Bone mineral density in correlation to biochemical profile in beta-thalassemic patients

Thesis

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in Clinical Haematology

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INTRODUCTION

Thalassemia syndromes are heterogeneous inherited disorders that arise from mutations in the globin genes that reduce or totally abolish synthesis of one or more of the globin chains. They result in hypochromia and microcytosis, and in the more severe forms, anaemia. As a group they compromise the most common single gene disorder known ***(Weatherall et al., 1994; Forget et al., 2000).***

The defect in globin synthesis results in abnormal as well as decreased quantity of globin chains. Ineffective erythropoiesis, hemolysis, and increased red blood cell turnover ensue ***(Mohamed, Jackson 1998).***

Cooley and Lee (1925) described the first series of splenomegaly in non-transfused children with anaemia and peculiar bone changes with mongoloid appearance caused by the enlargement of the cranial and facial bones. This enlargement was thought to be due to anaemia and the consequent counter- balance mechanism of ineffective erythropoiesis, thus resulting in the dramatic expansion of the bone marrow, almost 30–40 times more than the normal. Marrow expansion causes mechanical interruption of bone formation, leading to cortical thinning, and is hitherto considered as a main reason of distortion and

fragility of the bones in thalassaemia patients which may lead to increased incidence of fractures ***(Pootrakul et al., 1981; Ruggiero et al., 1998)***.

Other bone abnormalities have also been described in patients with thalassaemia, such as spinal deformities, scoliosis, nerve compression, osteopenia and osteoporosis.

Regular blood transfusion in conjunction with iron-chelation therapy has substantially increased the life expectancy of patients.

Therefore, osteopenia became an increasingly recognized cause of skeletal complications in young adults of both genders with thalassemia major or thalassaemia intermedia (***Pootrakul et al., 1981; Michelson and Cohen, 1988; Johanson, 1990; Orvieto et al., 1992; Jensen et al., 1998; Vichinsky, 1998).***

The mechanism of reduced bone mass in thalassemic patients is still uncertain. Increased bone resorption, indirectly demonstrated by an elevation in urinary marker of bone resorption, has been proposed as a major mechanism of bone loss in thalassemia.***(Pollak et al., 2000; Voskaridou et al., 2001).***

Iron overload, a common finding in thalassemic patients, has been suggested as another contributing factor

for the development of reduced bone mass ***(Singer, Vichinsky 1999; Bordat et al., 1993).*** In highly transfused patients, osteoporoses partly occur due to incessant but unexpected marrow hyperplasia ***(Vichinsky, 1998)*** or in others due to use of iron chelation therapy ***(Chan et al, 2000).***

Thus, multiple factors associated with the reduction in bone mass have been described, including delay puberty, ***(Perrotta et al., 2000)*** hypogonadism, ***(Molyvda-Athanasopoulou et al., 1999)*** vitamin D deficiency, ***(Pollak et al., 2000)*** desferrioxamine therapy, ***(Chan et al., 2000)*** and iron overload ***(Singer,Vichinsky 1999)***.

Furthermore, trace elements have been considered to play critical roles in bone metabolism. Although zinc is a minor building component in bone, it plays important functional roles in bone metabolism and bone turnover ***(Okano, 1996).*** Zinc plays an important role in connective tissue metabolism, acting as a cofactor for several enzymes, such as alkaline phosphatase (necessary for bone mineralization) and collagenase (essential for the development of the collagenous structure of bone) ***(Ilich and Kerstetter, 2000).***

AIM OF THE WORK

The present work aims to assess bone mineral density (BMD) in patient with beta-thalassaemia disease in correlation with biochemical parameters namely serum calcium, phosphorus, magnesium, zinc, serum ferritin level which may affect BMD. Also BMD will be correlated with frequency of blood transfusion, patient's compliance to iron chelators and patient's life style. This may be a step on the way to identify efficacious long term therapies to improve management of bone disease due to thalassemia.

