requires regular transfusion. Indeed, globally HbE/ β-thalassaemia is one of the most important varieties of thalassaemia especially in Asia (Weatherall, 2000) (Fig.1.1).

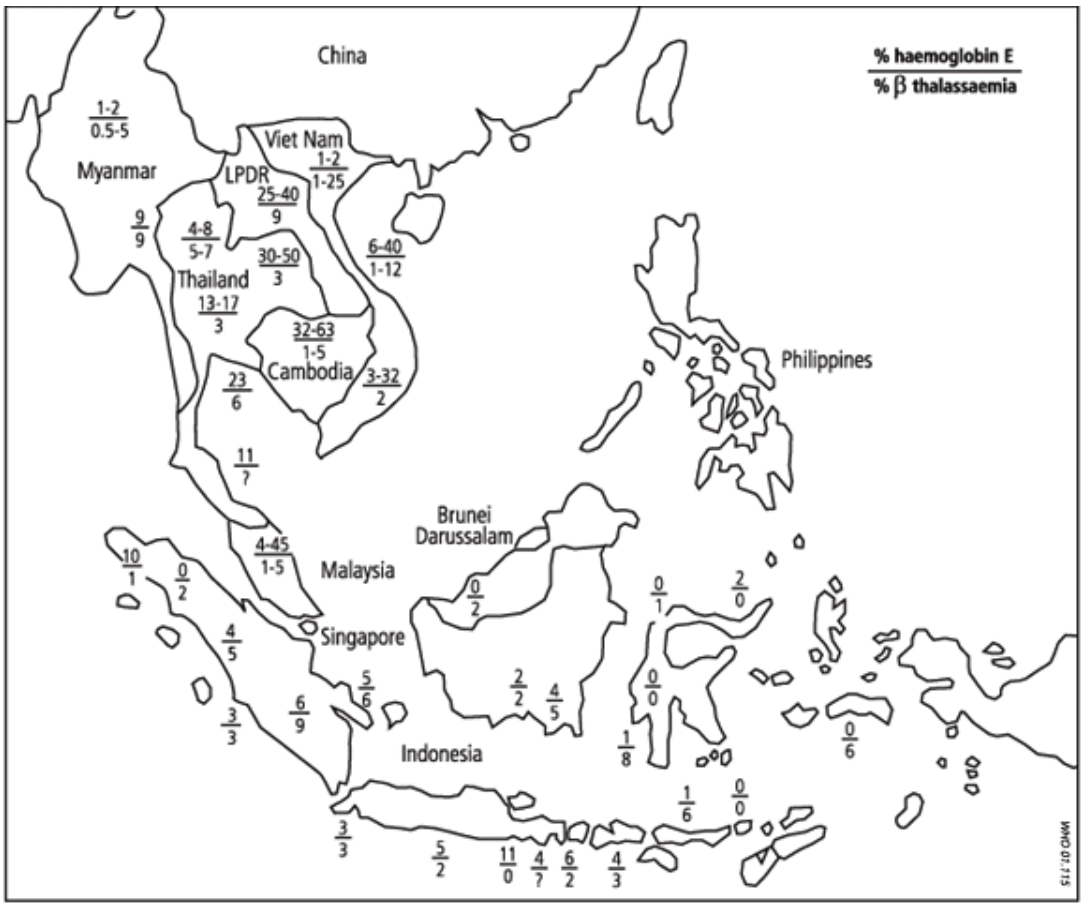

Figure 1.1 Distribution of Hb E and β-thalassaemia in Southeast Asia (Source: Weatherall and Clegg, 2001 a).

In Malaysia, the most recent data showed 4541 affected thalassaemia patients (Malaysian Health Technology Assessment Section, 2009). Nearly 73% of them presented with transfusion-dependent β-thalassaemia major and HbE/ β-thalassaemia, 10% with thalassaemia intermedia, 9% with HbH disease and the rest with other types of thalassaemia. The highest percentage of registered patients was found in East Malaysian state of Sabah (28%) while only 3% in Sarawak. In peninsular Malaysia, the distribution pattern followed the population density except for predominance of HbE/ β-thalassaemia in the Northern states (Malaysian Health Technology Assessment Section, 2009).

Management of β-thalassaemia major and some other varieties of thalassaemia requires lifelong medical treatment with blood transfusion and iron chelation. Bone marrow transplantation is the only chance of a cure, but the availability is limited and the risks are considerable (La Nasa *et al.*, 2005). Consequently, these disorders constitute an increasing drain on health resources, particularly in those countries that go through demographic transition from poverty into stronger economies (WHO, 2002, WHO, 2005). In such countries, the required treatment for survival of thalassaemia patients is becoming available thus, many patients are now able to reach their second or third decade of life (Weatherall, 2005). However, their life course is interrupted with many complications.

One of the important aspects of thalassaemia is the skeletal changes affecting cranial and facial bones. These changes occur in the form of hypertrophy of the maxilla, protrusion of anterior teeth, depression of the bridge of the nose and prominent malar eminences producing a characteristic facial feature known as ‘chipmunk face’ or as ‘rodent face’. Bossing of the skull, open bite, malocclusion, puffiness of eyelids and a mongoloid slant of eyes are other common features of this problem (Duggal *et al.*, 1996, Abu Alhaija *et al.*, 2002). Skeletal complications are commonly seen in inadequately treated patients and less in patients with optimal treatment (Tyler *et al.*, 2006).

