MODELING BONE LOSS IN EGYPTIAN PATIENTS WITH β-THALASSAEMIA MAJOR: A COMPARATIVE STUDY

THESIS

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Arabic Summary

LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
AHSP	Alpha Hemoglobin Stabilizing Protein
β	Regression Coefficient
BD	Bone Disease
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BMU	Bone Metabolism Unite
BUA	Broadband Ultrasound Attenuation
CS	Compton Scattering
CTx	C- Terminal Telopeptides of type I Collagen
DFO	Deferoxamine
DXA	Dual-energy X-Ray Absorptiometry
EPO	Erythropoietin
FM	Fat Mass
GH	Growth Hormone
HbA	Hemoglobin
HRT	Hormone Replacement Therapy
I_o	Incident Intensity
I_t	Transmitted Intensity
IGF	Insulin Growth Factor
LBFM	Lean Fat-Free Mass
μCT	Computed Microtomography
M	Mass of the Medium
μ_l	Linear Attenuation Coefficient
μ_m	Mass Attenuation Coefficient
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Cell Volume
MRI	Magnetic Resonance Imaging
OPG	Osteoprotegrin

p	Significance Level
PE	Photoelectric Effect
PTH	Parathyroid Hormone
QCT	Quantitative Computed Tomography
QUS	Quantitative Ultrasound
R	Correlation Coefficient
R^2	Determination Coefficient
RBC	Red Blood Cell
RANKL	Receptor Activator of Nuclear Factor k Ligand
S.ALP	Bone-Specific Alkaline Phosphatase
SEE	Standard Error of Estimation
SMI	Structural Model Index
SOS	Speed of Sound
sTFR	Soluble Transferrin Receptor
TM	β-Thalassaemia Major
TBFM	Tissue Bone-Free Mass
TFR	Transferrin Receptor
WHO	World Health Organization
WSSE	Weighed Sum of Squared Errors

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Chapter I

INTRODUCTION

I.1: BACKGROUND

Thalassaemia is an inherited disorder. It leads to the decreased production and increased destruction of red blood cells. Hemoglobin in the red blood cells carries oxygen for all organs in the body. The loss of red blood cells results in low hemoglobin. This leads to anemia. The decreased oxygen will impair the ability to maintain normal functions.

The cause of growth retardation in the inherited blood disorder β-Thalassaemia major has long been a subject of debate. This has become an issue in the last few years since children with β-Thalassaemia are undergoing hypertransfusion and iron chelation therapy, and now living well into their thirties and forties. Therefore, in addition to growth retardation, many of the endocrinopathies such as hypogonadism, hypothyroidism, hypoparathyroidism and diabetes mellitus, which were not apparent before, are now being diagnosed and treated. Despite the fact that much has been learned in the past few years about the etiology of growth retardation in children with β-Thalassaemia, treatment is still difficult in many cases.[1] β-Thalassaemia is found in people of Mediterranean, Middle Eastern, African, South Asian (Indian, Pakistani, etc.), Southeast Asian and Chinese descent. According to recent data collected through the Hereditary Disease Program of the World Health Organization (WHO) and based on local surveys and reports by visiting experts, the carriers of hemoglobin disorders in the world are estimated to be 269 million.[2] In addition, Thalassaemia can cause other health problems such as delayed puberty and slow growth rate, bone problems (including osteoporosis, brittle or deformed bones particularly in the facial area), enlarged spleen, severe anemia, heart disease, and liver problems.

Osteoporosis is emerging as a major cause of morbidity in patients with Thalassaemia major who, because of optimal treatment with blood transfusions and iron-chelation therapy from infancy, are now living into teenage and adult life without developing other major complications (e.g. cardiomyopathy) of the disease. Sensitive techniques are now available for assessing the degree of osteoporosis, which often presents clinically as lower backache, cord compression or fractures on minor trauma. There are probably several genetic and acquired factors relevant to the development of osteoporosis.[3]

Several sensitive techniques are now available for the quantitative assessment of the degree of osteoporosis and total bone mass. Bone density measurement by dual X-ray absorptiometry (DXA) of the lumbar spine, femoral neck and forearm is recommended as one of the most reliable and non-invasive techniques).[4, 5] Determination of bone markers, determining bone formation or bone resorption also contributes to the assessment of bone loss. Bone disease in Thalassaemia has been addressed in a few studies only and most of these concern prepubertal thalassaemic children.[6]

I.2: LITERATURE REVIEW

I.2.1: β-THALASSAEMIA

Thalassaemias are a group of congenital anemias that have in common deficient synthesis of one or more of the globin subunits of the normal human hemoglobins (Hbs).[7] β-Thalassaemia is a genetic disorder with mutations in the β-globin gene (Figure 1) that reduce or abolish β-globin protein production. Patients with β-Thalassaemia major (Cooley's anemia) become severely anemic by 6 to 18 months of age, and are transfusion-dependent for life, while those with Thalassaemia intermedia, a less-severe form of Thalassaemia, are intermittently or rarely transfused.[8]

I.2.2: EPIDEMIOLOGY

Thalassaemia is among the most common genetic disorders worldwide; 4.83% of the world's population carry globin variants, including 1.67% of the population who are heterozygous for α-Thalassaemia and β-Thalassaemia. In addition, 1.92% carry sickle hemoglobin, 0.95% carry hemoglobin E, and 0.29% carry hemoglobin C. Thus, the worldwide birth rate of people who are homozygous or compound heterozygous for symptomatic globin disorders, including α-Thalassaemia and β-Thalassaemia, is no less than 2.4 per 1000 births, of which 1.96 have sickle cell disease and 0.44 have Thalassaemias.[9]

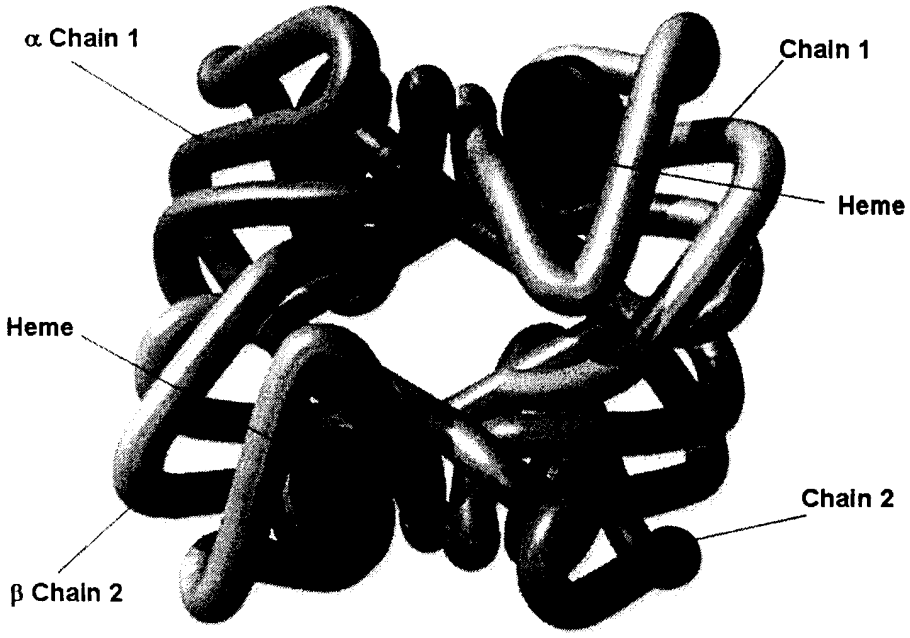

Figure 1. Molecular arrangement of a red blood cell showing α and β globin chains in attachment to Heme molecules.[8]

I.2.3: MOLECULAR PATHOLOGY

The β-globin gene is located in the short arm of chromosome 11 (Figure 2) in a region containing also the δ gene, the embryonic ψ gene, the fetal Gγ and Aγ genes, and the pseudogene ψβ1.[10] β-Thalassaemias are among the most common single-gene defects worldwide, and pose a severe health and economic burden to patients and families at risk. They are caused by different mutations in the β-globin gene cluster, resulting in reduced or absent adult hemoglobin (HbA), which is composed of two α- and two β-globin chains (α2β2) (Figure 1), leading to severe anemia. This disorder is characterized by reduced HbA production (β+ Thalassaemia) or complete absence of β-globin synthesis (β0 Thalassaemia).[8]

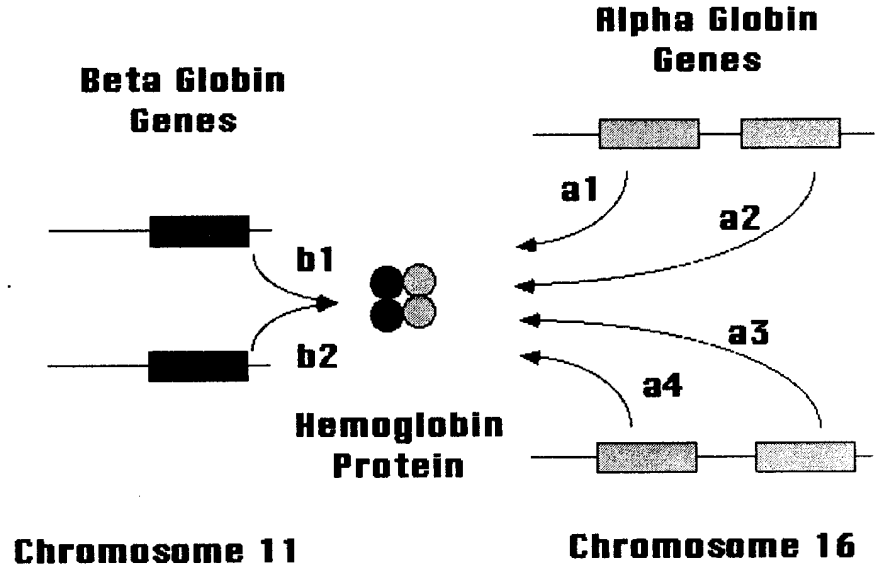

Figure 2. Genes on chromosome 16 are responsible for α-subunits, while genes on chromosome 11 control the production of β-subunits.[10]

Impaired synthesis of β-globin subunit leads to an excess production of the α-globin. Therefore, in β-Thalassaemia, the excess α-globin chains form tetramers that accumulate and precipitate in the erythroid progenitors, forming inclusion bodies that cause oxidative membrane damage within the red blood cells and immature developing erythroblasts in the bone marrow. This leads to premature death of many late erythroid progenitors in the bone marrow and spleen. The profound anemia that results from production of only a few hypochromic and microcytic red blood cells leads to a dramatic increase in erythropoietin (EPO) levels that ultimately drive an uncontrolled expansion of additional early erythroid progenitors inducing massive extramedullary haematopoiesis. These erythroid progenitors have an enhanced proliferative and survival capacity, but eventually they fail to differentiate, contributing to the process of ineffective eythropoiesis.[11, 12]

I.2.4: PATHOPHYSIOLOGY OF β-THALASSAEMIA

β-Thalassaemia occurs when there is a deficiency of β-globin; typically, it is caused by a direct down-regulation in the synthesis of structurally normal β-chains. However, α-Thalassaemia phenotype can also arise from structural β-chain variants if they are synthesized at a reduced rate, e.g., HbE. Alternatively, the variants are produced at a normal rate but are so unstable that they are rapidly destroyed giving rise to a functional deficiency. The former group of variants is also referred to as β-thalassemic hemoglobinopathies. The hyper-unstable β-chain variants act in a dominant negative fashion, causing a disease phenotype even when present in a single copy, and hence, have been referred to as dominantly inherited β-Thalassaemia.[13]

In contrast, typical β-Thalassaemia is inherited as a haplo-insufficient Mendelian recessive disease [13]. In β-Thalassaemia the disease does not manifest itself until the switch from γ-chain to β-chain synthesis has been completed. This usually occurs several months after birth. Thus, the clinical presentation of a patient with this disease usually occurs during the first year of life. There often is a compensatory increased production of γ-chains and δ chains resulting in an increased level of hemoglobin F and hemoglobin A2.[14]

The deficiency in β-globin leads to decreased hemoglobin content per red blood cell (RBC) (mean corpuscular hemoglobin, MCH), resulting in hypochromic cells. The RBC volume (mean cell volume, MCV) decreases, resulting in microcytosis.[14] The excess unmatched α-globin chains accumulate at the membrane and its skeleton, forming rounded precipitates called Heinz Bodies where they lead to membrane alterations consisting of reduced incorporation, misincorporation or clustering of some membrane protein components in the plasma membrane,[15] affecting RBC deformability, stability and hydration. This results in many abnormalities in RBC morphology and physiology, including fragmented cells), and abnormal osmotic properties.[14] Abnormal RBCs have a reduced half-life, and will be sequestered more rapidly than normal in the spleen resulting in splenomegaly. A highly expressed protein called alpha hemoglobin stabilizing protein (AHSP) acts as a chaperone for some free alpha chains and prevent their precipitation,[16] and the absence of which results in more severe (3-thalassemic phenotype).

The bone marrow shows intense erythroid hyperplasia, and 60-80% of the thalassemic erythroid precursors die in the marrow (normal value: 10-20%) or in sites of extramedullary erythropoiesis due to increase in both apoptosis and phagocytosis,[17] commonly known as ineffective erythropoiesis. The decreased RBC half life and the ineffective erythropoiesis result in decreased number of RBCs in the peripheral blood and subsequent aggravation of anemia.