MOLECULAR PATHOLOGY, PATHOPHYSIOLOGY AND CLINICAL FORMS OF THALASSEMIA

MOLECULAR PATHOLOGY OF THALASSEMIA

Normal Human Hemoglobin:

Human haemoglobin is formed of two pairs of globin chains. To each of which is attached one molecule of heme. Six variants are normally formed: three are transient embryonic haemoglobin referred to as Hb Gower 1, Hb Gower 2, and Hb Portland, Fetal haemoglobin HbF is predominant haemoglobin of fetal life, and HbA (more than 95%)and HbA**2** (1-3.5%) are characteristic haemoglobin of adult ***(Sir john, 1996).***

Human adult Hb is a heterogeneous mixture of a protein consisting of a major component A, and a minor component A2.

Except for some of the embryonic haemoglobins, all the normal human haemoglobins have one pair of α chain. The individual chain formed in post natal life are designed α, β, γ and δ.

HbA is formed of two α chains and two β chains (α2 β2). HbF is formed of two α chains and two γ chains (α2 γ2) and HbA2 is formed of two α chains and two δ chains (α2 δ2) the α chain thus common to all three types of haemoglobin molecules ***(Thein,1993).***

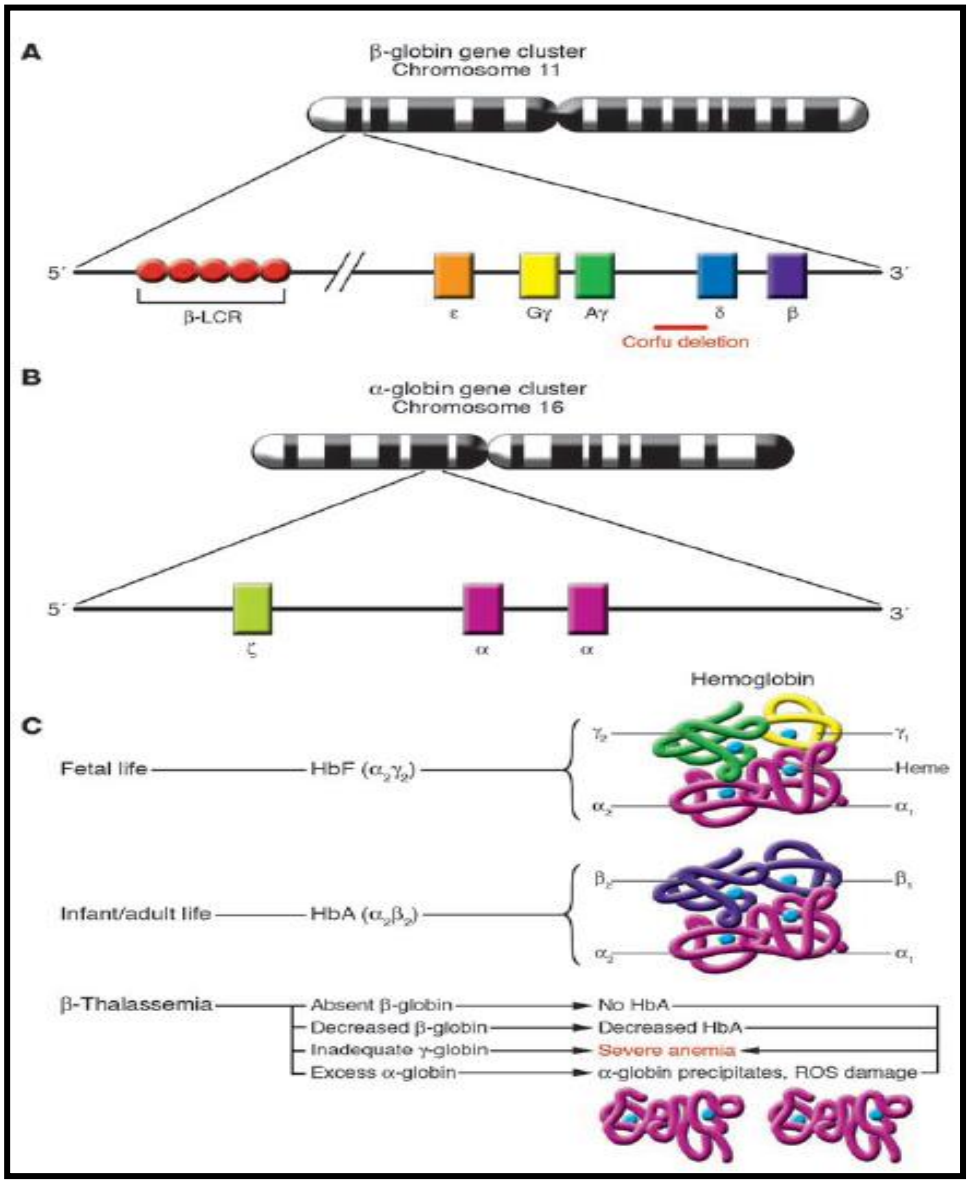


Fig. (**): The human globin loci and their role in β-thalassemia. (A) The β-LCR and structural genes (ε, Gγ, Aγ, δ, and β) within the β-globin locus on chromosome 11 are shown. The Corfu deletion,which includes part of the structural δ-globin gene and γ–δ intergenic sequences, is also shown. (B) The α-globin locus is shown with the ζ- and 2 α-globin genes on chromosome 16. (C) In early fetal life, the α- and γ-globin chains combine to form HbF (α2γ2), the main β-globin–like globin during the remainder of fetal life and early postnatal life. In late postnatal and adult life, normal hemoglobin (HbA, α2β2) predominates. In homozygous β-thalassemia, decreased or absent β-globin production leads to decreased or absent HbA levels, respectively. The synthesis of γ-globin does not increase enough to compensate for the reduced or absent β-globin level. As a result, excess α-globin accumulates and precipitates in erythroid cells and causes damage due to the action of ROS and apoptosis of the damaged cells. Severe anemia results ***(Bank, 2005).***

Human haemoglobin shows further heterogeneity, particularly in fetal life, and this has important implications for understanding the thalassemia and for possible approaches to their prenatal diagnosis. Human fetal haemoglobin is a mixture of molecular species with the formulas α**2** γ**2** 136 Gly and α**2** γ**2** Ala at birth the ratio of molecules containing γ Gly chain to those containing γ Ala chain is about 3:1 ***(Weatheral et al., 1994)***