Another frequent aspect of thalassaemia is the retarding effect on the general growth and skeletal maturation of the affected patients (De Sanctis *et al.*, 2004, Christoforidis *et al.*,

2007). Such complications have negative physical, psychological and social impact on thalassaemia patients and their parents (Khurana *et al.*, 2006, Ismail *et al.*, 2006).

It is important to conclude that dealing with the problem of thalassaemia requires efforts to develop adequate clinical and laboratory programs for their control. Informations about frequency, different forms, severity and complications of the disease must also be defined in order to help in providing best diagnostic and therapeutic services for thalassaemia patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical background

The period between 1925 and 1940 saw the first descriptions of the clinical features of different form of thalassaemias (Ranney, 2001). The first description of the severe form of thalassaemia is accounted to the American paediatrician Thomas B. Cooley in 1925, who described four children with anaemia, splenomegaly, enlargement of the liver, discoloration of skin and sclera together with a peculiar mongoloid appearance caused by enlargement of the cranial and facial bones (Weatherall and Clegg, 2001 a). Indeed, Cooley was describing the severe life-threatening form of β thalassaemia which is still known as Cooley's anaemia. Several Italian clinicians described at about the same time, the milder forms of thalassaemia (Bunn and Forget, 1986, Weatherall and Clegg, 2001 a). The term ‘thalassaemia’ was first used in 1932 which is derived from a Greek word ‘thalassa’ meaning the sea, because all early cases were reported in children of Mediterranean origin (Bunn and Forget, 1986).

Theoretical model for the genetic basis of thalassaemia has been established by 1960 (Ranney, 2001). Afterwards, it was apparent that thalassaemia is not one disease, but a very diverse group of genetic disorders, all of which result from abnormal haemoglobin synthesis. Furthermore, it became clear that thalassaemia have a widespread distribution and not localized to the Mediterranean region. However, a relatively complete

understanding of the molecular basis of thalassemia was only available in 1980s (Ranney, 2001, Weatherall and Clegg 2001 a).

2.2 Thalassaemia in general

Thalassaemia is one of the inherited haemoglobin disorders 'haemoglobinopathies' in which there is inadequate synthesis of globin chain subunits of haemoglobin (Weatherall and Clegg, 2001 a). However, the globin chain produced-if any-are generally normal in structure (Provan and Gribben, 2000). Haemoglobinopathies include also the structural variants of haemoglobin such as haemoglobin S and C that, in contrast with thalassaemia, are produced in normal quantities but have altered structure and function. In addition, there are variants such as haemoglobin E and Lepore which are both quantitavely and qualitatively abnormal (Provan and Gribben, 2000). These variants are sometimes called 'thalassaemia variants' because they share thalassaemia in the reduced rate of globin chain synthesis and hence having the same thalassaemia phenotype (Dispenzieri, 2001). .

Indeed, because these diseases are all so common and occur together in particular populations, it is not uncommon for an individual to inherit a gene for one or other form of thalassaemia and a structural haemoglobin variant (Provan and Gribben, 2000, Dispenzieri, 2001, Weatherall and Clegg, 2001 b). Haemoglobinopathies are most easily understood on the basis of an elementary understanding of haemoglobin structure.

2.2.1 Human haemoglobin structure and function

Human haemoglobins are tetrameric molecules containing two pairs of globin chains, each of which is associated with an iron-containing heme group. The heme groups allow the haemoglobin molecules to transport the oxygen to the tissues of the body (Clark and Higgins, 2000, Dispenzieri, 2001).

The structure of human haemoglobin (Hb) changes during development. Normal adult (HbA) and fetal (HbF) haemoglobins have α-chains that are combined with β- (HbA, $\alpha_2 \beta_2$), δ- (HbA_2, $\alpha_2 \delta_2$) or γ-chains (HbF, $\alpha_2 \gamma_2$), whereas in the embryo, α-like chains called ζ-chains combine with γ- (Hb Portland, $\zeta_2 \gamma_2$) or ε-chains (Hb Gower 1, $\zeta_2 \varepsilon_2$), and α- and ε-chains form Hb Gower 2 ($\alpha_2 \varepsilon_2$). Embryonic haemoglobin is confined to the yolk-sac stage of development and thereafter is replaced by HbF until shortly before term. After birth, HbA and HbA2 replace HbF over the first year of life while in normal adults small amounts of HbF, constituting nearly 1% of the total haemoglobin, continue to be produced. The α-like genes are encoded on chromosome 16, whereas the β-like genes form a cluster on chromosome 11(Clarke and Higgins, 2000, Weatherall, 2001) (Fig. 2.1).

The main function of haemoglobin is to carry oxygen to the tissues. Other functions include the transport of carbon dioxide (CO_2) and a buffering action (reduction of the changes in PH) in a red cell (Bain, 2001).