Iron overload frequently develops in thalassemic patients as a consequence of blood transfusions,[18] and increased iron absorption.[14] As iron loading progresses, the capacity of the its main transport protein, serum transferrin, to bind and detoxify it may be exceeded and a non-transferrin bound fraction of plasma iron may promote the generation of free hydroxyl radicals, resulting in oxygen related damage. In the absence of chelating therapy, the accumulation of iron results in progressive dysfunction of many organs including the heart, liver, endocrine glands and pancreas.[19]

I.2.5: CLINICAL MANIFESTATIONS

In many developed countries, neonatal screening programmes will first identify infants with more severe forms of β-Thalassaemia, before the development of any symptoms; in some cases, antenatal screening of the parents and possibly prenatal diagnosis will have identified the infant to be at high risk of β-Thalassaemia before birth. Mutations in the β-globin gene almost never cause clinical symptoms in utero or neonatally due to the predominance of β-globin at this stage. In many countries, neonatal screening programs do not exist and diagnosis in the child will depend on their symptomatic presentation. Severe β-Thalassaemia usually presents in the first year of life. Typically there is failure to thrive, with poor weight gain and growth with developmental delay. The parents may have noticed that the infant is pale and jaundiced, with a protruding abdomen. There may be a family history of severe anaemia, and typically the family will not be of northern European origin. Examination confirms the pallor and jaundice, with palpable hepatosplenomegaly. There may be evidence of marked erythroid hyperplasia, with signs of the typical 'thalassaemic facies' including expansion of the skull vault and maxillary bones. The symptoms and signs are not specific and differential diagnoses include gastrointestinal or hepatic disease, and malignancy.[20]

I.2.6: BONE DEFORMITIES IN β-THALASSAEMIA

Increased survival in patients with β-Thalassaemia major allowed for several complications of the disease and its treatment to manifest, one of which is bone disease.[21] Untransfused or poorly transfused patients with Thalassaemia develop typical bone abnormalities that were described already in the first reports of the disease and that are due to the extremely increased erythropoiesis, with consequent expansion of the bone marrow to 15 to 30 times normal (Figure 3A).[22]

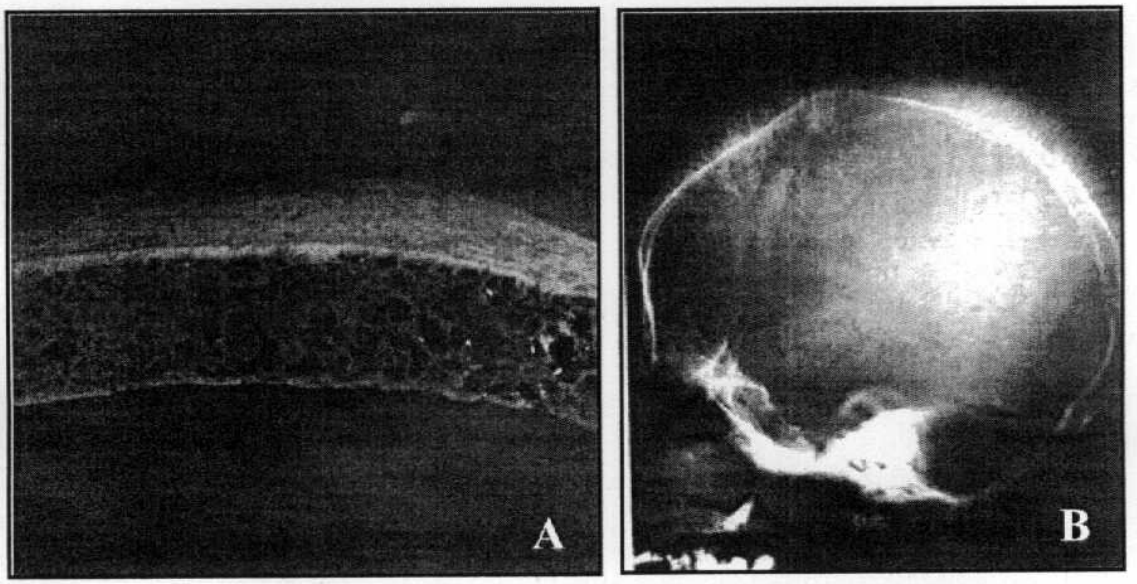

Figure 3. β-Thalassaemia patients develop typical bone abnormalities due to the extremely increased erythropoiesis, with consequent expansion of the bone marrow (A) and deformed skull by frontal and posterior bossing with the diploe increased in thickness (B).[22]

The skull is large and deformed by frontal and posterior bossing with the diploe increased in thickness to several times normal (Figure 3B). The outer and inner tables are thin and the trabeculae are arranged in vertical striations, resulting in a "hair-on-end" appearance. A peculiar, stratified appearance of the skull has been reported. The zygomatic bones are prominent, the base of the nose is depressed, and pneumatization of the sinuses is delayed. Overgrowth of the maxilla produces severe malocclusion, with a rodentlike appearance.[22] Metatarsal and metacarpal bones are the first to expand as a consequence of increased erythropoiesis. The measurement of the size of the metacarpal bones has been proposed as an indicator of time for initiating transfusion therapy.[22] The ribs are broad, often with a "rib-within-rib" appearance, and the vertebral bodies are square. The trabeculation of the medullary space gives the bones a mosaic pattern. Shortening of long bones is frequent, as a result of premature fusion of the humeral and femoral epiphyseal lines.[23, 24] Extramedullary erythropoiesis gives rise to masses that protrude from bones where red marrow persists. Overgrowth from the vertebral bodies has been reported to cause cord compression and paraparesis . Ear impairment due to extramedullary marrow growing in the middle ear and progressive visual loss caused by compressive optic neuropathy have been reported.[25, 26] This kind of picture is more often present in patients with Thalassaemia intermedia, in whom transfusions are avoided at the price of intense autologous marrow hyperactivity. Improvement in the radiologic bone appearance in the cohorts of patients who have, since an early age, undergone regular transfusions has been striking. The lack of severe skull deformities is reflected in the mildness of thalassemic features that are now observed in most patients. However, bone lesions of a different nature are often observed as a consequence of excessive deferoxamine (DFO) therapy.

I.2.7: OSTEOPOROSIS AND OSTEOPENIA IN β-THALASSAEMIA

Osteoporosis is a universal medical problem, affecting both genders. It is generally accepted that its main causes are aging, genetic disorders of osteogenesis, lack of certain nutritional elements or physical activity, and endocrine disorders mainly estrogen deficiency. Other causes include neoplastic disorders, gastrointestinal disorders causing malabsorption, liver diseases, inflammatory conditions, and drugs. Osteopenia and osteoporosis represent prominent causes of morbidity in patients of both genders with Thalassaemia.[27] During the last decade, the presence of osteopenia and osteoporosis in well-treated thalassaemics has been described in different studies with high prevalence up to 50%.[28]

The mechanism of pathogenesis of bone disease in Thalassaemia Major is multifactorial and complicated. Peak bone mass is achieved shortly after completion of puberty and normally remains stable until the third decade of life when age-related bone mass begins. Growth hormone (GH) and sex steroids play a crucial role in bone remodeling and in the maintenance of skeletal architecture during adult life. GH and insulin growth factors (IGFs) have anabolic effect in bone formation. Sex steroids act probably by increasing the expression of receptor activator of nuclear factor k ligand (RANKL) by osteoblastic cells and alterations in the RANK/RANKL/OPG system in favor of osteoclasts. Impaired GH secretion and lack of sex steroids in thalassemic patients due to pituitary damage, contribute to failure of achieving optimal peak bone mass. Other endocrine complications such as hypoparathyroidism and vitamin D deficiency have also a detrimental role on bones in Thalassaemia Major.[29] Although delay in onset of puberty is a common cause of

growth failure in adolescent thalassemic patients, growth retardation could also be due to iron overload, or the toxic effects of desferrioxiamine,[30] Iron chelation has been correlated with growth failure and bone abnormalities, and high desferrioxamine dosage has been associated with cartilage alterations.[28]

I.2.8: ACQUIRED FACTORS CONTRIBUTING TO REDUCED BMD IN β-THALASSAEMIA

Previous studies have demonstrated that multiple acquired factors are involved in the pathogenesis of osteopenia/ osteoporosis in Thalassaemia Major. They include the primary disease, itself causing bone marrow expansion,[31, 32] and several secondary factors, such as hormonal deficiency,[33] iron overload , desferrioxamine toxicity,[34] calcium, zinc and vitamin D deficiencies, and inadequate physical activity.[35] Most of these factors act through the imbalance in bone remodelling; they inhibit osteoblast activation and/or increase osteoclast function, leading to bone loss and osteoporosis.

I.2.8.1:BONE MARROW EXPANSION

Bone marrow expansion due to ineffective erythropoiesis is a typical finding in patients with Thalassaemia Major and has been considered as a major cause of bone destruction.[31, 32] Marrow expansion causes mechanical interruption of bone formation, leading to cortical thinning, increased distortion and fragility of the bones. Transferrin receptor studies have demonstrated increased bone marrow activity even in patients with low reticulocyte count or marrow hypoplasia.[36] However, no direct correlation was found between serum levels of soluble transferrin receptor (sTFR) and the severity of osteoporosis.

I.2.8.2:ENDOCRINE COMPLICATIONS

Hypothyroidism, hypoparathyroidism, diabetes mellitus and mainly hypogonadism (as delayed puberty and/or secondary hypogonadism) are the main causes of osteopenia/osteoporosis in Thalassaemia Major.[37] Haemosiderosis of the pituitary gonadotrophic cells and iron deposition in the testes and ovaries are involved in the pathogenesis of endocrine complications in Thalassaemia Major.[38] Hypogonadism is a well-recognized cause of osteoporosis and osteopenia, not only in patients with Thalassaemia Major but also in the general population, and is characterized by high bone turnover with an enhanced resorptive phase .

Oestrogen and progesterone appear to inhibit osteoclast activity and promote bone formation, while testosterone has a direct stimulatory effect on osteoblast proliferation and differentiation.[39] As mentioned in bone physiology, IGFs play also an important role in bone remodelling. Low serum IGF levels decrease osteoblast proliferation and bone matrix formation and reduce the activation of osteoclasts.[40] Several studies have demonstrated a positive correlation between the BMD of the lumbar spine and the IGF-I concentration. It is well documented that the GH-IGF axis is defective in Thalassaemia Major. Thalassaemia patients have significantly lower circulating levels of IGF-I and the corresponding binding protein (IGFBP-III) than normal individuals, thus leading to increased bone resorption, decreased bone formation and finally to bone loss.[41]

I.2.8.3: IRON OVERLOAD AND DESFERRIOXAMINE

Although endocrine dysfunction has a major role in the development of osteoporosis in transfused and non-transfused Thalassaemia patients, the transfusion volume and chelation dose also influence the bone mass. Iron deposition in the bone impairs osteoid maturation and inhibits mineralization locally, resulting in focal osteomalakia. The mechanism by which iron overload interferes in osteoid maturation and mineralization includes the incorporation of iron into crystals of calcium hydroxyapatite, which consequently affects the growth of hydroxyapatite crystals and reduces the bone metabolism unite (BMU) tensile strength.[42] On the other hand, desferrioxamine inhibits DNA synthesis, osteoblast and fibroblast proliferation, osteoblast precursors differentiation, and collagen formation, while it enhances osteoblast apoptosis, especially in patients who receive inappropriately high doses of desferrioxamine.[43]

I.2.8.4: VITAMIN AND TRACE MINERALS DEFICIENCIES

Vitamin C deficiency in iron-overloaded patients with low levels of serum ascorbic acid induces the risk of osteoporotic fractures. Vitamin D deficiency is also implicated in the pathogenesis of osteoporosis in Thalassaemia Major patients due to the regulatory effect of vitamin D in both osteoclasts and osteoblasts. Adequate calcium intake and small amounts of vitamin D administration during skeletal development can increase bone mass in adolescents and decrease bone loss in adult life.[44] However, most studies have failed to show reduced serum levels of 25-hydroxyvitamin D in Thalassaemia Major patients. There is adequate data indicating that Thalassaemia patients have also zinc deficiency, which may lower their BMD.[35] It is well-known that zinc and copper deficiencies are associated with osteoporosis;[45] thus, zinc supplementation may be administered in Thalassaemia patients with this trace mineral deficiency.

I.2.8.5: PHYSICAL ACTIVITY

Patients with Thalassaemia Major have reduced physical activity due to the complications of the disease and their overprotective parents, who do not encourage muscle activity. The association between mechanical stress and bone mass was first recorded by Galileo in 1683, who noted the relationship between body weight and bone size. Whedon,[46] reported that immobility or prolonged bed rest leads rapidly to hypercalciurea, negative calcium balance and bone loss. He also mentioned that the duration and force of the muscle activity on bone are important in maintaining bone mass. In athletes the positive osteogenic effect of exercise has been proven by several studies. Lane et al.,[46] studied male and female athletes, over 50 years old, who had been long distance runners for many years and found that their lumbar bone mass was higher when compared with sedentary controls. The above data suggest that lack of physical activity is another predisposing factor for osteoporosis in Thalassaemia Major patients and muscle activity has to be encouraged in these patients. Despite the major role of the above acquired factors in the development of Thalassaemia-induced bone loss, there are thalasasemia patients who continue to present osteopenia and/ or osteoporosis despite adequate transfusion and chelation programmes, hormonal replacement, and absence of other factors that contribute to the development of reduced BMD. It seems that there are underlying genetic factors playing a significant role in the imbalance of bone remodelling.

I.2.9: HYPOPARATHYROIDISM AND VITAMIN D DEFICIENCY

Hypoparathyroidism is another endocrine complication in Thalassaemia, which may develop in late adolescence and contribute to osteopenia and subsequently osteoporosis. A recent study has reported prevalence up to 13.5% with no sex differences.[47] Iron overload with deposition on parathyroid cells and tissue fibrosis are the main causes of hypoparathyroidism while chronic anemia is an additional factor causing parathyroid dysfunction.[48] The condition presents with the typical biochemical picture of hypoparathyroidism of low calcium and high phosphate levels. PTH may be normal or low and vitamin D is low. Low calcium and phosphorus are found in 24-hour urine collection.

Vitamin D deficiency may start early in thalassemics, before hypoparathyroidism is established. Vitamin D deficiency potentially contributes to low bone mass in Thalassaemia. Notably, Thalassaemia Major patients progressively develop iron overload, and it is possible that a deficiency in liver hydroxylation of vitamin D, or in vitamin D absorption, appears in older thalassemic patients.

I.2.10: GENERAL ASPECTS IN MANAGEMENT OF OSTEOPOROSIS IN TM

The corner stone in the management of osteoporosis in patients with thalassemia major, in whom loss of bone mass starts early, is prevention. Treatment of anemia with regular transfusions and the management of iron overload with prompt chelation are mandatory for every patient with Thalassaemia in order to avoid adverse events of the disease on bones. Additional lifestyle measures should be encouraged such as physical activity and smoking quitting. Adequate calcium and vitamin D intake during skeletal development can increase bone mass in adult life with the final goal to prevent bone loss and fractures. Early recognition and management of endocrine complications of Thalassaemia are essential for eliminating the risk of bone disease (BD). Induction of puberty at a proper age and treatment of hypogonadism with hormone replacement therapy (HRT) seem to be the most effective ways for preventing osteoporosis and other bone deformities in Thalassaemia. Calcitonin, a potent inhibitor of osteoclasts, has also been tried in combination with calcium and vitamin D.[49] Bisphosphonates are worldwide used in patients with postmenopausal osteoporosis to increase BMD and prevent bone fractures. The reduction in fractures may be related not only to the increase in bone mass arising from the inhibition of bone resorption and reduced activation frequency of boneremodeling units but also to enhanced osteomineralization.