Before the eight week of intrauterine life there are three embryonic haemoglobins: Gower 1(ζ2 ε1), Gower 2 (α2 ε2) and haemoglobin Portland (ζ2 γ2). The ζ and ε chains are the embryonic counterparts of the adult α and β chains respectively. During the fetal development, there is orderly switch from ζ- to α- and from ε-to γ chain production, followed by β- and δ- chain production after birth ***(Bunn et al., 1986).***

The α chain is directed by two α genes,α1 and α2 on chromosome 16, and the β and δ chains by single gene on chromosome 11. The γ chain is directed by two genes γ Gly and γAla also on chromosome 11 ***(Lewis et al., 1996).***

The four chains are associated in the form of tetramer. The α**1** β**1** contact is the strongest and involves many amino acids with many interlocking side chain, the α**1** β**2** contact is less extensive, while the contacts between like chains are relatively week. The binding of molecule of heme into a heme "pocket" in each chain is vital for the oxygen-carrying capacity of the molecule, if the hem attachment is weakened, the globin chain dissociated into dimmers and monomers ***(Weatheral et al., 1981).***

It is now realized that many naturally occurring genetically determined (inherited) variants of human haemoglobin exist and although many are harmless, some have serious clinical effects. Collectively, the clinical syndromes resulting from disorder of haemoglobin are referred to as "hemoglobinopathies" they can be grouped into three main categories:

(1) Those due to structural variants of haemoglobin such as HbS.

(2) Those due to failure to synthesize haemoglobin normally, as thalassemias

(3) Those due to failure to complete the normal neonatal switch from fetal haemoglobin (Hb F) to adult haemoglobin (HbA). These comprise a group of disorder referred to as hereditary persistent of fetal haemoglobin (HPFH) ***(Sir John et al., 1996).***

The β-like globin genes, a linked cluster on chromosome 11, are arranged over approximately 60,000 nucleotide bases. Promoter elements upstream from the initiation codon of each active gene are involved in the initiation of transcription. The cluster also contains other regulatory elements that interact to promote erythroid-specific gene expression and to coordinate the developmental regulation of each gene.