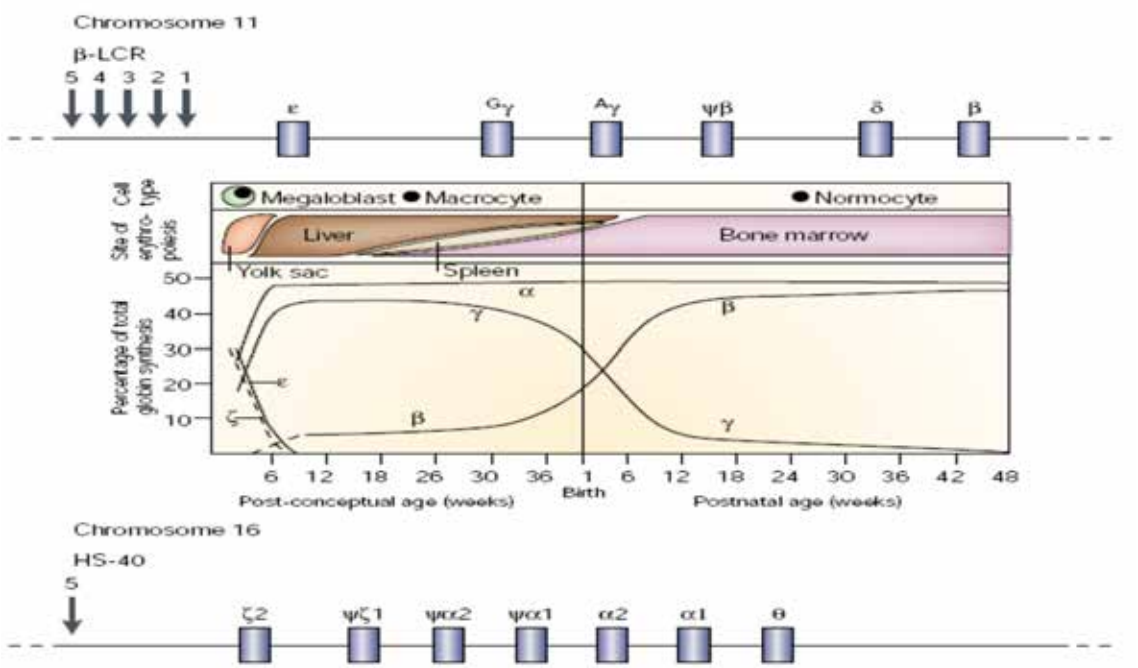

Figure 2.1 The α-globin gene cluster on chromosome 16 and the β-globin gene cluster on chromosome 11 (Source: Weatherall, 2001).

2.2.2 Classification of thalassaemia

Thalassaemias can be subdivided into two main groups α and β thalassaemia. Although each can be further classified into different subgroups, all these disorders have one thing in common; there is always imbalanced globin synthesis (Necheles *et al.*, 1969, Clarke and Higgins, 2000). The differences are summarized in figure 2.2.

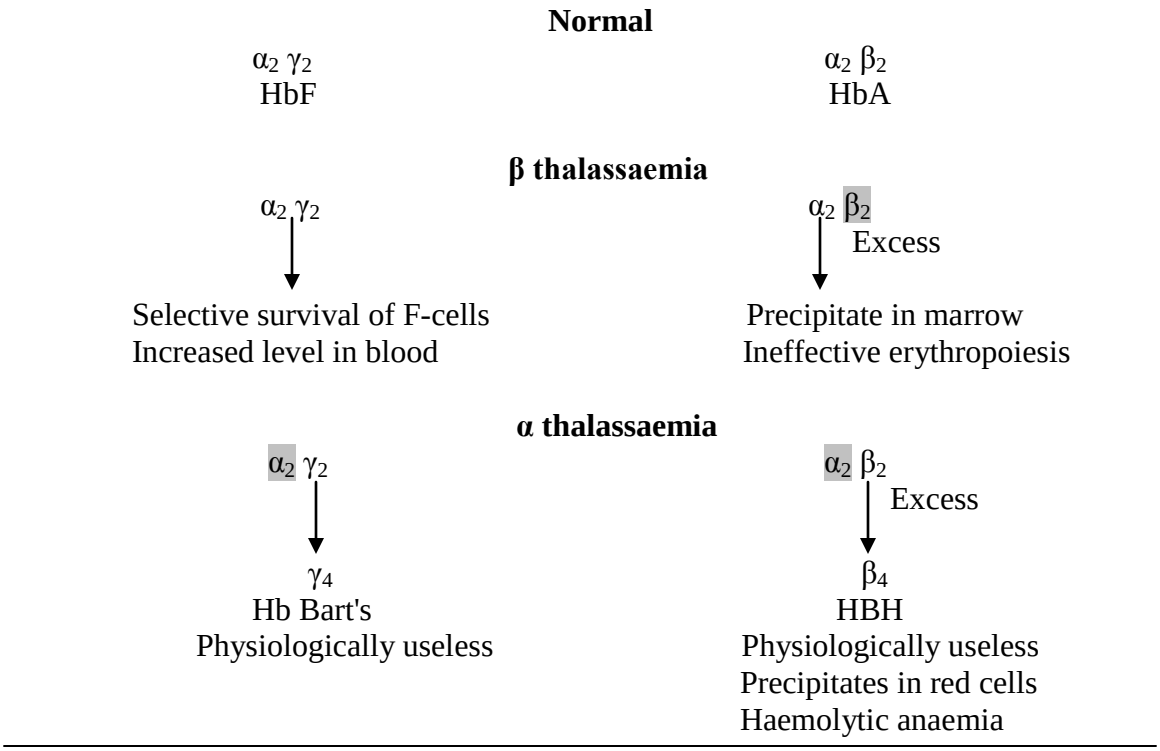

Figure 2.2 A simplified representation of the differences in the haemoglobin patterns between α and β thalassaemias. Shaded boxes indicate defective globin synthesis (Source: Weatherall & Clegg, 2001 a).