I.2.11: BIOCHEMICAL MARKERS OF BONE TURNOVER

Bone is a metabolically active tissue that undergoes continuous remodelling by two counter acting processes, namely bone formation and bone resorption. These processes rely on the activity of osteoclasts (resorption), osteoblasts (formation) and osteocytes (maintenance). Under normal conditions, bone resorption and formation are tightly coupled to each other, so that the amount of bone removed is always equal to the amount of newly formed bone (Figure 4).[50] This balance is achieved and regulated through the action of various systemic hormones [e.g., parathyroid hormone (PTH), vitamin D, and other steroid hormones] and local mediators (e.g. cytokines, growth factors). In contrast, somatic

growth, ageing, metabolic bone diseases, states of increased or decreased mobility, therapeutic interventions and many other conditions are characterised by more or less pronounced imbalances in bone turnover. The results of such uncoupling in bone turnover are often changes in bone structure, strength and mass. While bone structure and strength are difficult to measure in vivo, bone mass can be assessed by densitometric techniques (e.g., DXA).[50, 51] Along with clinical and imaging techniques, biochemical marker play an integral role in the assessment and differential diagnosis of metabolic bone disease.[50, 51]

These biochemical markers have greatly enriched the spectrum of analytes used in the assessment of skeletal pathologies. They are non-invasive, comparatively inexpensive and, when applied and interpreted correctly, helpful tools in the diagnostic and therapeutic assessment of metabolic bone disease. They are usually classified according to the metabolic process they are considered to reflect into marker of bone formation and bone resorption.

Bone formation markers are products of active osteoblasts expressed during different phases of osteoblast development. They are considered to reflect different aspects of osteoblast function and of bone formation. All markers of bone formation are measured in serum or plasma, and urine they include.[51] Total alkaline phosphatase, bone alkaline phosphatase, osteocalcin and procollagen type I propeptides (i.e., PINP, PICP).

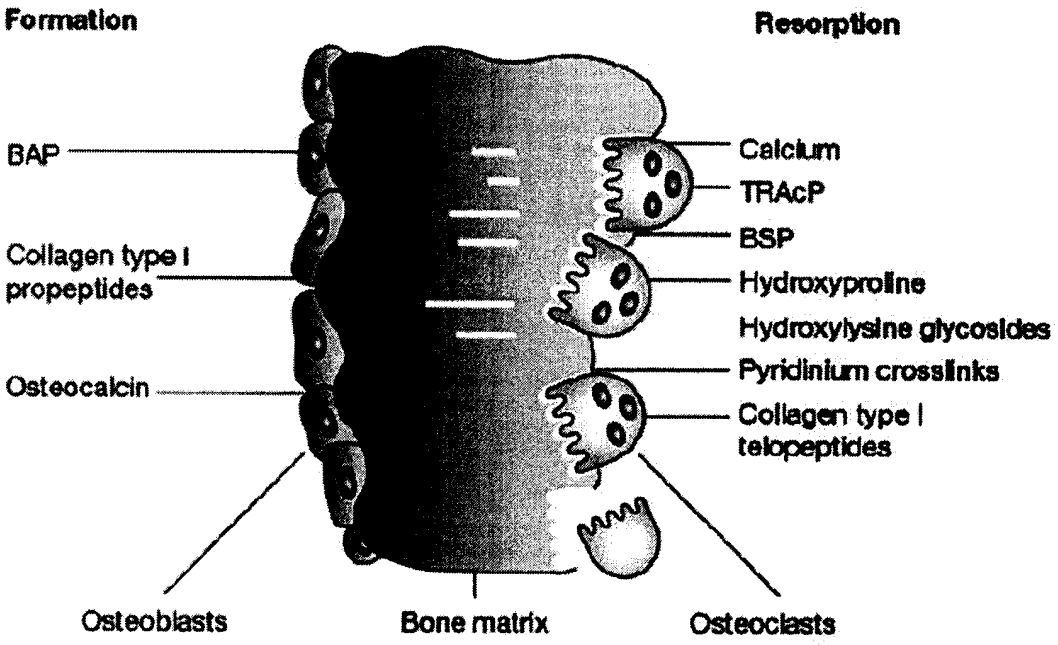

Figure 4. Schematic representation of the various bone markers currently in use.[50]

Total serum alkaline phosphatase (ALP) is a ubiquitous, membrane-bound tetrameric enzyme attached to glycosyl-phosphatidylinositol moieties located on the outer cell surface. It plays an important role in osteoid formation and mineralisation.[52] The total AP serum pool consists of several dimeric isoforms, which originate from various tissues: liver, bone, intestine, spleen, kidney, and placenta.[53, 54]

In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone.[55] In children and adolescents the bone-specific isoenzyme predominates (up to 90%) because of skeletal growth.[56, 57]

Serum total ALP is the most widely used marker of bone metabolism due to the wide availability of inexpensive and simple methods. Once liver disease is ruled out, serum levels of total ALP provide a good impression of the extent of new bone formation and osteoblast activity.[58, 59] From a clinical perspective, detection of the bone-specific ALP isoenzyme is increasingly preferred because of its higher specificity.[55, 60, 61]

Many techniques have been developed to differentiate between the two main isoforms of circulating ALP, including heat denaturation, electrophoresis, precipitation, selective inhibition and, more recently, immunoassays.[62, 63] In healthy adults, most methods show a good correlation between bone specific and total ALP.

Regarding to markers of bone resorption, the majority of them are degradation products of bone collagens including:[51] hydroxyproline, collagen cross-links, pyridinolines (pyridinoline, deoxypyridinoline) and cross-linked telopeptides (i.e., NTx, CTx). More recently, non-collagenous proteins such as bone sioloprotein BSP, and osteoclast-derived enzymes such as cathepsin K and L have been investigated as markers of bone resorption.

Bone resorption markers are extremely useful for metabolic bone disease diagnosis and for early indications of treatment responsiveness. Their use in drug development is advantageous, as changes in clinical endpoints, such as bone mineral density and bone fracture, require months to years, respectively, to manifest.

Desoxypyridinoline and cross-link telopeptides of type I collagen are the two markers of resorption most frequently investigated. Telopeptides are distinguished by their terminals: amino- (NTx, Osteomark) and carboxy- (CTx, Crosslaps). Variations in diurnal rhythm and the effect of meals must be taken into account when results of these tests are evaluated. C-terminal telopeptides of type I collagen (CTx) is a bone resorption biomarker that has been shown to be a highly effective biomarker tested for the detection of high bone turnover.[64] It has been used as a biomarker for clinical development of drugs for osteoporosis and metastatic bone disease.[65, 66] It shows a significant response to antiresorptive therapy in postmenopausal womem and in individual diagnosed with osteopenia .It can be also used in predicting skeletal response (bone mineral density) in post menopausal women undergoing anti resorptive therapies.

I.3: TECHNIQUES FOR MEASUREMENT OF BONE DENSITY

I.3.1: DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA)

Dual-energy X-ray absorptiometry (DXA) is a method by which the body composition of fat, lean and BMD can be measured. As indicated by the name, DXA utilizes two X-ray beams of two different energies. If a medium is placed in the path of the beam, the X-rays will interact with the medium atoms through Compton scattering and photoelectric effect (Figure 5). As a result, the X-ray beam will be attenuated (reduced in intensity) when measured on the other side of the medium. The extent to which the beam is attenuated is a function of the linear attenuation coefficient (μ_l) and the thickness (x) of the attenuating medium. The fraction of radiation intensity that is transmitted through a homogeneous medium is given as:

$$\frac{I_t}{I_o} = e^{-\mu_l x} \qquad \text{(Eq.1)}$$

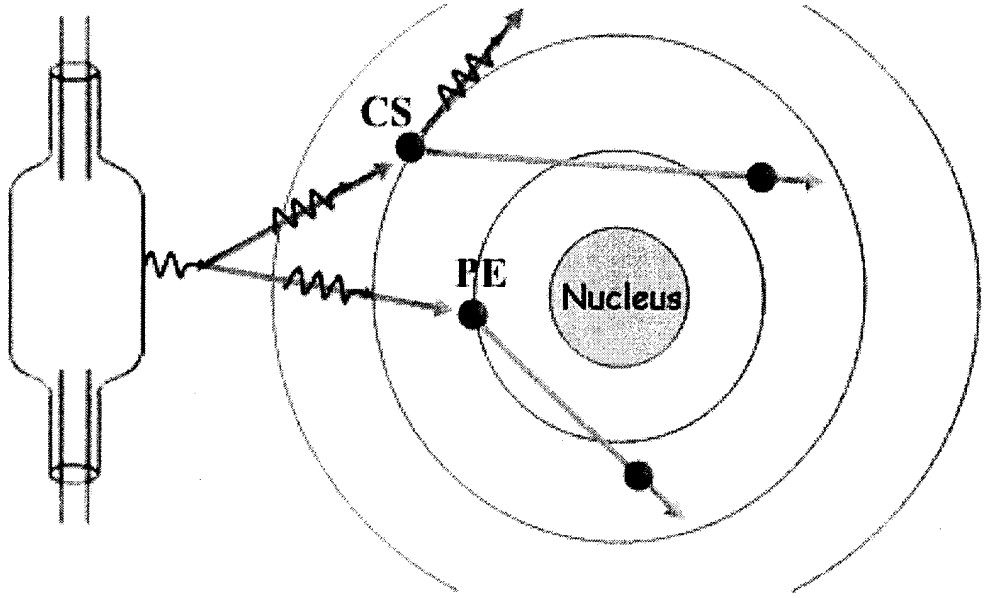

Figure 5. Compton scattering (CS) and photoelectric effect (PE) reduces the intensity of a penetrating X-ray beam.

where I_t is the transmitted intensity, and I_o is the original (incident) intensity. μ_l is dependent on the atomic number and mass per unit area (or density) of the attenuating medium. To compare the attenuation capability of the materials regardless of their densities, the mass attenuation coefficient (μ_m) is used. μ_m is given as:

$$\mu_m = \frac{\mu_l}{density} \qquad \text{(Eq.2)}$$

Using μ_l, the fraction of radiation intensity that is transmitted through a medium is given as:

$$\frac{I_t}{I_o} = e^{-\mu_m M} \qquad \text{(Eq.3)}$$

where M is the mass of the medium in kg.

Both μ_l and μ_m are dependent on the energy of the radiation beam (Table 1). They are lower at higher energies, and higher at lower energies. I_t and I_o can be measured using appropriate radiation detectors. μ_m, according to the material type and energy of the used radiation, is known and listed in reference tables. Therefore, the mass of the attenuating material is the only unknown variable, which can be calculated as in the following:

$$\ln \frac{I_t}{I_o} = -\mu_m M \qquad \text{(Eq.4)}$$

If the attenuating medium is composed of two or more materials then the composite μ_m is the sum of the individual μ_m values weighted for their fractional contribution to the total mass.[67] Here, M is the total mass, not the individual masses, of the different media.

Table 1. Mass attenuation coefficient (μ_m) of some materials for 40 and 70-keV X-ray photons.

COMPONENT	μ_m (m^2 kg^{-1})	
	40 keV	*70 keV*
Ca	1.7920	0.5059
Water	0.2636	0.1942
Protein	0.2363	0.1831
Fat	0.2273	0.1872
Bone mineral	0.9039	0.3159

The DXA technique measures fat, lean and BMD utilizing two X-ray beams: one of low energy (e.g., 40 keV), and the other of higher energy (e.g., 70 keV) (Figure 6). In a bone-free region, the low-energy beam is attenuated by both components of the soft tissue (lean and fat), while the high-energy beam is attenuated primarily by lean. The mass of lean measured by the high-energy beam is then used to measure the portion of fat in the total mass measured by the low-energy beam. Therefore, in a bone-free region, the two radiation beams measure the individual masses of the two components of soft tissue, namely lean and fat.

In a bone-containing region, the low-energy beam is attenuated by both bone and soft tissue, while the high-energy beam is attenuated primarily by bone. The mass of bone measured by the high-energy beam then is used to measure the portion of soft tissue in the total mass measured by the low-energy beam. Therefore, in a bone-containing region, the two radiation beams measure the individual masses of the bone and soft tissue. Combining the measurements of the two radiation beams in the two regions, with the assumption that lean-to-fat ratio is the same in the two regions, allows mass measurement of three components: lean, fat and bone.

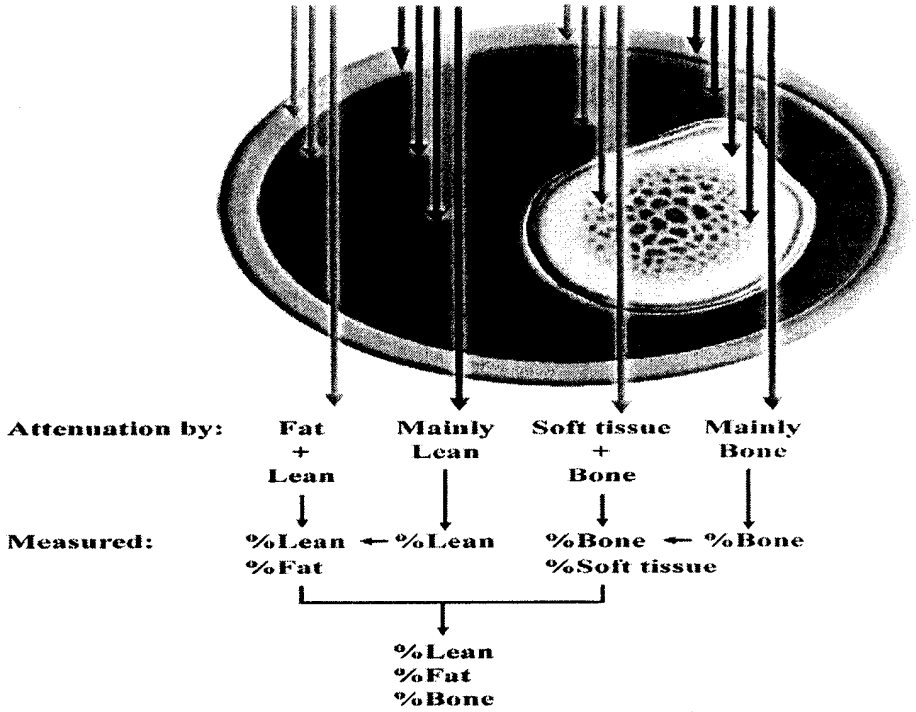

Figure 6. The basic interactions and calculation on which DXA measurements of lean, fat and bone content are based. Low-energy and high-energy beams are represented by the blue and red arrows, respectively.