Hemoglobin Switching

As an adaptation to changing oxygen requirements, different hemoglobins, all composed of two different pairs of globin chains each attached to a heme moiety, are synthesized in the embryo, fetus, and adult ***(Wood et al., 1983).*** Severe β-thalassemia usually becomes manifest as a result of the decline in the synthesis of fetal haemoglobin ($\alpha_2\ \gamma_2$) during the first year of life. The precise mechanisms that control the switch from the production of fetal hemoglobin to that of adult haemoglobin ($\alpha_2\ \beta_2$) are not fully understood. ***(Orkin SH et al., 1995;Wood WG et al., 1983; Wood WG. et al., 1993; Grosveld F et al., 1993).***

PATHOPHYSIOLOGY

Mechanisms of Anemia

In severe untreated β-thalassemia, erythropoiesis may be increased by a factor of up to 10, more than 95 percent of which may be ineffective. Ineffective erythropoiesis, the hallmark of β-thalassemia, is a result of the myriad deleterious effects of a relative excess of α-globin chains ***(Nathan et al., 1966).***

This relative excess interferes with most stages of normal erythroid maturation: both intramedullary death of red-cell precursors through arrest in the G_1 phase of the cell cycle ***(Wickramasinghe, 1976)*** and accelerated intra-medullary apoptosis of late erythroblasts ***(Yuan et al., 1993; Schrier, 1997)*** have been demonstrated. Studies of the consequences of the accumulation of excess α-globin chains and their degradation products within the red-cell membrane and its skeleton ***(Schrier, 1997; Shinar et al., 1993; Grinberg et al., 1995)*** have also demonstrated abnormalities in the ratio of spectrin to band 3 and in the function of band 4.1.

The observation that the presence of excess membrane iron may aggravate membrane changes ***(Grinberg et al., 1995)*** has led to interest in the red-cell

membrane as a potential therapeutic target in β-thalassemia. In a mouse model, increased cellular rigidity and decreased stability in connection with membrane-associated α-globin chains ***(Sorensen et al, 1990)*** have reportedly been ameliorated during exposure to agents that bind membrane iron ***(Shalev et al., 1995).*** Further understanding of these processes may guide future therapies.

Clinical Consequences of Anemia

The severe ineffective erythropoiesis results in erythroid marrow expansion to as much as 30 times the normal level. Both an increase in plasma volume as a result of shunting through expanded marrow and progressive splenomegaly, exacerbate anaemia. Increased erythropoietin synthesis may stimulate the formation of extramedullary erythropoietic tissue, primarily in the thorax and paraspinal region. Marrow expansion also results in characteristic deformities of the skull and face, as well as osteopenia and focal defects in bone mineralization, ***(Rioja et al., 1990; Orvieto et al., 1992)*** and may aggravate a painful periarticular syndrome characterized histologically by microfractures and osteomalacia ***(Gratwick et al., 1978).***

Marrow hyperplasia leads ultimately to increased iron absorption and progressive deposition of iron in tissues.