The disease can be described at several levels. First, there is a clinical (phenotypic) classification based on its severity. Second, thalassaemias can be classified according to their genetic basis by describing the globin subunits which is synthesized at a reduced rate. Finally, it is now possible to subclasssify many thalassaemias according to the particular mutation that is responsible for defective globin synthesis (Old, 2003).

A. Clinical classification

Based on the severity of the disease, thalassaemia can be classified into major, minor and intermedia. Thalassaemia major is severe and transfusion dependant while thalassaemia minor is symptomless condition that can be only identified

haematologically. It is usually presents the carrier state or trait. Thalassaemia intermedia includes a wide spectrum, ranging from disorders which are not as severe as major forms to asymptomatic conditions which are only slightly more severe than the trait. In addition, some heterozygotes for thalassaemia mutations are clinically and haematologically normal; they are sometimes designated 'silent' carriers (Weatherall & Clegg, 2001 a).

B. Genetic classification

According to genetic classification, thalassaemia can broadly divided into α, β, γ, δβ, δ, and εγδβ varieties depending on which globin or globins are under produced.

2.3 Different forms of thalassaemia

2.3.1 Beta (β) Thalassaemias

β thalassaemias are characterized by reduced synthesis of the β-globin chain and a subsequent imbalance in α/β-globin chain ratio that results in chronic hemolytic anemia (Aessopos *et al.*, 2007). The beta thalassaemia include four clinical syndromes of different severity: two conditions are generally asymptomatic, the silent carrier state and β thalassemia trait which usually results from inheritance of one mutant β-globin gene, and two require medical management, thalassaemia intermedia and thalassaemia major (Olivieri, 1999). The latest two will be discussed in details in this text due their clinical importance and relevance to this study.

A. β Thalassaemia Major

β thalassaemia major patients are those with homozygosity or compound heterozygosity for β thalassaemia who are dependent on blood transfusions to maintain life beyond early childhood (Bain, 2001). Thalassaemia major (TM) usually causes profound anaemia with the haemoglobin value in the range of 2.0 to 6.5 g/dl which starts during the first year of life (Olivieri, 1999, Weatherall, 2001). The Thalassaemia Clinical Research Network (TCRN) has defined TM as homozygous or compound heterozygous β thalassaemia requiring 8 or more transfusions in one year. By TCRN definition, patients with β thalassaemia required fewer than 8 transfusions annually are considered thalassaemia intermedia (Cunningham *et al.*, 2004).

Typical features of under treated thalassaemia major children include impaired growth, pallor mucous membrane, brown skin pigmentation, poor musculature, hepatosplenomegaly and characteristic skeletal and maxillofacial deformities (Abu Alhaija *et al.*, 2002, De Sanctis, 2002, Cunningham *et al.*, 2004, Rund and Rachmilewitz, 2005). The childhood of these children is interrupted with numerous complications which include pathological fractures, recurrent infections and extramedullary haemopoietic masses (Wonke, 1998, Cunningham *et al.*, 2004).

On the other hand, well-transfused thalassaemic children often remain asymptomatic until the early childhood. Their future course then depends on weather they have received adequate iron chelation. If not, they begin to show signs of hepatic, endocrine and cardiac complications (Borgna-Pignatti *et al.*, 2004, Borgna-Pignatti *et al.*, 2005, Charafeddine *et*

al., 2008). Children who are adequately transfused and are compliant to chelation therapy have better survival and less complications (Borgna-Pignatti *et al.*, 2004, Borgna-Pignatti *et al.*, 2005, Charafeddine *et al.*, 2008). However, in some cases they may suffer from the side effect of long-term chelation therapy (Cunningham *et al.*, 2004).

B. β Thalassaemia Intermedia

β Thalassaemia intermedia (TI) encompasses a wide clinical spectrum. Mildly affected patients are completely asymptomatic until adult life, experiencing only mild anemia and maintaining hemoglobin levels between 7 and 10 g/dl. These patients require only occasional blood transfusions, if any. Patients with more severe thalassemia intermedia generally present between the ages of 2 and 6 years, and although they are able to survive without regular transfusion therapy, growth and development can be retarded (Ho *et al.*, 1998, Taher *et al.*, 2006). In few words, Aessopos *et al.*, (2005) described thalassaemia intermedia patients as those who have clinical symptoms present after the first year of life with a milder anaemia than thalassaemia major that do not require regular transfusions and a longer life expectancy.

Thalassaemia intermedia is equally very heterogeneous at the genotype level. These patients are most commonly homozygotes or compound heterozygotes for β-thalassemia, having both β-globin loci affected. Less frequently only a single β-globin locus is mutated, the other being completely normal. The mild clinical characteristics of thalassaemia intermedia compared to thalassaemia major result from three different mechanisms (Galanello and Cao, 1998, Ho *et al.*, 1998):

- Inheritance f a mild or silent beta-chain mutation.
- Co-inheritance of determinants associated with increased γ-chain production.
- Co-inheritance of α-thalassaemia.