I.3.2: OTHER TECHNIQUES

Quantitative ultrasound (QUS) techniques have been developed over the past 10 years to determine bone quality and skeleton status. Studies have shown that sonographic parameters were able to provide information not only about bone density but also about its structure and elastic properties. The QUS devices currently available can be applied to different peripheral sites of the skeleton (i.e., the calcaneus, the proximal phalanges of the hand and the tibial shaft). Interest in this technique stems from practical, economic and health safety aspects, since it is much faster, simpler and more portable than DXA; moreover it is less expensive, and it does not employ ionizing radiation. These features suggest a role for QUS as an effective screening tool for osteoporosis in postmenopausal women.[68]

QUS has therefore gained interest, since QUS parameters [i.e., speed of sound (SOS), broadband ultrasound attenuation (BUA), and stiffness index (SI)] enable estimation of both the amount of bone mass and possibly the skeletal quality beyond the amount of bone mineral.[69] Several trials have shown that the fracture-predictive ability of QUS is similar to the predictive ability of DXA in both adults and children,[70] that QUS has a predictive ability that is independent of the amount of bone mineral measured by DXA, and that SOS has been reported to be lower in children with fractures compared to children without.[114] The changes in BUA during growth have also been suggested to mimic the changes in BMC estimated by both DXA and peripheral quantitative computed tomography (pQCT). In addition, QUS has been used to assess bone status in children with chronic diseases known to influence bone metabolism.[115] With the advantages of not using ionizing

radiation, easy performance, short scan time, and cost–effectiveness, QUS has been recommended by many as an ideal technique when monitoring the skeleton during growth.

Quantitative Computed Tomography (QCT) is another technique that has been also demonstrated to be a useful for measuring visceral fat, in addition to evaluating apparent volumetric BMD and can distinguish between the trabecular and cortical components of bone.[71] Spinal QCT has been shown to be useful in detecting bone loss and predicting fracture risk in patients with normal renal function.[71] In HD patients, Lechleitner et al.,[72] found reduced BMD of the spine assessed with QCT, with no change in trabecular bone of the spine and increased cortical BMD on follow-up QCT performed one year after the initial study. Tsurusaki et al.,[73] used peripheral QCT and found that there was decreased BMD that primarily affected cortical bone. However, the number of measurements that can be performed on one individual is limited because of the radiation exposure involved in CT scanning, thus making this method unsuitable for studies requiring repeated measurements on the same subject. MRI compares well with CT-measured adipose tissue areas. However, MRI has no risk of radiation exposure unlike CT.[74] On the contrary; it has the disadvantage of high cost and less availability.

The most expensive procedure for determining total body mass and its components is neutron activation. The neutron activation instruments can provide measurements of potassium, sodium, chloride, calcium, nitrogen and carbon as well as a number of minor elements. Based on neutron activation analysis one can calculate a four or six compartment model with knowledge about the proportion of Ca in the hydroxyapatite of bone crystal and the fact that nitrogen represents 16% of protein mass. The important limitation of this method is its high radiation dose.[75]

Electrical and dielectric parameters might also provide diagnostically valuable information on trabecular bone microstructure, not extractable from BMD measurements. For this reason, structural properties of human trabecular bone samples harvested from the distal femur and proximal tibia were investigated using computed microtomography (μCT) in humans.[76] Quantitative parameters [e.g., structural model index (SMI) and trabecular bone volume fraction (BV/TV)] were calculated together with electrical parameters (i.e., relative permittivity, loss factor, conductivity, phase angle, specific impedance and dissipation factor), measured over a wide frequency range (50 Hz–5 MHz) using a two-electrode impedance spectroscopy setup. Significant linear correlations were found between the dissipation factor and both BV/TV and SMI. Principal component analyses showed that the high frequency principal component of the dissipation factor was significantly related to SMI. The linear combination of high and low frequency relative permittivity predicted 73% of the variation in BV/TV. Thus, relative permittivity and dissipation factors were significantly and specifically related to a trabecular bone microstructure as characterized with μCT. This may constitute a useful basis for theoretical and experimental work towards the development of impedance spectroscopy techniques for detection of bone quality *in vitro* or in special cases of open surgery.

Compared to all the abovementioned methods, DXA systems are affordable, practical, require no active subject involvement and involve minimal radiation levels. The other methods, on the other hand, are costly, require highly trained staff for their operation and implementation, depend in part on subject participation, or involve exposure to moderate radiation doses. Furthermore, DXA permits quantification of multiple whole body and segmental components. For these reasons, DXA is gaining international acceptance as a body-composition reference method.[77]

Chapter II

AIM OF THE STUDY

The objectives of the present study were to: evaluate total and segmental BMD for patients with β-Thalassaemia major; evaluate the biochemical marker changes that may affect the BMD for these patients; and mathematically model total and segmental BMD for these patients on bases of age, sex, and ethnicity-specific reference parameters.

Chapter III

SUBJECTS AND METHODS

III.1: SUBJECTS

The study population included 60 subjects, who were divided into two groups. The first (n = 30) is the patients group, which consisted of patients with β-Thalassaemia major referred to the Hematology Department, Medical Research Institute, Alexandria University; for routine follow up and regular blood transfusion. The second (n = 30) is the Control group, which were matched with the study group for age, sex, and socioeconomic level. All study participants were asked to volunteer to the study and provide signed informed consent prior to their admission to the study.

Complete history was obtained for all participants with emphasis on categorizing bone aches and history of fractures, and complete physical examination with emphasis on bone, joints, and neurological examination. None of the Thalassaemia patients presented with any endocrine disorder or was receiving any kind of hormonal replacement therapy. All patients were receiving blood transfusions to maintain a hemoglobin concentration above 9 g/dl. Patients were also receiving chelating therapy, which included desferrioxamine, deferiprone or a combination of both.

Exclusion criteria from the study protocol were: age below 16 years, rheumatic bone disease, history of rickets or osteomalacia, congenital liver and kidney diseases, and corticosteroid therapy for more than 6 months.

III.2: BLOOD BIOCHEMICAL MEASUREMENTS

Blood samples were obtained from fasting Thalassaemia patients and healthy Controls. Samples were immediately centrifuged and 50 µl of plasma were mixed with 100 µl of 5% metaphosphoric acid. Samples were aliquoted and stored at –80°C until assayed. Serum levels of calcium (Ca), ionized calcium (Ca^{2+}), inorganic phosphate (P), alkaline phosphatase (ALP), bone-specific alkaline phosphatase (S.ALP), and C-Telopeptide of Collagen Type I (CTx) were assessed for all participants using standard methods.[78]

III.3: BODY-COMPOSITION AND BONE DENSITOMETRIC MEASUREMENTS

Complete history was obtained for all participants with emphasis on categorizing bone aches and history of fractures. Complete physical examinations were also done with emphasis on bone, joints, and neurological examination. We measured anthropometric and body-composition variables for all participants. Specifically, body weight (*kg*) (participants clothed in underwear, bare feet) was measured using a sensitive digital scale (to the nearest 0.01 *kg*) (Electronic Body Scale, TCS–200–RT, China). Height (*m*) was measured using a stadiometer. BMI was expressed as Weight/Height^2 (kg/m^2). Segmental and total body bone mineral content (BMC), BMD, fat mass (FM), fat-free mass (FFM), bone-free tissue (BFT) and T- and Z-Scores were assessed for all participants using a DXA total body

scanner (Lunar DXP Pro, GE Health Care, USA) (Figures 7 and 8), at the Department of Medical Biophysics, Medical Research Institute; Alexandria University, EGYPT.

III.4: STATISTICAL ANALYSIS

Analyses of all data were carried out using the statistical software package StatView® 5.0 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics including frequencies, proportions, and means ± SD were calculated for all relevant variables. Two-tailed *t*-test of significance of independent two samples was used to compare the Thalassaemia patients to the healthy Control groups using relevant variables. Differences were considered to be significant at $P < 0.05$. The associations between each BMD variable (i.e., lumbar spine, pelvis, and total) and anthropometric variables (i.e., Sex, Age, Weight, and Height) were modeled using multiple linear regression analysis. Statistical significance and fraction of explained variability of BMD were the main criteria for selecting independent variables. Significance was also accepted at $p < 0.05$ for single terms. The regression coefficient (β), standard error of estimation (*SEE*), and significance level (P) were determined for independent variables added simultaneously. Separate prediction equations for lumbar spine BMD, pelvis BMD, and total BMD of each group based on the independent variables: Sex, Age, Weight and Height were developed and their cumulative correlation coefficient (R), coefficient of determination (R^2), and *SEE* were calculated. The prediction equations were used to simulate BMD measurements and the difference between predicted and observed values was given as mean squared difference. Errors generated by the prediction equations were calculated as the weighed sum of squared errors (*WSSE*) and the Bland-Altman test was applied for assessing residual statistics and goodness of fit.

Figure 7. Typical arrangement for a total body scan, for one of the healthy Controls, using the Dual-energy X-ray Absroptiometry (DXA) at the Department of Medical Biophysics, Medical Research Institute, Alexandria University, Alexandria, EGYPT.

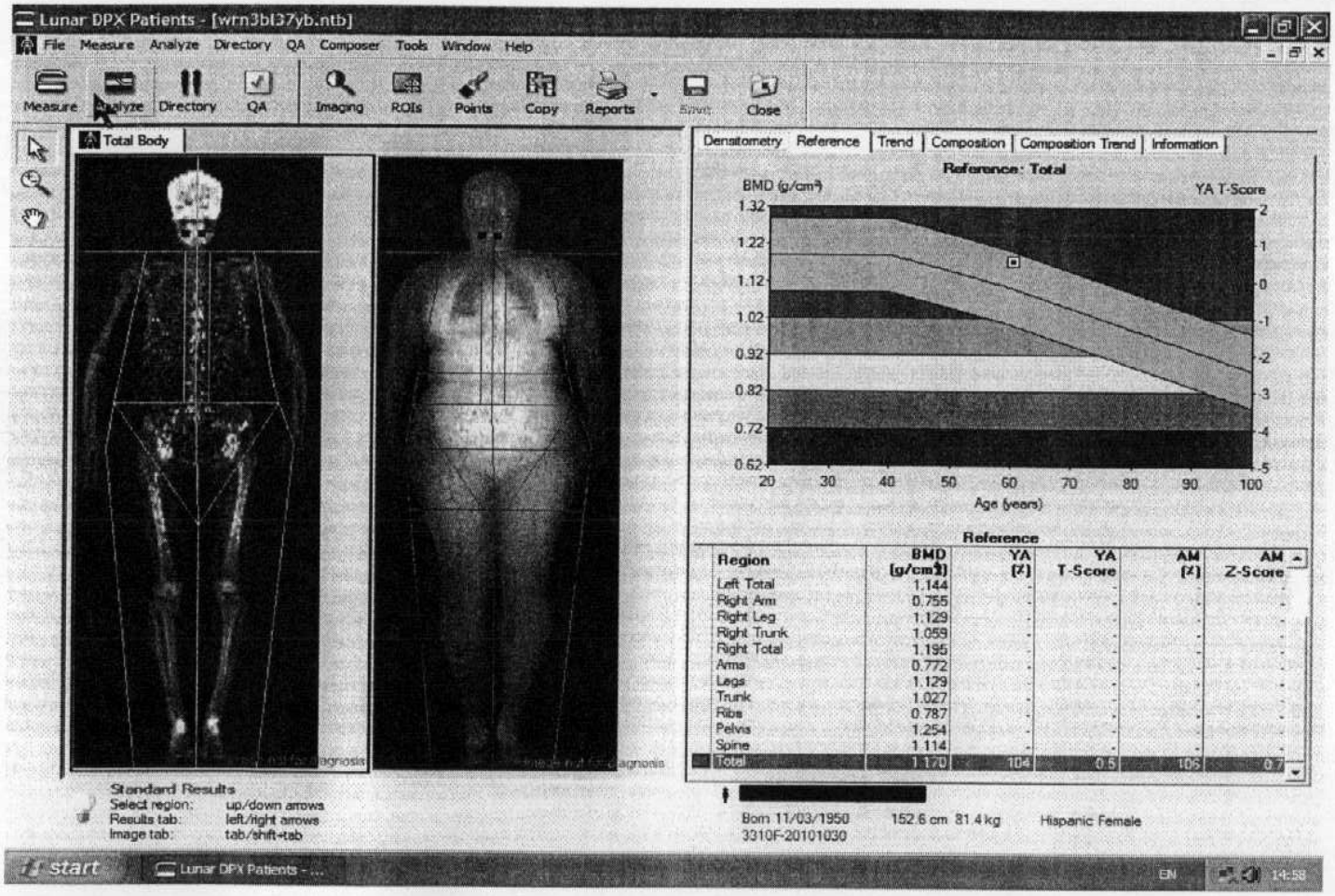

Figure 8. Typical Output Report after a total body scan, for one of the healthy Controls, using the Dual-energy X-ray Absroptiometry (DXA) at the Department of Medical Biophysics, Medical Research Institute, Alexandria University, Alexandria, EGYPT.

Chapter IV

RESULTS

The anthropometric and body-composition variables of the two study groups are presented in Table 2 and Figures 9 through 13. The average age (±SD) for Thassaemia patients and healthy Control participants were 24.92 ± 4.93 and 25.41 ± 6.04 yr, which were not statistically different; as shown in Table 2 respectively. However, the parameters weight, height, and BMI were all significantly lower for Thalassaemia patient as compared to the healthy Controls (i.e., 25.41 ± 6.04 vs. 79.81 ± 18.04 kg, 1.58 ± 0.08 vs. 1.71 ± 0.10 m, and 21.25 ± 2.06 vs. 27.02 ± 4.48 kg/m^2; respectively; $P < 0.001$ for all associations).

Moreover, advanced body-composition analysis for weight components, using the DXA technique, which its results are shown in Table 2 and Figures 9 through 13, showed that the total FM compartment was significantly different between Thalassaemia and Control groups (i.e., 14.03 ± 5.77 vs. 27.03 ± 10.32 kg, respectively, $P < 0.001$). In the same way, the LBFM and TBFM compartments were also significantly different between Thalassaemia and Control groups (i.e., 34.34 ± 8.61 vs. 48.36 ± 12.87 and 47.98 ± 8.68 vs. 75.39 ± 17.48 kg, respectively, $P < 0.001$). Thus, both body weight and its tissue components (i.e., FM, LBFM, and TBFM) were significantly lower for Thalassaemia patients as compared to the healthy Controls.