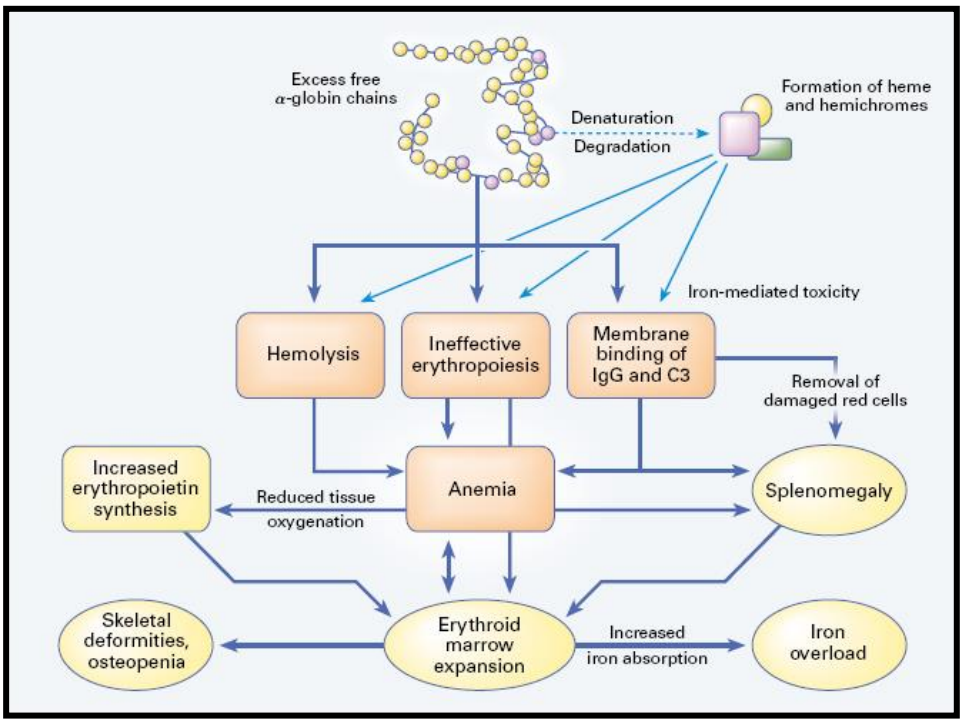


Fig. (**): Effects of Excess Production of Free *a*-Globin Chains. Primary disease processes are indicated in orange, and compensatory mechanisms in yellow. Excess unbound *a*-globin chains and their degradation products precipitate in red-cell precursors, causing defective maturation and ineffective erythropoiesis. Hemolysis, owing to the presence of inclusions in the red cells and damage to their membranes by *a*-globin chains and their degradation products, also contributes to the anemia. Anemia stimulates the synthesis of erythropoietin, leading to an intense proliferation of the ineffective marrow, which in turn causes skeletal deformities and a variety of growth and metabolic abnormalities. The anemia is further exacerbated by hemodilution, caused by the shunting of blood through the expanded marrow, and by splenomegaly resulting from entrapment of abnormal red cells in the spleen. Bone marrow expansion also results in characteristic deformities of the skull and face, severe osteopenia, and increased iron absorption ***(Olivieri, 1999)***.

CLINICAL FORMS OF β-THALASSEMIA

The β-thalassemias include four clinical syndromes of increasing severity: two conditions are generally asymptomatic: the silent carrier state and β-thalassemia trait, and usually result from the inheritance of one mutant β-globin gene, and two require medical management: thalassemia intermedia and thalassemia major. The more severe forms most often result from homozygosity or compound heterozygosity for a mutant β-globin allele and, occasionally, from heterozygosity for dominant mutations. ***(Thein et al., 1990)***

Homozygous or compound heterozygous β-thalassemia usually presents no diagnostic problems. The early onset of anemia, characteristic blood changes, and elevated fetal hemoglobin concentrations are found in no other condition. The diagnosis can be confirmed by the demonstration of the β-thalassemia trait in both parents. This condition is characterized by mild anemia, reduced mean cell volumes and mean cell hemoglobin concentrations ***(Weatherall DJ, Clegg JB. The thalassaemia syndromes. 4th ed. Oxford, England: Blackwell Scientific (in press)*** and elevated concentrations of the normal minor adult component of hemoglobin (usually exceeding 3.5 percent), hemoglobin A_2 ($\alpha_2 \delta_2$).

Thalassemia major and thalassemia intermedia have no specific molecular correlate but encompass a wide spectrum of clinical and laboratory abnormalities ***(Camaschella, et al., 1995)***

Patients referred to as having thalassemia major are usually those who come to medical attention in the first year of life and subsequently require regular transfusions to survive. Those who present later or who seldom need transfusions are said to have thalassemia intermedia ***(Alan R. Liss, 1988).***

After thalassemia is diagnosed, patients who appear not to require immediate transfusion may benefit from a period of observation and folate repletion, particularly if the disease is diagnosed after the age of one year. This approach will allow the identification of patients in whom early growth and development are normal and whose well-compensated anemia may be exacerbated only by infection, folate deficiency, or increasing hypersplenism ***(Camaschella et al., 1995; Alan R. Liss, 1988***; ***Rund et al.,1997; Ho et al., 1998).***