The clinical manifestations of β thalassaemia intermedia are extremely variable. In some cases the disorder presents early in life with anaemia, while in others it may not appear until later due to a complications such as hypersplenism. Growth and development may be normal or may be retarded. The major symptoms in early childhood are anaemia and jaundice. There is always some degree of splenomegaly and hepatomegaly (Weatherall and Clegg, 2001 a, Taher *et al.*, 2006, El Rassi *et al.*, 2008). The bone changes are extremely variable and range from almost none at all to severe skeletal deformity similar to that seen in poorly managed TM patients (Mohamed and Jackson, 1998, Mortazavi and Khojasteh, 2007) .

2.3.2 Haemoglobin E/β Thalassaemia

The interaction of thalassaemias with structural haemoglobin variants are of considerable clinical importance. Although the majority of them are rare, some forms such as sickle cell β thalassaemia and HbE/ β thalassaemia cause a considerable public health problem in some parts of the world. Indeed, because of the particularly high frequency of β thalassaemia and HbE in Asia, HbE / β thalassaemia is one of the most important varieties of thalassaemia (Weatherall and Clegg, 2001 a). It has replaced β thalassaemia as the most common thalassaemia disorder in many regions including coastal North America with at least million people affected worldwide most of them in

Asia (Lorey, 2000, Vichinsky, 2007). In many Southeast Asian countries, HbE/ β thalassaemia is the most common cause of transfusion-dependent thalassaemia (Hurst *et al.*, 1983, Weatherall and Clegg, 2001 b).The details of this disorder will be discussed next.

Haemoglobin E is a β-chain variant ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$) caused by a substitution of glutamic acid by lysine at codon 26 of the β-globin gene. The β^E chain is synthesized at a reduced rate compared with β^A. The result of the reduced rate of synthesis of β^E chain and therefore of haemoglobin E is that heterozygotes, compound heterozygotes and homozygotes show some thalassaemic features and thus is considered a thalassaemic haemoglobinopathies (Bain, 2001, Vichinsky, 2007). Heterozygous (HbE trait) or homozygotes (HbE disease) produces clinically mild condition while the most significant clinical consequences occur with the compound heterozygote state of Hb E/β thalassaemia, leading to a variable phenotype ranging from thalassaemia minor through thalassaemia intermedia to thalassaemia major with haemoglobin levels range from 3 to 13 g/dl. However, the majority of patients have a disease of moderate severity with average haemoglobin of 7.7 g/dl (Weatherall, 2000, Fucharoen *et al.*, 2000, Fucharoen and Winichagoon, 2000).

The clinical severity of Hb E/β thalassaemia is very variable. The most severely affected individuals are transfusion-dependent and have early onset of symptoms with marked anaemia, hepatosplenomegaly, intermittent jaundice, growth retardation and overexpansion of the bone marrow cavity leading to facial deformity and malpositioned

teeth. Less severely affected individuals may have later onset with mild anaemia, splenomegaly and facial deformity but do not require regular transfusions to maintain life. Occasional patients are only mildly affected. However, during pregnancy or intercurrent infection, patients who are not usually transfusion-dependent may become sufficiently anaemic to require transfusion (Fucharoen *et al.*, 2000, Fucharoen and Winichagoon, 2000, Premawardhena *et al.*, 2005, Panigrahi *et al.*, 2005).

2.3.3 Alpha (α) thalassaemias

The α thalassaemias are a group of inherited disorders of haemoglobin in which the production of α-globin chains is partially or completely suppressed (Bernini and Harteveld, 1998). The severity of the defect is very variable. At one extreme is a completely asymptomatic condition resulting from deletion or dysfunction of on of the four α genes. At the other extreme is haemoglobin Bart's hydrops fetalis, a condition generally incompatible with life resulting from deletion of all four α genes and a consequent total lack of α-globin synthesis which causes death in utero or shortly after birth (Bain, 2001, Chui *et al.*, 2003, Chui, 2005). The clinical phenotypes of α thalassaemia reflect the degree of defective α chain synthesis.

Hb H disease is one of the important varieties of α thalassaemia which constitutes a serious health problem in Southeast Asia and southern China (Cohen *et al.*, 2004). Patients with Hb H disease have only one active α-globin gene, often due to the deletion of three other α-globin genes (Chui, 2005). They are sometimes known as ‘deletional’ Hb H disease. Approximately 20% of patients with Hb H disease have deletion of two α-

globin genes plus inactivation of the third α-globin gene by non-deletional mutation such as Hb Constant spring (Hb CS), Pakse, or Quong Sze mutations. This group of disorders is known as 'non-deletional' Hb H disease (Chui, 2005). Hb CS is the most common non-deletional α thalassaemia mutation associated with Hb H disease (Vichinsky, 2005). Hb H, Hb H-Constant Spring and homozygous α thalassaemia affect at least a million people worldwide (Weatherall and Clegg, 2001 b).

Hb H disease is generally considered to be a clinically mild disorder. However, there is a marked phenotypic variability, ranging from asymptomatic, to need for periodic transfusions, to severe anaemia with haemolysis and hepatosplenomegaly, and even to fatal hydrops fetalis syndrome in utero (Chui *et al.,* 2003). The severity of Hb H disease is highly variable. A moderately severe anaemia and hepatosplenomegaly characterize it. Typically, the haemoglobin level is maintained at around 9.5 g/dl and chronic transfusion support is unnecessary. However, 29 to 50% of deletional Hb H disease patients require intermittent transfusion therapy. Extramedullary haemopoiesis is uncommon (Weatherall and Clegg, 2001 a, Vichinsky, 2005).