Segmental (i.e., arms, trunk and legs) and total FM are shown in Figure 9, which were significantly lower ($P < 0.001$) for the Thalassaemia patients as compared to the healthy Controls. In the same way, segmental (i.e., arms, trunk and legs) and total LBFM and TBFM, as shown in Figures 10 and 11 respectively, were all significantly lower ($P < 0.001$) for the Thalassaemia patients as compared to the healthy Controls.

The results for segmental (i.e., head, arms, legs, trunk, ribs, pelvis, and spine) and total BMC are shown in Figure 12, which were all significantly lower ($P < 0.001$) for the Thalassaemia patients as compared to the healthy Controls. Similarly, segmental (i.e., arms, legs, pelvis, and spine) and total BMD were significantly lower ($P < 0.001$) for the Thalassaemia patients as compared to the healthy Control group (Figure 13).

Table 2. Anthropometric and body-composition variables measured for Thalassaemia patients and healthy Control groups.

	Thalassaemia		*Controls*	
Number	30		30	
Sex (M/F)	15/15		15/15	
Age (*year*)	24.92 ± 4.93	(18.01 – 38.14)	25.41 ± 6.04	(18.48 – 42.62)
Weight (*kg*)	52.41 ± 6.04*	(40.00 – 62.30)	79.81 ± 18.04	(50.30 – 114.00)
Height (*m*)	1.58 ± 0.08*	(1.45 – 1.71)	1.71 ± 0.10	(1.57 – 1.92)
Body Mass Index (BMI, kg/m^2)	21.25 ± 2.06*	(16.13 – 24.20)	27.02 ± 4.48	(18.51 – 33.67)
Fat Mass (*kg*)	14.03 ± 5.77*	(5.11 – 24.34)	27.03 ± 10.32	(5.43 – 41.24)
Lean Bone-Free Mass (*kg*)	34.34 ± 8.61*	(18.42 – 50.99)	48.36 ± 12.87	(30.68 – 71.12)
Tissue Bone-Free Mass (*kg*)	47.98 ± 8.68*	(18.23 – 58.23)	75.39 ± 17.48	(47.57 – 109.16)
T-Score	-1.67 ± 1.21*	(-3.60 – 0.40)	0.60 ± 1.35	(-2.10 – 2.90)
Z-Score	-1.54 ± 1.12*	(-3.39 – 0.50)	0.08 ± 1.14	(-1.80 – 2.20)

Values are expressed as Mean ± SD and numbers in parenthesis are ranges (minimum – maximum).
Statistical analysis was carried out using unpaired Student's *t*-test of significance. *P < 0.001 versus healthy Control group.

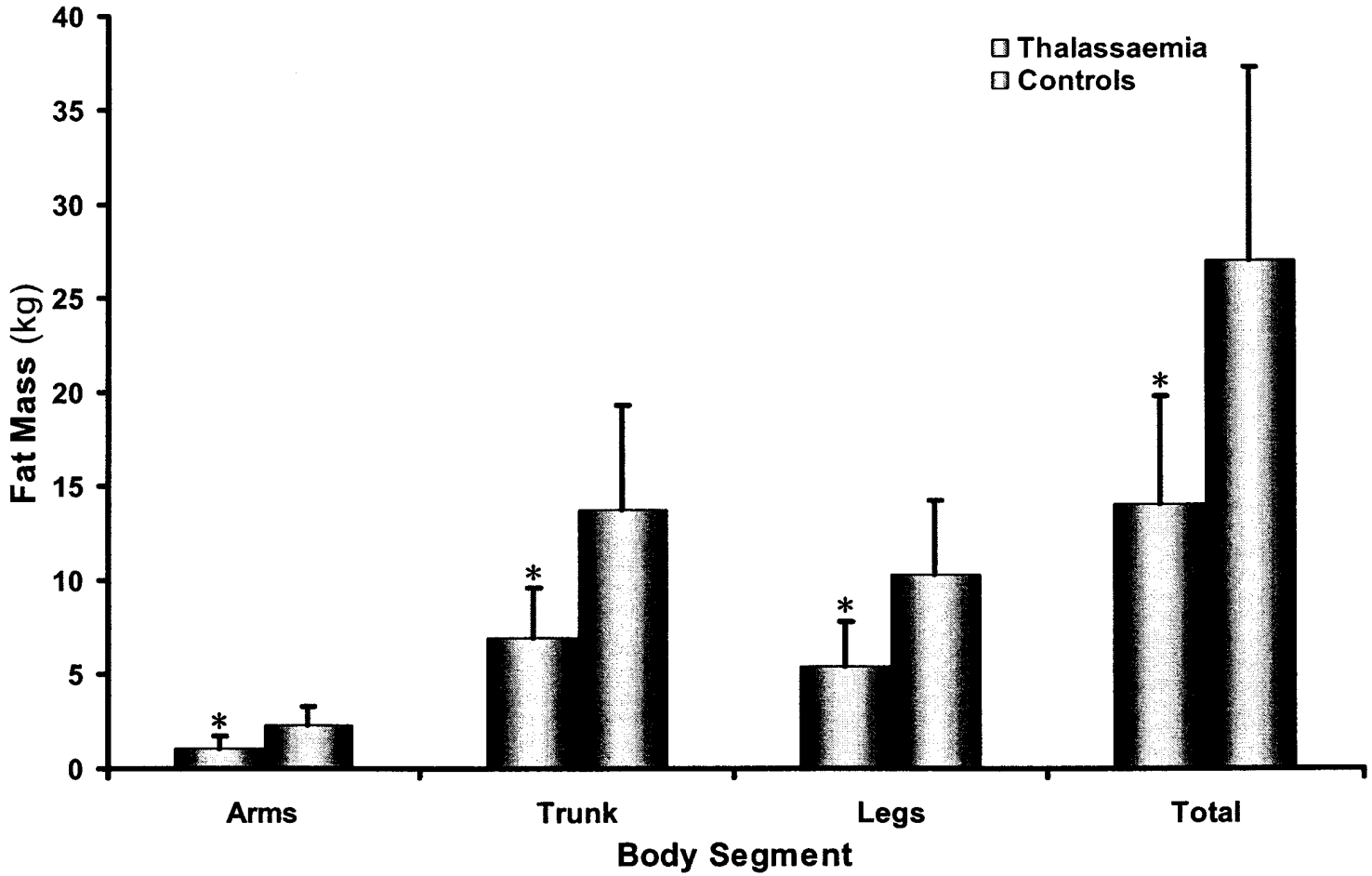

Figure 9. Graphical bar chart of segmental and total Fat Mass (FM, *kg*) measured using the Dual-energy X-ray Absroptiometry (DXA) for Thalassaemia patients and healthy Control groups. *P < 0.001 versus healthy Control group.

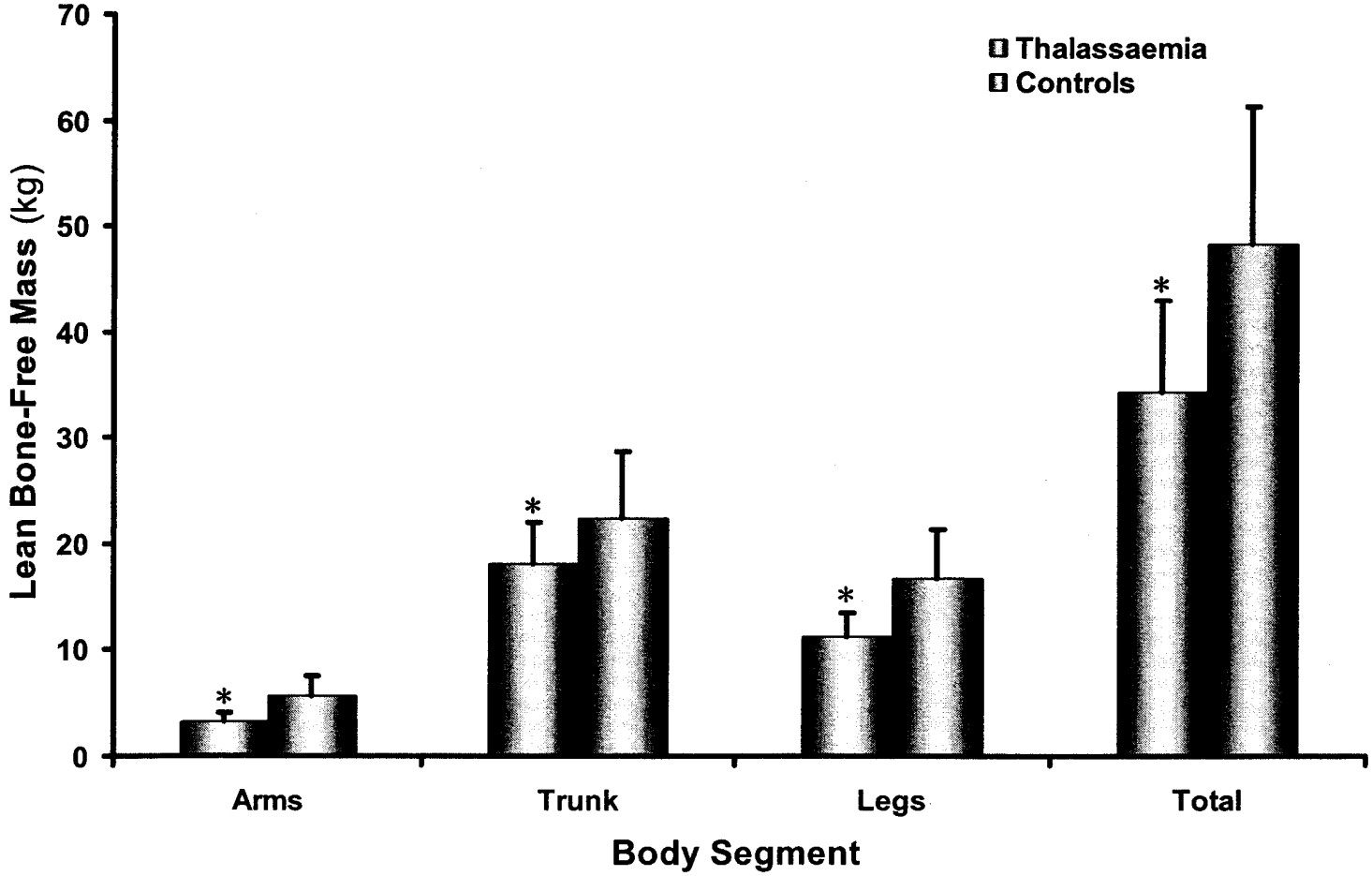

Figure 10. Graphical bar chart of segmental and total Lean Bone-Free Mass (LBFM, *kg*) measured using the Dual-energy X-ray Absroptiometry (DXA) for Thalassaemia patients and healthy Control groups. *P < 0.001 versus healthy Control group.

Figure 11. Graphical bar chart of segmental and total Tissue Bone-Free Mass (TBFM, *kg*) measured using the Dual-energy X-ray Absroptiometry (DXA) for Thalassaemia patients and healthy Control groups. *P < 0.001 versus healthy Control group.

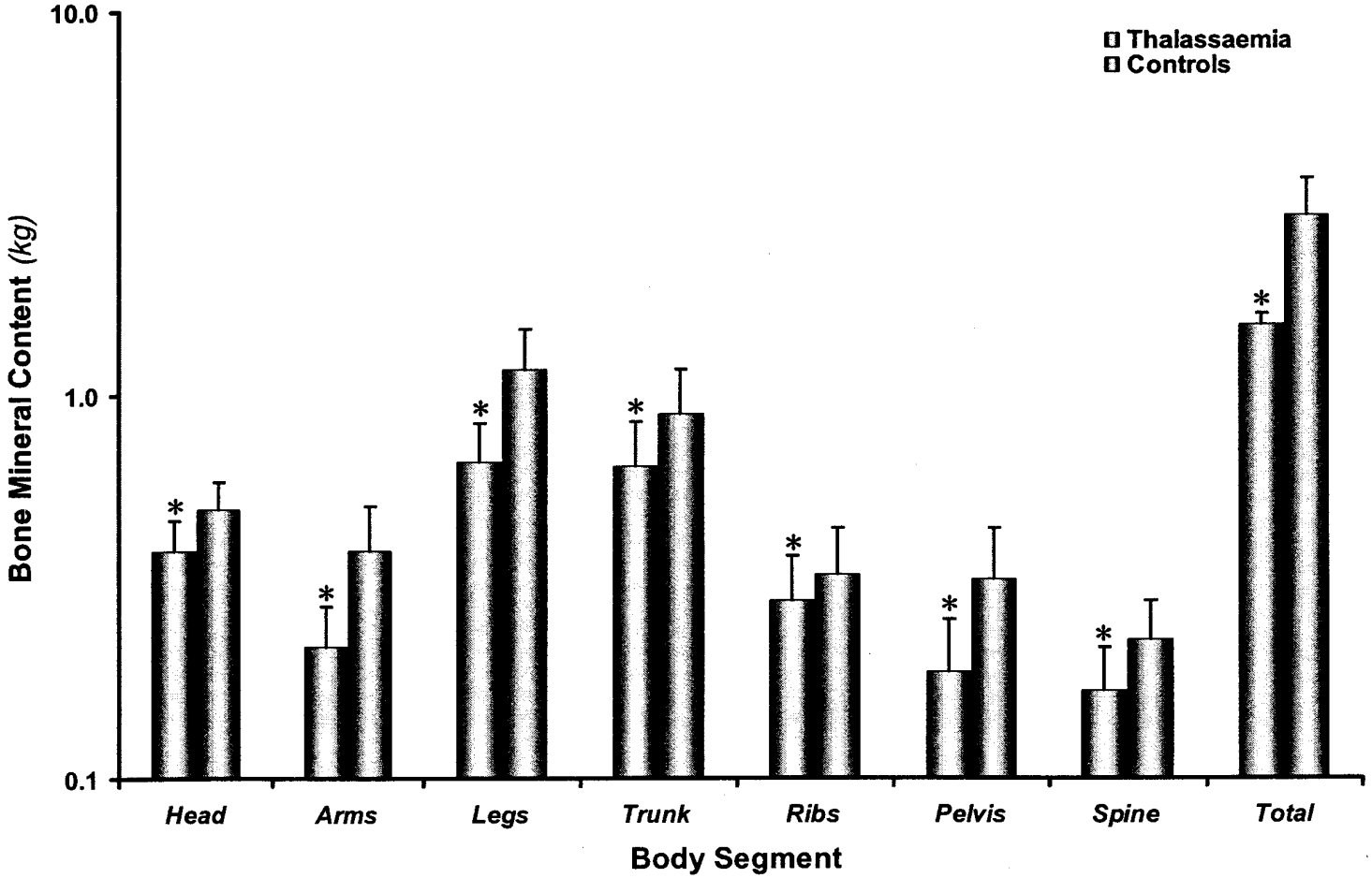

Figure 12. Graphical bar chart of segmental and total Bone Mineral Content (BMC, *kg*) measured using the Dual-energy X-ray Absroptiometry (DXA) for Thalassaemia patients and healthy Control groups. *P < 0.001 versus healthy Control group.