PATHOPHYSIOLOGY OF BONE DISEASE IN THALASSEMIA

OVERVIEW OF BONE FUNCTION AND REMODELLING

The skeleton provides the mechanical support of the body and a reservoir for normal mineral metabolism. Bone is an active tissue constantly being remodelled and changing metabolically through the balanced activity of osteoclasts and osteoblasts on trabecular surfaces. On a microscopic level, bone metabolism always occurs on the surface of the bone at focused sites, each of which is termed a bone metabolism unit (BMU). Osteoclasts and osteoblasts are the cells that carry out bone metabolism at the fundamental BMU site. Therefore, although these cells account for only a small fraction of bone volume, their function is essential ***(Mundy, 1999).***

Bone turnover is always initiated by osteoclasts eroding a mineralized surface. After their activation by different factors (mechanical load, growth factors, hormones and cytokines), osteoclasts are attracted to the new BMU site where they erode the bone matrix, forming a lacunae. Resorption is then halted, followed by the recruitment of osteoblast groups to the outer edge of the erosion cavity that secrete new bone matrix and gradually

fill in the resorption cavity. When the lacuna is filled with osteoid, this newly formed matrix is mineralized with hydroxyapatite, giving the BMU tensile strength ***(Christenson, 1997).***

Osteopenia/osteoporosis and bone mineral density:

Bone mass is the result of a balance between bone formation gained during growth and the subsequent bone loss. Several sensitive techniques are available for the quantitative assessment of the degree of total bone mass. Bone density measurement by dual X-ray absorptiometry (DEXA) of the lumbar spine, femoral neck and forearm is recommended as one of the most reliable and non-invasive technique for the assessment of bone mass ***(Grampp et al., 1997; Cefalu, 2004).***

The lumbar spine, which consist primarily of trabecular bone and wide bone marrow spaces, is most affected. The explanation of this finding is that increased erythropoiesis occurs mainly in the axial skeleton. Moreover, axial BMD increases more rapidly than appendiculer BMD during puberty ***(Rubin et al., 1993).***

According to the World Health Organization ***(WHO, 1994),*** osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequential

increase in fracture risk. The WHO based the diagnosis of postmenopausal osteoporosis on the presence of a BMD T-score that is 2.5 SD or greater below the mean for young women ***(Bonjour et al., 1999; Nguyen et al., 2000).*** The International Society of Clinical Densitometry (ISCD) used the same BMD criteria ***(Writing Group for the ISCD Position Development Conference, 2004)*** for the definition of osteoporosis in males, premenopausal women and children ***(Binkley et al., 2002; Hajjar & Kamel, 2004).***

ACQUIRED FACTORS CONTRIBUTING TO REDUCED BMD IN BETA-THALASSAEMIA

Previous studies have demonstrated that multiple acquired factors are involved in the pathogenesis of osteopenia/ osteoporosis in TM. They include the primary disease, itself causing bone marrow expansion ***(De Sanctis et al., 1998; Mahachoklertwattana et al., 2003a),*** and several secondary factors, such as hormonal deficiency ***(Anapliotou et al., 1995; Jensen et al., 1998; Raiola et al., 2003)***, iron overload ***(Anapliotou et al., 1995***; ***Wonke, 2001),*** desferrioxamine toxicity ***(Olivieri et al., 1992; De Sanctis et al., 1996***; ***Lala et al., 1998; Chan et al., 2002; Di Stefano et al., 2004)***, calcium, zinc and vitamin D deficiencies, and inadequate physical activity ***(Wonke, 1998; Bekheirnia et al., 2004).***

Bone marrow expansion:

Bone marrow expansion due to ineffective erythropoiesis is a typical finding in patients with TM and has been considered as a major cause of bone destruction

(De Sanctis et al., 1998; Mahachoklertwattana et al., 2003b).