Patients with Hb H-Constant Spring disease usually are more anaemic, more symptomatic, more prone to have significant hepatosplenomegaly, and more likely to require transfusions with average haemoglobin level of 2 g/dl less than in deletional Hb H disease (Mohamed and Jackson, 1998, Chui *et al.,* 2003). It was estimated that 90% of patients with Hb H-CS disease have been intermittently transfused, and up to 40% have

required repeated transfusions, particularly in early infancy and in later adulthood (Cohen *et al.*, 2004).

2.4 Pathophysiology of thalassaemia

Systemic manifestations of thalassaemia and their remarkable clinical diversity can be easily understood once the pathophysiological mechanism of the disease identified. Indeed, the fundamental disease process in both α and β-thalassaemia, which is imbalanced globin synthesis, is the same. However, their pathophysiology differs because of the different properties of the particular globins that are produced in excess (Weatherall and Clegg, 2001 a).

The pathophysiology of β thalassaemia is reasonably well understood, at least in outline (Weatherall, 1998). The basic defect is the reduction in β-globin output ranging from a minimal deficit (β^+) to complete absence (β^0). This leads to imbalanced globin chain synthesis and to the production of an excess α chains. The free α-globin chains precipitate in the red cell precursors causing their premature destruction in the bone marrow; a process known as 'ineffective erythropoiesis. Red cells that survive to reach the peripheral blood are prematurely destroyed in the spleen. Anaemia in β thalassaemia thus results from a combination of ineffective erythropoiesis, peripheral haemolysis and an overall reduction in haemoglobin synthesis (Weatherall, 1998, Thein, 2005).

The anaemia in turn results in increase in the erythropoietin production, which leads to intense proliferation and expansion of the bone marrow with the resulting skeletal

deformities (Wonke, 1998, Thein, 2004, Thein, 2005). The hyperplasia of bone marrow leads to increased iron absorption and iron loading, often exacerbated by the need of regular blood transfusion, which lead to iron deposition in tissues, organ failure and finally death if excess iron is not removed (Weatherall and Clegg, 2001 a, Thein, 2004, Borgna-Pignatti *et al.*, 2005). Generally, factors which reduce the degree of chain imbalance and the magnitude of α chain excess in red cell precursors, have an ameliorating effect on the β thalassemia phenotype (Thein, 2004). Figure 2.3 summarize the pathophysiology of β thalassemia.

On the other hand, the fundamental defect in α thalassaemia is like β thalassaemia, imbalanced globin synthesis. However, there are two major pathophysiological differences. First, the α thalassaemias, unlike β thalassaemias, are manifest in fetal life because α chains are shared by both fetal and adult haemoglobin (Fig. 2.2). Second, the properties of the excess γ and β chains that are synthesized in α thalassaemia are quite different from the excess α chains that are produced in β thalassaemia (Weatherall and Clegg, 2001 a). Unlike excess α chains in β thalassaemia, they do not precipitate in red cell precursors, but they form the soluble homotetramers, haemoglobin Bart's (γ_4) and Hb H (β_4). These homotetramers often become attached to the cell membranes of circulation erythrocytes causing local oxidative damage, membrane dysfunction and shortened red cell survival (Weatherall, 1998, Chui *et al.*, 2003). It appears that the ability to produce these soluble tetramers allows some effective erythropoiesis to occur and hence haemolysis is the major cause of anaemia in α thalassaemia, although ineffective erythropoiesis plays a pathogenic role. In contrast, ineffective erythropoiesis is the

dominant cause of the anaemia in β thalassaemia major and intermedia (Weatherall, 1998, Weatherall and Clegg, 2001 a, Schrier, 2002, Chui *et al.*, 2003).