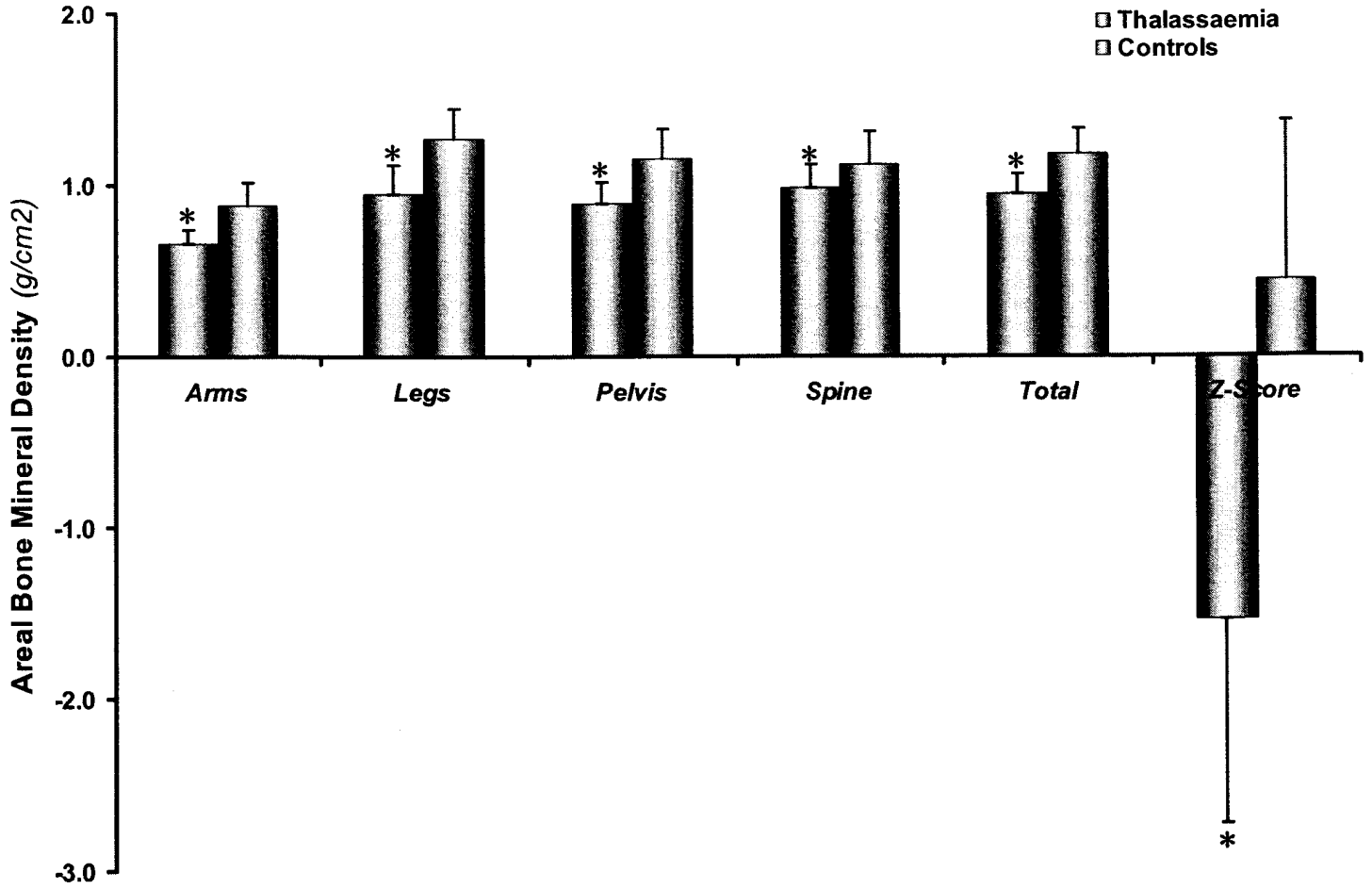

Figure 13. Graphical bar chart of segmental and total Bone Mineral Density (BMD, g/cm^2) measured using the Dual-energy X-ray Absroptiometry (DXA) for Thalassaemia patients and healthy Control groups. *P < 0.001 versus healthy Control group.

Moreover, Thalassaemia patients were found to have significantly lower T- and Z-Scores (-1.67 ± 1.21 vs. 0.60 ± 1.35 and -1.54 ± 1.12 vs. 0.08 ± 1.14, respectively, $P < 0.001$ for all associations) as compared to the healthy controls, as shown in Table 2 and Figure 13. This implies a direct effect of β-Thalassaemia Major on patients' bone health status, which can be clinically evaluated by the significant changes in both T- and Z-Scores for diagnosing osteopenia and osteoporosis.

In depth analysis of biochemical markers in the serum of Thalassaemia and Control groups showed that although both groups had comparable levels of Ca, its ionized counterpart levels (i.e., Ca^{2+}) were significantly lower for Thalassaemia patients as compared to the healthy Controls (i.e., 1.02 ± 0.12 vs. 1.21 ± 0.11 nmol/l, $P < 0.001$, respectively). However, both P and ALP were significantly higher for Thalassaemia patients than for healthy Controls (i.e., 4.65 ± 1.26 vs. 3.44 ± 0.61 mg/dl and 168.21 ± 49.53 vs. 78.82 ± 24.05 iU/l, respectively, $P < 0.001$). More importantly, both markers of bone formation (i.e., S.ALP) and of bone resorption (i.e., CTx), were significantly lower for Thalassaemia patients in comparison with that for healthy Controls (i.e., 31.45 ± 10.15 vs. 43.33 ± 6.43 µg/l and 0.48 ± 0.33 vs. 1.26 ± 0.53 ng/ml, respectively; $P < 0.001$).

In this study, we used results of segmental BMD for the sites more susceptible to fracture risks (i.e., spine BMD and pelvis BMD) in addition to total BMD measurements to develop mathematical equations to predict bone density in β-Thalassaemia Major patients. To this end, an initial multiple regression model was used to determine the effect of simultaneously adding the covariates: Sex, Age, Weight and Height on lumbar spine BMD, pelvis BMD, and total BMD, respectively, as the dependent variables, as shown in Tables 4 through 6.

Tables 4 through 6 show results of regression coefficients (β), standard error of estimation (*SEE*) together with the significance level (*P*) for the independent variables: Sex, Age, Weight and Height in association with lumbar spine BMD, pelvis BMD and total BMD for Thalassaemia patients, respectively. Statistical analysis showed all residuals to be normally distributed and the covariates Sex, Age, Weight, and Height to be significan

Table 3. Biochemical variables measured in serum for Thalassaemia patients and healthy Control groups.

	Thalassaemia		*Controls*	
Number	30		30	
Calcium (Ca, *mg/dl*)	8.64 ± 0.74	(6.80 – 9.30)	8.97 ± 0.58	(8.10 – 10.30)
Ionized Calcium (Ca^{2+}, *nmol/l*)	1.02 ± 0.12*	(0.84 – 1.19)	1.21 ± 0.11	(0.90 – 1.40)
Inorganic Phosphate (P, *mg/dl*)	4.65 ± 1.26*	(3.10 – 7.20)	3.44 ± 0.61	(2.80 – 4.60)
Alkaline Phosphatase (ALP, *iU/l*)	168.21 ± 49.53*	(87.00 – 237.00)	78.82 ± 24.05	(41.00 – 127.00)
Bone Specific Alkaline Phosphatase (S.ALP, *µg/l*)	31.45 ± 10.15*	(20.00 – 54.00)	43.33 ± 6.43	(36.00 – 48.00)
C-Telopeptide of Collagen Type I (CTx, *ng/ml*)	0.48 ± 0.33*	(0.06 – 1.25)	1.26 ± 0.53	(0.33 – 2.11)

Values are expressed as Mean ± SD and numbers in parenthesis are ranges (minimum – maximum).
Statistical analysis was carried out using unpaired Student's *t*-test of significance. *P < 0.001 versus healthy Control group.

Table 4. Coefficients of initial multiple linear regression of predictors of lumbar spine bone mineral density (BMD) for Thalassaemia patients added simultaneously.

	β	*SEE*	P-*Value*
Sex (*M/F*)	0.173	0.044	0.0004
Age (*year*)	0.001	0.004	0.0859
Weight (*kg*)	0.006	0.002	0.0014
Height (*m*)	0.237	0.080	0.0058

β: regression coefficient and SEE: standard error of estimation.

Table 5. Coefficients of initial multiple linear regression of predictors of pelvis bone mineral density (BMD) for Thalassaemia patients added simultaneously.

	β	*SEE*	P-*Value*
Sex (*M/F*)	0.103	0.039	0.0126
Age (*year*)	– 0.006	0.004	0.1040
Weight (*kg*)	0.009	0.001	< 0.0001
Height (*m*)	0.267	0.072	0.0008

β: regression coefficient and SEE: standard error of estimation.

Table 6. Coefficients of initial multiple linear regression of predictors of total bone mineral density (BMD) for Thalassaemia patients added simultaneously.

	β	*SEE*	P-*Value*
Sex (*M/F*)	0.105	0.030	0.0015
Age (*year*)	– 0.006	0.003	0.0446
Weight (*kg*)	0.008	0.0001	< 0.0001
Height (*m*)	0.341	0.055	< 0.0001

β: regression coefficient and SEE: standard error of estimation.

Table 7 shows the continuous prediction equations developed using the four independent variables: Sex, Age, Weight and Height for lumbar spine BMD, pelvis BMD, and total BMD for Thalassaemia patients, where Sex was coded as 1 for men and 2 for women, Age was in years, weight was in kilograms and Height was in meters. Values of the determination coefficient (R^2) for all prediction equations showed that the independent variables: Sex, Age, Weight and Height explained at least 99% of the variability in each BMD site predicted values.

The analysis of simulations carried out using these prediction equations for each BMD site observed values, showed that the standard error of estimations (*SEE*) of the lumbar spine BMD for Thalassaemia patients was 0.11 g/cm^2. Moreover, the *SEE* of the pelvis and total BMD were both as low as 0.09 g/cm^2. The weighed sum of squared errors (*WSSE*) of the lumbar spine, pelvis and total BMD predictions for Thalassaemia patients were comparable among each other.

Scatter plots of predicted (i.e., fitted using equations in Table 7) versus experimentally measured values by DXA of the lumbar spine, pelvis and total BMD are shown in Figures 14 through 16, respectively. We can appreciate the linear behavior in the three plots as shown by the identity line passing through the origin.

Table 7. Final prediction equations based on four independent variables for lumbar spine, pelvis, and total bone mineral density (BMD) for Thalassaemia patients.

Prediction Equation	R^2	SEE	WSSE
BMDspine $= 0.173 \times Sex + 0.001 \times Age + 0.006 \times Weight + 0.237 \times Height$	0.99	0.11	18.31
BMDpelvis $= 0.103 \times Sex - 0.006 \times Age + 0.009 \times Weight + 0.276 \times Height$	0.99	0.09	19.69
BMDtotal $= 0.105 \times Sex - 0.006 \times Age + 0.008 \times Weight + 0.341 \times Height$	0.99	0.09	18.52

Sex was coded as 1 for men and 2 for women, Age is in years, weight is in kilograms and Height is in meters. R^2: coefficient of determination; SEE: standard error of estimation; WSSE: weighed sum of squared errors.

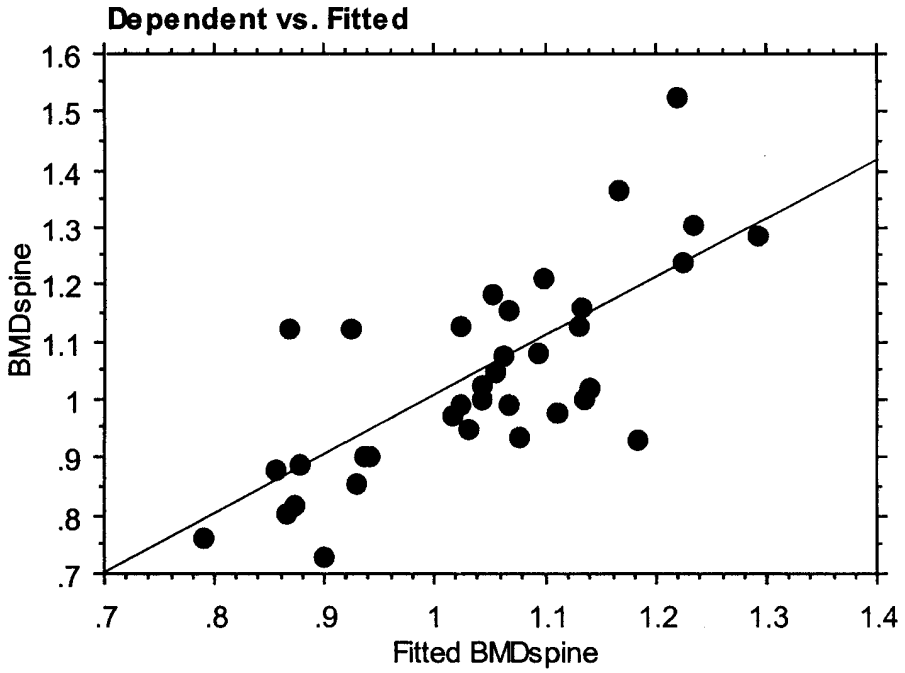

Figure 14. Scatter plot chart of fitted versus experimental lumbar spine bone mineral density (BMDspine, g/cm^2) for Thalassaemia patients.

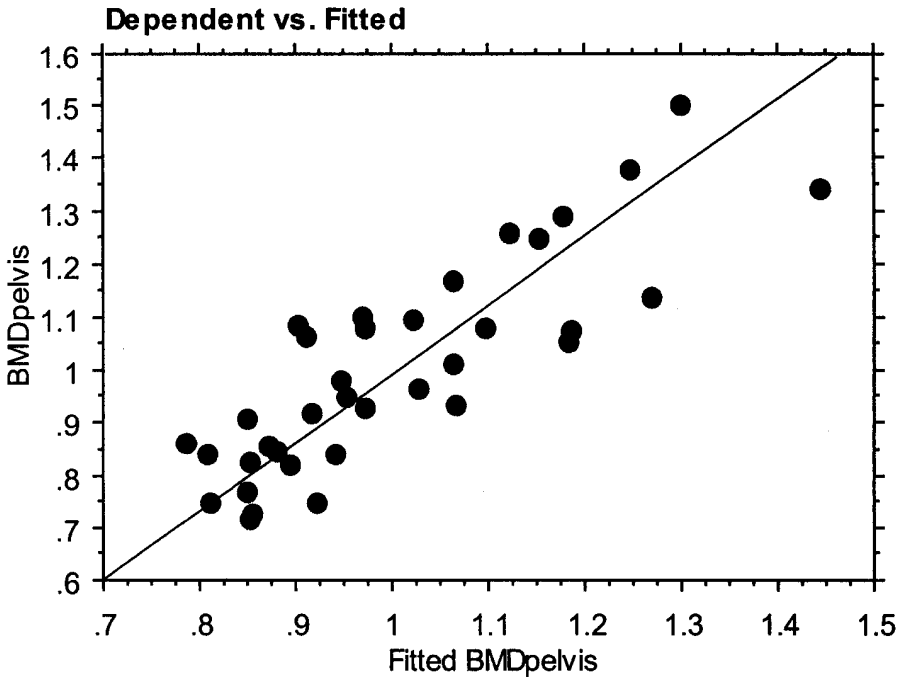

Figure 15. Scatter plot chart of fitted versus experimental pelvis bone mineral density (BMDpelvis, g/cm^2) for Thalassaemia patients.

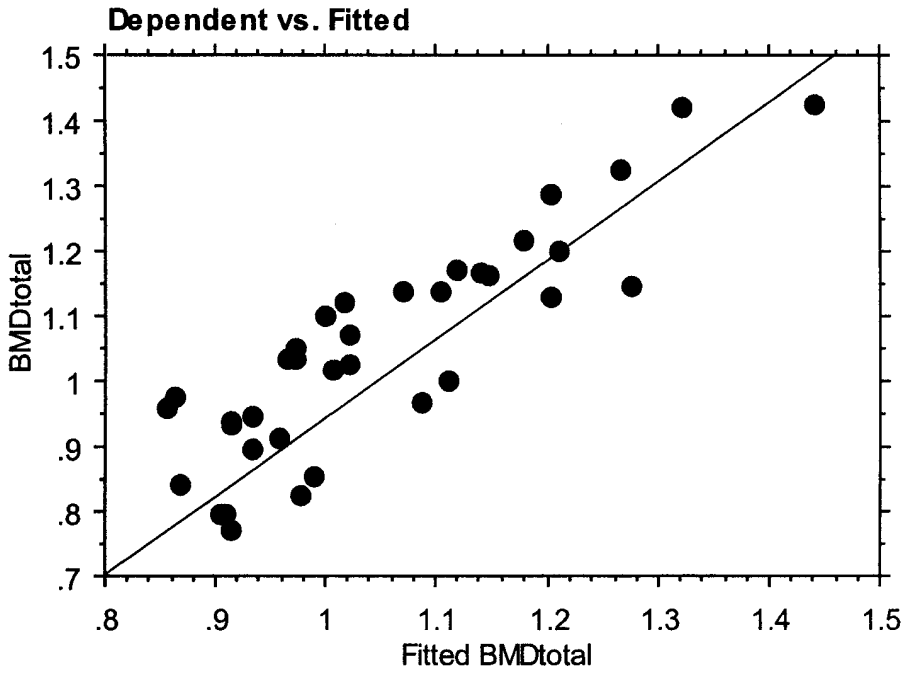

Figure 16. Scatter plot chart of fitted versus experimental total bone mineral density (BMDtotal, g/cm^2) for Thalassaemia patients.

Chapter V

DISCUSSION

The demographic and body-composition soft-tissue data of Table 2 showed that Thalassaemia patients were generally short and underweight, which was evidenced by significantly lower height, weight and consequently BMI, as compared to healthy Controls. Individual analysis of BMI showed that except for two males (i.e., 4%), Thalassaemia patients had normal BMI values, with an average 21.25 ± 2.06 and range 16.13 - 24.20 kg/m^2. In accordance with these findings, many studies have shown that although conventional therapy allows Thalassaemia children to grow normally during the first decade of life, growth retardation is observed in a significant proportion during adolescence.[33, 79-81] Further detailed body-composition analysis for Thalassaemia patients showed that their segmental (i.e., arms, legs, and trunk) and total FM were significantly lower ($P < 0.001$) by about 50% as compared to healthy Controls. In the same way, segmental and total LBFM and TBFM for Thalassaemia patients were also significantly lower ($P < 0.001$), yet by a lower percentage, in comparison with their healthy Controls.

In their historical description, Cooley and Lee,[82] assigned bone deformities to be a characteristic feature of Thalassaemia patients, which were attributed to marrow expansion and consequent reduction of trabecular bone tissue and cortical thinning due to increased but ineffective haemopoiesis. In line with this, we found that segmental (i.e., head, arms, legs, trunk, ribs, pelvis, and spine) and total BMC values for Thalassaemia patients were significantly ($P < 0.001$) lower than those for HC (Figure 12). Moreover, segmental and total areal BMD for Thalassaemia patients were also significantly ($P < 0.001$) lower than for healthy Controls (Figure 10). As DXA measurements are influenced by bone size, the small stature observed in Thalassaemia patients (Table 2) has been postulated as contributing to their overall decreased BMD.[83]

The mean BMD total Z-Score was −1.54 ± 1.12 for Thalassaemia patients and 0.08 ± 1.14 for healthy Controls (Table 2 and Figure 13). Further analysis showed that, 9 patients (30%) were in the osteoporotic range (Z-score < −2.5), 10 (33%) were in the osteopenic range (−1.0 > Z-Score > −2.5), while the resting 11 patients (37%) were in the normal range (Z-Score > −1.0). In line with these findings are the observations by Vogiatzi et al.,[84] who showed that the overall fracture prevalence among all Thalassaemia syndromes was 12.1%, being equally distributed between females (11.5%) and males (12.7%). On average, BMD Z- and T-Scores were 0.85 lower among patients with a history of fractures (mean Z/T Score −2.78 vs. −1.93, 95% CI: −0.49 - −1.22, P = 0.02). They concluded their study by stating that fractures remain a frequent complication among the aging patients with both Thalassaemia Major and Thalassaemia intermediate.

While none of Thalassaemia patients of this study had any clinical sign of endocrinopathy or presented with any kind of pubertal disorder, 9 (30%) had complained of general bone aches and 3 (10%) had been previously treated for fractures following minor trauma. However, none of them were receiving, or had received, any calcium and/or vitamin D supplements. Moreover, their biochemical profile showed normal intact parathyroid hormone concentrations and thyroid function tests in every patient. Their average ferritin level was 1393 ± 804 µg/L as compared to 62.3 ± 21.4 for healthy Controls. Thalassaemia

patients had significantly higher ($P < 0.01$) plasma P and ALP levels in comparison with healthy Controls (Table 3). Recent advances in transfusion management and chelation therapy of Thalassaemia patients have achieved an improvement in skeletal development and cosmetic bone appearance. However, despite optimal conventional treatment and lack of endocrine complications, as is the case in this study, low BMD is still reported in Thalassaemia patients.[85-89] As Thalassaemia patients get older, bone diseases such as rickets, scoliosis, spinal deformities, nerve compression, fractures and severe osteoporosis become a serious cause of morbidity. In early stages, patients may be asymptomatic but can present with back pain, a limp, dyspnea, neurological emergencies, or sudden fractures.[5]

The pathogenesis of skeletal disorders in Thalassaemia patients is multifactorial; which may be due to hormonal deficiency, compromised nutritional status, bone marrow expansion due to erythroid hyperplasia, increased iron stores or desferrioxamine toxicity.[90] Regression of marrow expansion due to regular transfusions, prevention of endocrine complications resulting in normal growth and pubertal development and reduction of desferrioxamine toxicity are the major contributors to this effect. In this way, low BMD is not observed at least for the first two decades of life in patients with Thalassaemia. Studies concerning desferrioxamine-induced bone dysplasia in Thalassaemia patients have been reported in the literature since the early 1990s.[91, 92] Chan et al.,[43] have retrospectively reviewed radiographs of 41 children with homozygous Thalassaemia. The radiographic findings showed an increased prevalence of thinned metacarpal cortices, which the authors claim that it was due to desferrioxamine-induced bone dysplasia. In addition, desferrioxamine's possible interference with metals such as zinc or calcium may influence normal bone acquisition. Overly vigorous chelation is associated with deferoxamine-induced bone dysplasia, which can slow growth rate in children and may be only partially reversible.[93] During the last 5 years, a combination of desferrioxamine and deferiprone were mainly used as chelation therapy in one group of our patients. This not only provides better synergistic chelation effects and enhances compliance but also reduces the dose–dependent toxic effects of desferrioxamine. Yet, we were not able to reveal any differences between those who used desferrioxamine and those who used combination chelation in terms of segmental or total BMD. This could be attributed partially to the high doses of desferrioxamine used in combination in order to lower the serum ferritin levels.

Thalassaemia patients are in greater risk of developing predisposing factors for osteoporosis thus, close surveillance and preventive intervention are essential. Regular screening and proper management of possible endocrine complications can possibly secure normal bone health, an extremely important consideration as life prolongation is nowadays an important achievement in patients with Thalassaemia. That is why we thought of developing easy prediction formulae for lumbar spine and pelvis BMD, which are the sites most susceptible to fracture risks, together with the total BMD of Thalassaemia patients. Multiple linear regression analysis showed that the covariates Sex, Age, Weight and Height were significantly associated with spine, pelvis and total BMD independently. Figures 11-13 show the scatter plots for the dependent versus fitted BMD components. The continuous prediction equations developed for spine, pelvis and total BMD based on these four independent variables are given in Table 7.

Chapter VI

CONCLUSIONS & RECOMMENDATIONS

In conclusion, the mathematical formulae developed on basis of the individual variables Sex, Age, Weight and Height were capable of predicting lumbar spine, pelvis, and total BMD by DXA for Thalassaemia patients. These simple mathematical formulae will allow the clinician to monitor bone health status of his patients thus, being able to manage bone loss in Thalassaemia patients. Moreover, bone-disease management includes also careful monitoring of chelation, lifestyle adjustments mainly by increasing Ca and vitamin D intake, physical activity, and hormonal therapy, in addition to refraining from smoking. Osteoclast inhibitors (e.g., bisphosphonates) have the potential to reduce bone resorption and may be a valuable treatment approach for Thalassaemia. Both transfused and non–transfused patients should be educated about risk factors and early symptoms of skeletal disorders and all patients should be screened on regular time–basis for bone densitometry by DXA. To validate these mathematical formulae, multicenteral studies involving larger study populations of different ethnic groups will be necessary. We can sum up with the major benefits of this study as:

- These equations gave estimations, which were on average < 0.70% for lumbar spine BMD, < 0.30% for pelvis BMD, and < 0.20% for total BMD of all participants, which did not result in false negative or positive diagnosis of BMD status.

- The use of these equations may permit estimating BMD deficiency accompanied with Thalassaemia at the specific sites lumbar spine and pelvis, which are most subject to fracture risk in Thalassaemia pateints, predicting fractures.

- In addition, the mathematical equations predicting total BMD may provide a better measure for the presence of osteoporosis or low BMD.

- Thus, we believe these mathematical equations may be of important practical use to clinicians in the diagnosis of osteoporosis prior to ordering a DXA total body scan.

Chapter VII

SUMMARY

Thalassaemias refers to a spectrum of inherited hemoglobinopathies characterized by the reduced or absent synthesis of one or more globin chains, of which Thalassaemia Major (also known as β-Thalassaemia, Cooley's anemia and Mediterranean anemia) is the most familiar and sever disorder. Thalassaemia is caused by a defect in the gene responsible for the synthesis of β-chain of adult hemoglobin, which results in ineffective erythropoiesis and increased peripheral hemolysis. According to the WHO, approximately 7.0% of the world population carries a globin gene mutation (nearly 200 different mutations have been found in association with Thalassaemia phenotype alone), and in the vast majority of cases it is inherited as an autosomal recessive trait. Furthermore, Thalassaemia represents a major public health problem in Egypt, since it constitutes about 85% of the chronic hemolytic anemias, with a gene frequency of 0.03% and a carrier rate of 9.0-10.5%. Thus, more than 1000 affected cases are expected to be born every year in the country.

Conventional management of Thalassaemia by regular blood transfusions and adequate chelation therapy has greatly improved the life expectancy of patients over the last decade. However, Thalassaemia is often accompanied by a series of serious bone diseases manifested by diffuse bone pain, scoliosis, spinal deformities, nerve compression, spontaneous fractures, and severe osteoporosis, the etiology of which is multifactorial and is still under investigation. Factors such as hormonal deficiency and especially gonadal failure, bone marrow expansion, increased iron stores, and Calcium/vitamin D deficiency have been shown to take part in the impaired bone metabolism of this disease. Moreover, chelation therapy with desferrioxamine has been shown to inhibit DNA synthesis, osteoblast and fibroblast proliferation, osteoblast precursor's differentiation and collagen formation, whereas in high doses it enhances osteoblast apoptosis.

Osteoporosis is a progressive disorder characterized by low bone mass, leading to enhanced bone fragility and increased fracture risk, which has been reported to be an important cause of morbidity in patients. Thus, monitoring bone health status in Thalassaemia patients is of utmost importance for proper medical treatments. Quantification of bone disease in Thalassaemia is traditionally performed using Dual-energy X-ray Absorptiometry, which is the "gold standard" for bone mineral density, and many studies have reported a high prevalence of severe osteoporosis. Repeated examinations by Dual-energy X-ray Absorptiometry are costly and are not always accessible for every patient, depending on the health services provided and the socioeconomic level of patients. Thus, there is a need for a practical and easy method for the frequent monitoring of bone mineral density in these patients, so that the clinician can have an idea about progression of the disease and/or the accompanying therapies.