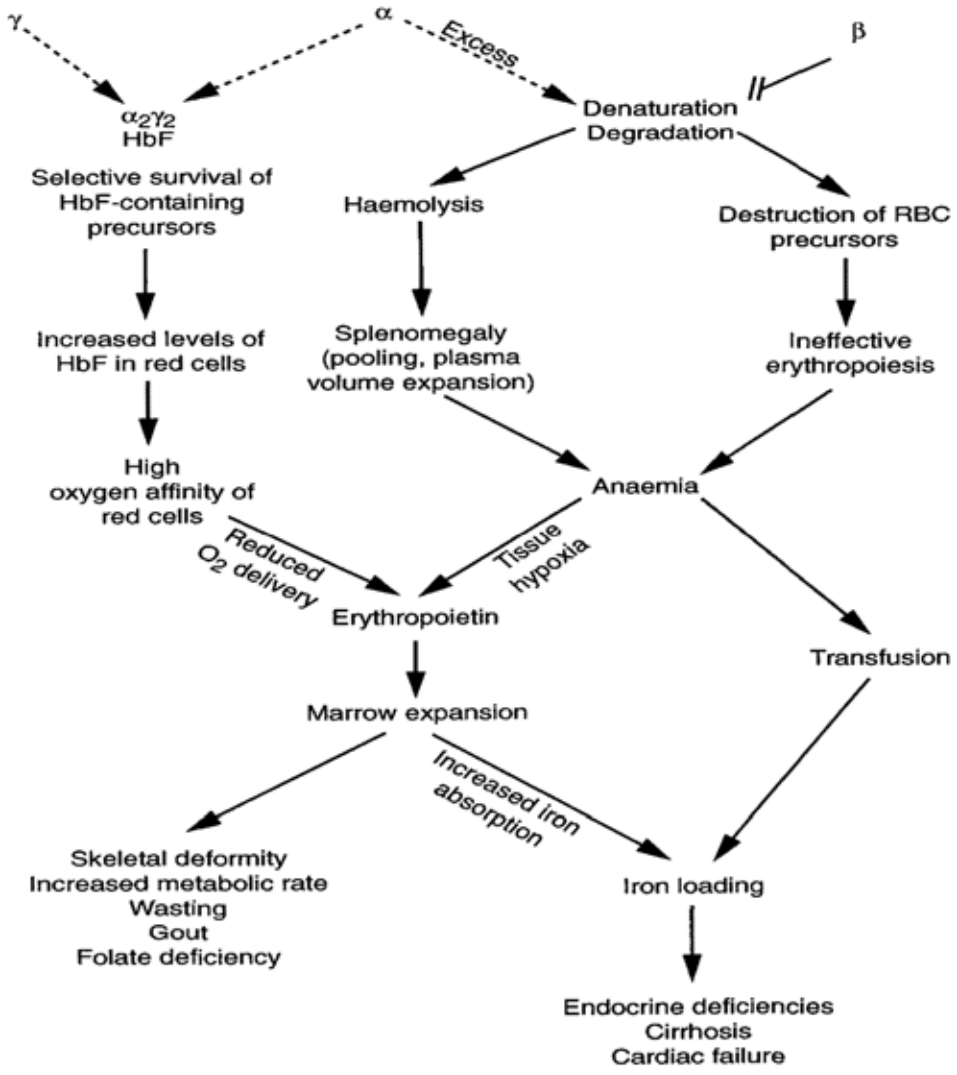

Figure 2.3 A summary of the main pathophysiological features of β thalassaemia (Source: Weatherall, 1998).

Indeed, the interactions of thalassaemias with the structural haemoglobin variants result in a variety of diseases with much more complex pathophysiological mechanisms. As an example, the extraordinary clinical variation in Hb E/β thalassaemia is still one of the unsolved pathophysiological issues in human thalassaemia (Schrier, 2002, Vichinsky, 2007). The instability of Hb E plays a minor role in its pathophysiology while the interaction between Hb E and β thalassaemia alleles is the main determinant. The globin chain imbalance that results from these mutations correlates with the severity of the disease (Fucharoen and Winichagoon, 2000, Vichinsky, 2007). However, this does not explain the whole problem because some patients with the same mutations within a family may show significant clinical severity. The variability is probably due to weather the patient is heterozygotes for β^0 thalassaemia or β^+ thalassaemia mutations (Vichinsky, 2007). Co-inheritance of α thalassaemia mutations may also modulate severity (Cohen *et al.*, 2004).

It has been stated that oxidant injury could be the cause of haemolysis and that accelerated apoptosis is responsible for ineffective erythropoiesis (Schrier, 2002). Pootrakul *et al.,* (2000) study has found that Hb H disease have minimal ineffective erythropoiesis and apoptosis comparing to Hb CS which have a distinct increase in ineffective erythropoiesis and apoptosis. This study has also found that patients with Hb E/β thalassaemia had the most ineffective erythropoiesis and the most erythroid apoptosis among the three groups.

In summary, it is now clear that the cardinal feature of all thalassaemias is imbalanced globin synthesis. Thus, any factor that lessens the degree of imbalance will have a beneficial effect on the clinical phenotype (Thein, 2004).

2.5 Complications of thalassaemia

Complications of thalassaemia can be arranged under three main headings according to etiology. Indeed, many of the complications are multifactorial with complex etiology and cannot be easily categorized under a specific cause. Only complications related to this study are discussed.

2.5.1 Ineffective erythropoiesis and anaemia related complications

2.5.1.1 Erythroid hyperplasia

Erythroid hyperplasia is a common complication in un-transfused or undertransfused thalassaemia patients. It is a compensatory mechanism for ineffective erythropoiesis and haemolysis that leads to expansion of the haemopoietic bone marrow and extramedullary erythropoiesis with their subsequent complications (Drew and Sachs, 1997, Mohamed and Jackson, 1998, Weatherall and Clegg, 2001 a). Main problems due to erythroid hyperplasia are:

A. Craniofacial and skeletal deformity

The characteristic deformities of the skull and face and other skeletal features of severe thalassaemia were emphasized in many early and late descriptions of the disease

(Poyton and Davey, 1968a, Jackson *et al.*, 1987, Cannell, 1988, Bassimitci *et al.*, 1996, Abu Alhaija *et al.*, 2002, Amini *et al.*, 2007). These deformities are unusual before six months of age and are most commonly seen in children over one year old with the homozygotes develop more pronounced skeletal abnormalities and earlier symptoms than heterozygotes (Tyler *et al.*, 2006).

In untreated or under treated patients, the resulting anaemia causes expansion of the bone marrow up to 15-30 times normal and affects every part of the skeleton (Wonke, 1998, Tyler *et al.*, 2006). The most prominent and well-known orofacial features of β thalassaemia are prominent cheekbones and a protrusive premaxilla with distinct depression of the bridge of the nose, often referred to as 'rodent' or 'chipmunk' faces (Cannell, 1988, Bassimitci *et al.*, 1996, Abu Alhaija *et al.*, 2002).