Thirty patients with Thalassaemia undergoing regular blood transfusions and 30 sex- and age-matched healthy controls volunteered in the study protocol. Serum calcium, ionized calcium, inorganic phosphate, bone-specific alkaline phosphatase, and ferritin levels were monitored for all participants. Moreover, segmental and total body-composition (i.e., fat mass, lean bone-free mass, and tissue bone free mass) bone mineral content, and bone mineral density were determined by Dual-energy X-ray Absorptiometry technique for all participants

Analysis of biochemical markers in the serum of Thalassaemia and Control groups showed that although both groups had comparable levels of calcium, its ionized counterpart levels (i.e., Ca^{2+}) were significantly lower for Thalassaemia patients as compared to the healthy

Controls. However, both Inorganic Phosphate and Alkaline Phosphatase were significantly higher for Thalassaemia patients than for healthy Controls. More importantly, both markers of bone formation (i.e., Bone-Specific Alkaline Phosphatase) and of bone resorption (i.e., C-Telopeptide of Collagen Type I), were significantly lower for Thalassaemia patients in comparison with that for healthy Controls.

Thalassaemia patients were found to have significantly lower T- and Z-Scores as compared to the healthy controls. This finding implies a direct effect of β-Thalassaemia major on patients' bone health status, which can be clinically evaluated by the significant changes in both T- and Z-Scores for diagnosing osteopenia and osteoporosis. We developed mathematical formulae on basis of the individual variables Sex, Age, Weight and Height for predicting lumbar spine, pelvis, and total bone mineral density of Thalassaemia patients. These simple mathematical formulae will allow the clinician to monitor bone health status of his patients thus, being able to manage bone loss in Thalassaemia patients. Moreover, bone-disease management includes also careful monitoring of chelation, lifestyle adjustments mainly by increasing calcium and vitamin D intake, physical activity, and hormonal therapy, in addition to refraining from smoking. Osteoclast inhibitors (e.g., bisphosphonates) have the potential to reduce bone resorption and may be a valuable treatment approach for Thalassaemia. Both transfused and non–transfused patients should be educated about risk factors and early symptoms of skeletal disorders and all patients should be screened on regular time–basis for bone densitometry by Dual-energy X-ray Absorptiometry. To validate these mathematical formulae, multicenteral studies involving larger study populations of different ethnic groups will be necessary.

Chapter VIII

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PROTOCOL

نمذجة نقص كثافة العظام في مرضى مصريين مصابين بأنيميا البحر الأبيض المتوسط: دراسة مقارنة

Modeling Bone Loss in Egyptian Patients with β-Thalassaemia Major: A Comparative Study

Protocol of thesis submitted to the
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Ph.D. in Medical Biophysics

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Background

β-thalassaemia major is a hereditary disease that occurs when both of the two genes responsible for producing the β-globin chain of hemoglobin are altered.[1] The clinical profile and severity of illness in β-thalassaemia major are manifested by a chronic anemia, increased ineffective erythropoiesis, and expansion of bone marrow cavities, therefore reducing trabecular bone tissue.[1-3] Moreover, bone disease in thalassaemia is manifested by diffuse bone pain, scoliosis, spinal deformities, nerve compression, spontaneous fractures, and severe osteoporosis.[4, 5] The etiology of osteoporosis is multifactorial and is still under investigation.[2].

Factors such as hormonal deficiency and especially gonadal failure, bone marrow expansion, increased iron stores, and calcium / vitamin D deficiency seem to take part in the impaired bone metabolism of this disease.[6, 7] The life expectancy of patients with thalassemia has greatly improved over the last decade as a result of regular transfusions and increased compliance with iron chelation therapy. However, this improvement is often accompanied by a series of serious complications including osteopenia and osteoporosis.[8]

Dual-energy X-ray absorptiometry (DXA) is the method of choice for the diagnosis of osteopenia / osteoporosis based on estimating bone mineral density (BMD) as recommended by the World Health Organization (WHO).[9] There are also other approaches for determining various markers of bone formation or resorption [10]. Osteoporosis is a progressive disorder characterized by low bone mass, leading to enhanced bone fragility and increased fracture risk.[11, 12] There exists only one study that has investigated bone turnover processes for Egyptian children with β-thalassemia, yet without examining or modeling its direct effects on their total and segmental body BMD.[8]

Aim

The objectives of the present study are to: evaluate total and segmental BMD for patients with β-thalassaemia major; evaluate the biochemical marker changes that may affect the BMD for these patients; and mathematically model total and segmental BMD for these patients on bases of age, sex, and ethnicity-specific reference parameters.

Materials and Methods

A. Subjects

The study population will comprise 60 subjects, who will be divided into two groups. The first (n = 30) is the patients group, which will consist of patients with β-thalassaemia major referred to the Hematology Department, Medical Research Institute, Alexandria University; for routine follow up and regular blood transfusion. The second (n = 30) is the control group, which will be matched with the study group for age, sex, and socioeconomic level. All study participants will be asked to volunteer to the study and provide signed informed consent prior to their admission to the study.

Patients with age below 16 years, rheumatic bone disease, history of rickets or osteomalacia, history of congenital liver and kidney diseases, and history of corticosteroid therapy for more than 6 months will be excluded from the study protocol.

B. Methods

1. All participants will be subjected to complete history taking, stressing on bone aches and history of fractures, and complete physical examination stressing on bone, joints, and neurological examination.
2. All participants will be subjected to the following biochemical investigations:
    a. Serum electrolytes (i.e., calcium and phosphorus).[13]
    b. Bone specific alkaline phosphatase by ELISA technique.[14]
    c. C-Telopeptide of collagen type I by ELISA technique (CTx - I).[14]
3. Segmental and total body bone mineral content (BMC), BMD, and fat and lean contents will be measured for all participants using the DXA technique (DXP Pro. GE Health Care, USA).[11]

Analysis of Results

Data will be grouped in sheets and electronic database containing anthropometric, clinical, and blood biochemical data, and analysis of all participants will be constructed. Statistical analysis of all data will be performed using a statistical software package StatView® 5.0 (SAS Institute Inc., Cary, NC, USA) and will be further tabulated and plotted.

Data of clinical and body compartments will be modeled, simulated, and analyzed for all participants using electronic spread sheets and sophisticated mathematical packages (SAAM II, version 1.2, SAAM Institute, Washington University, USA).[15]

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ARABIC SUMMARY

الملخص العربى

تعتبر أنيميا البحر الأبيض المتوسط واحدة من أمراض الدم الموروثة التى تتميز بانخفاض أو نقصان فى انتاج واحدة أو أكثر من سلاسل الجلوبين، وتعرف أيضا بالثلاسيميا الكبرى أو أنيميا كولى، وتعد الأكثر انتشاراً والأشد اضطراباً. وتنتج الثلاسيميا من خلل في الجينات المسؤولة عن تصنيع سلسلة الهيموجلوبين من نوع بيتا الموجود فى البالغين، مما يؤدى الى تكون الكريات الحمراء الغير فعالة و إلى زيادة انحلال الدم المحيطى. ووفقا لمنظمة الصحة العالمية فإن حوالى ٧,٠٪ من سكان العالم يحملون طفرات وتحور في جين الجلوبين (فقد وجد على ما يقرب من ٢٠٠ نوع من الطفرات المختلفة تعود للثلاسيميا وحدها)، وأن الغالبية العظمى من الحالات يتم توارثها جينيا. وعلاوة على ذلك، فإن مرض الثلاسيميا يمثل مشكلة صحية عامة رئيسية فى مصر، نظرا لأنه يشكل حوالي ٨٥٪ من امراض فقر الدم الانحلالي والمزمن، مع تواتر الجينات بحوالى ٠,٠٣٪ ومعدل النقل من ٩,٠-١٠,٥٪، ولذا فمن المتوقع ظهور أكثر من ١٠٠٠ حالة متضررة من المولودين في مصر في كل عام.

غير أن تحسن طرق علاج مرض أنيميا البحر الابيض المتوسط بفضل عمليات نقل الدم واستخدام علاجات استخلاب الحديد الملائمة قد انعكس على زيادة متوسط العمر المتوقع للمرضى على مدى العقد الماضى. هذا وغالبا ما يترافق مرض أنيميا البحر الابيض المتوسط مع سلسلة من أمراض العظام الخطيرة مثل آلام العظام المنتشره، كالجنف وتشوهات العمود الفقرى، وضغط الأعصاب، والكسور العفوية، وهشاشة العظام الحادة، ولا زال الموضوع قيد دراسات عديدة لمعرفة الأسباب. وقد تبين ان عوامل كثيرة مثل نقص الهرمونات وكذلك خصوصا فشل الغدد التناسلية والتمدد في نخاع العظام وزيادة تجمع الحديد ونقص الكالسيوم / فيتامين (د) قد تؤثر بشكل كبيرفي عمليات الايض فى تكوين بناء العظام، علاوة على ذلك فقد تبين انه مع استخدام علاج استخلاب الحديد "ديفيروكسامين" لتثبيط تخليق الحمض النووى وفى بناء العظم، وانتشار الخلايا الليفية، بالإضافة إلى التمايز فى بناء العظم وتكوين مادة الكولاجين، فى حين استخدام جرعات عالية منه قد اثبتت تعزيز بناء العظم نفسه.

ان هشاشة االعظام هو اضطراب تقادمى ويوصف بانه انخفاض في كتلة العظام، مما يؤدى إلى ترقق العظم وبالتالى زيادة خطورة حدوث الكسور بتقدم السن وفى الأمراض المصحوبة بانخفاض كثافة العظام بصفة عامة، أو فى مرضى أنيميا البحر الابيض المتوسط بصفة خاصة. الأمر الذى يستدعى المتابعة والملاحظة المستمرة للحالة الصحية لهم من اجل تقديم افضل سبل العلاج. هذا ويتم تشخيص حالات امراض العظام المختلفة باستخدام جهاز قياس امتصاص الاشعة السينية ثنائية الطاقة، والذى يعتبر بأنه "المعيار الذهبي" لقياس كثافة العظام المعدنية. غير أن القياس المتكرر باستخدام جهاز قياس امتصاص الأشعة السينية ثنائية الطاقة مكلف مادياً وليس دائما فى متناول كل مريض، ويعتمد على توافره وعلى مستوى الخدمات الصحية المقدمة و كذلك على المستوى الاجتماعى والاقتصادى للمرضى. وبالتالى، فهناك حاجة إلى طريقة عملية وسهلة لقياس كثافة العظام المعدنية في العظام باستمرار لهؤلاء المرضى، بحيث يمكن أن تكون للطبيب المعالج فكرة عن تطور المرض و/أو تأثير العلاج المستخدم.

اجريت الدراسة الحالية على ثلاثين مريضا من الذكور والإناث المصابين بأنيميا البحر الأبيض المتوسط، ممن يخضعون لعمليات نقل دم دورى بانتظام، بالإضافة إلى ثلاثين شخصاً سليماً يمثلون المجموعة الضابطة مطابقين لهم من ناحية السن والجنس والمستوى الإجتماعى، تطوعوا جميعاً لاكمال بروتوكول الدراسه. تم إجراء الكشف الطبى على جميع المشاركين فى الدراسة وسحب عينات دم صائم منهم لقياس مستويات المتغيرات الكيميائية الحيوية فى بلازما الدم مثل: الكالسيوم والكالسيوم المتأين، الفوسفات الغير عضوى، إنزيم الفوسفات القلوى، إنزيم الفوسفات القلوى المميز للعظام، بالإضافة إلى سى تيلوبيتايد للنوع الأول من الكولاجين والفيريتين. كما تم قياس الكثافة المعدنية للعظام المقطعية والكلية لجميع المشاركين باستخدام تقنية قياس امتصاص الأشعة السينية ثنائية الطاقة.

لقد قمنا باستنتاج صيغ رياضية متقدمة على اساس المتغيرات الفردية كالجنس والعمر والوزن والطول للتنبؤ بكثافة العظام المعدنية المقطعية فى كلٍ من الحوض والعمود الفقرى بالإضافة إلى كثافة العظام المعدنية الكلية للمرضى. وبأستخدام هذه الصيغ الرياضية البسيطه يمكن للطبيب المعالج مراقبة الوضع الصحى وحالة العظام فى المرضى، مما يمكنه معرفة مقدار النقص فى كثافة العظام المعدنية لمرضى أنيميا البحر الأبيض المتوسط نتيجة التقدم فى السن و/أو نتيجة العلاج المستخدم. وقد أعطت هذه المعادلات تقديرات كانت فى المتوسط أقل من ٠,٧٠٪ لكثافة المعادن بعظام الفقرات القطنية، أقل من ٠.٣٠٪ لكثافة المعادن بعظام الحوض، وأقل من ٠,٢٠٪ لكثافة المعادن بالعظام الكلية لجميع المشاركين، والتي لم تسفر عن تشخيص زائف سلبى أو إيجابى لحالة كثافة المعادن بالعظام. إن استخدام هذه المعادلات يمكن أن يمكننا من تقدير نقص كثافة المعادن بالعظام المتزامن مع مرض أنيميا البحر الأبيض المتوسط فى مواقع محددة مثل الفقرات القطنية، والورك، التي هى الأكثر عرضة لخطر الكسر، وتوقع الكسور. و بالإضافة إلى ذلك، فإن

هذه المعادلات الرياضية قد تمكننا من التنبؤ بكثافة المعادن بالعظام الكلية مما يمكننا من توفير أفضل قياس لوجود مرض ترقق بالعظام أو نقص كثافة المعادن بالعظام.

وعلاوة على ذلك فان علاج أمراض العظام تتطلب ايضا تسجيل ومعرفة مستوى استخلاب الحديد فى الدم وتعديل نمط الحياة عن طريق زيادة تناول الكالسيوم وفيتامين (د) وممارسة النشاط البدنى الرياضى واستخدام العلاج الهرمونى بالاضافة الى الامتناع عن التدخين. ان مثبطات هدم العظام مثل "البايفوسفونيت" لديها القدرة على الحد من ارتشاف العظام وربما يكون نهجا مناسبا وقيما في علاج مرض أنيميا البحر الأبيض المتوسط. كما ينبغى ايضا تثقيف المرضى سواء الذين ينقل اليهم الدم أم لا، عن مدى عوامل الخطر والاعراض المبكرة لأضطرابات الهيكل العظمى وعلاوة على ذلك، يجب فحص وقياس كثافة العظام عن طريق جهاز قياس امتصاص الأشعة السينية ثنائية الطاقة بصورة منتظمة. هذا وإنه للتحقق من صحة هذه الصيغ الرياضية المستنتجة فانه يلزم إجراء دراسة كبيرة تشمل شريحة عريضة من مجاميع عرقية مختلفة.

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كإيفاءٍ جزئى لشروط الحصول على درجة

دكتوراة الفلسفة فى

الفيزياء الحيوية الطبية

من

خالد غانم مجيد

ماجيستير الفيزياء (١٩٩٨)

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