Indeed, chipmunk face (Fig. 2.4 B) usually refers to the severest distinguishable form of thalassaemia mainly involves maxillary area that shows overgrowth in all directions with resultant overbite and protrusion of the exposed anterior teeth (Jackson *et al.*, 1987, Abu Alhaija *et al.*, 2002). Whereas, in some patients, a milder deformity described as 'mongoloid face' (Fig.2.4 A) and characterized by prominent frontal and parietal bones, sunken nasal bridge, retruding zygomas and upward slants of eyes (Jackson *et al.*, 1987, Abu Alhaija *et al.*, 2002).

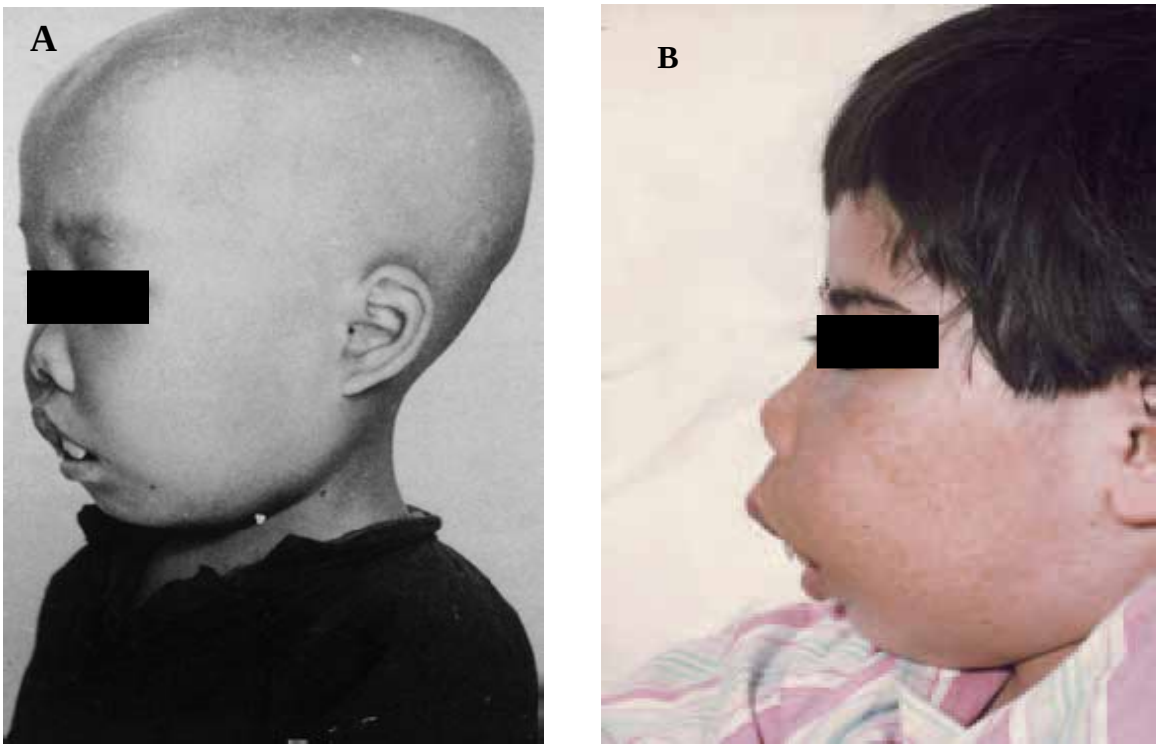

Figure 2.4 Facial appearances in severe β thalassaemia. A. Mongoloid face. B. Chipmunk face (Source: Weatherall and Clegg, 2001 a)

These skeletal changes are mirrored by characteristic radiological changes of the skull, long bones, ribs and hands. Cephalometric radiographs shows dilatation of the diploic space, with subperiosteal bone grows in a series of radiating striations, giving a typical 'hair on end' appearance (Fig.2.5). Marrow overgrowth in frontal, temporal and facial bones consistently impedes pneumatization of paranasal sinuses while in the maxillary bone it may causes lateral displacement of the orbits and ventral displacement of the central incisors (Tunaci *et al.*, 1999, Weatherall and Clegg, 2001 a, Tyler *et al.*, 2006). Panoramic and intraoral radiography may show a generalized loss of bone density similar to that seen in osteomalacia or osteoporosis, and a thin cortex of the mandible. The trabeculae of jaws appear coarse in pattern with enlarged marrow spaces described as 'Chicken-wire' (Poyton and Davey, 1968b, Hes *et al.*, 1990).

Figure 2.5 X-ray showing typical hair on end appearance in severe β thalassaemia (Source: Weatherall & Clegg 2001 a).

The radiographic picture of other parts of the skeleton include bulbous expansion of posterior and anterior segments of the rib, rib within rib pattern, spine deformities and vertebral collapse. Long bones show loss of normal concave outline together with cortical thinning and porous rarefaction. Similar changes are seen in the small bones of the hands and feet (Tunaci *et al.*, 1999, Weatherall and Clegg, 2001 a, Tyler *et al.*, 2006). Children who are not transfused until late childhood or adolescence commonly show premature fusion of the growth plates in the tubular bones of extremities (Tunaci *et al.*, 1999). Pathological fractures are also a major feature of inadequately transfused patients (Basanagoudar *et al.*, 2001).