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 ***(Vichinsky, 1998***; ***Ma et al., 2003).***

However, no direct correlation was found between serum levels of soluble transferrin receptor (sTFR) and the severity of osteoporosis ***(Wonke et al., 1998).***

Endocrine complications

Hypothyroidism, hypoparathyroidism, diabetes mellitus and mainly hypogonadism (as delayed puberty and/or secondary hypogonadism) are the main causes of osteopenia/osteoporosis in TM ***(Anapliotou et al., 1995***; ***Jensen et al., 1998; Wonke, 1998; Carmina et al., 2004).***

Haemosiderosis of the pituitary gonadotrophic cells and iron deposition in the testes and ovaries are involved in the pathogenesis of endocrine complications in TM ***(Berkovitch et al., 2000***; ***Wonke, 2001).***

Hypogonadism is a well-recognized cause of osteoporosis and osteopenia, not only in patients with TM but also in the general population, and is characterized by high bone turnover with an enhanced resorptive phase ***(Riggs et al., 1998).***

Oestrogen and progesterone appear to inhibit osteoclast activity and promote bone formation ***(Riggs, 2000),*** while testosterone has a direct stimulatory effect on osteoblast proliferation and differentiation ***(Olszynski et al., 2004).***

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 ***(Geusens & Boonen, 2002).***

Several studies have demonstrated a positive correlation between the BMD of the lumbar spine and the IGF-I concentration ***(Mahachoklertwattana et al., 2003a***; ***Rucker et al., 2004).***

It is well documented that the GH-IGF axis is defective in TM. 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 ***(Soliman et al., 1998; Lasco et al., 2002***; ***Morabito et al., 2004; Perifanis et al., 2001).***

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 BMU tensile strength ***(Mahachoklertwattana et al., 2003b)***.

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 ***(De Sanctis et al., 1996; Chan et al., 2002)***.

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 ***(Michelson & Cohen, 1988).***

Vitamin D deficiency is also implicated in the pathogenesis of osteoporosis in TM patients due to the

regulatory effect of vitamin 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 ***(Johnston et al., 1992)***.

However, most studies have failed to show reduced serum levels of 25- hydroxyvitamin D in TM patients. There is adequate data indicating that thalassaemia patients have also zinc deficiency ***(De Virgiliis et al., 1988; Arcasoy et al., 2001)***, which may lower their BMD ***(Bekheirnia et al., 2004).***

It is well-known that zinc and copper deficiencies are associated with osteoporosis ***(Cohen & Roe, 2000);*** thus, zinc supplementation may be administered in thalassemia patients with this trace mineral deficiency.

Physical activity

Patients with TM 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 (1984) 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 ***(Todd & Robinson, 2003).***

Lane et al. (1986) 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 TM 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.

BONE REMODELLING IN THALASSAEMIA-INDUCED STEOPOROSIS

Most of the acquired factors described earlier act mainly through the inhibition of osteoblastic activity. There is evidence of reduced osteoblast function in TM.

Morabito et al. (2004) have shown decreased levels of serum osteocalcin, a protein produced by osteoblasts, in patients with TM. Although osteoblast dysfunction is hitherto thought to be the major pathogenetic mechanism for osteoporosis in TM, there is also evidence of increased osteoclast activation in these patients.

Both ***Dresner Pollack et al. (2000)*** and ***Voskaridou et al. (2003)*** have shown that patients with TM and osteoporosis have elevated markers of bone resorption, such as urinary levels of N-telopeptides of collagen type I (NTX), which is a specific marker of bone resorption, and increased serum levels of tartrate resistant acid phosphatase isoform 5b (TRACP-5b), an enzyme that is produced only by activated osteoclasts ***(Voskaridou et al., 2001, 2003***).

Furthermore, both NTX and TRACP-5b levels correlated with BMD of the lumbar spine in these patients ***(Dresner Pollack et al., 2000***; ***Voskaridou et al., 2003)***. In accordance with these data, ***Lasco et al. (2002)*** and

Morabito et al. (2004) have reported that pyridinoline and deoxypyridinoline, other markers of bone resorption, are increased in patients with TM and osteoporosis compared with normal controls.

The RANK/RANKL/OPG system seems to be of great importance for the activation and proliferation of osteoclast precursors. ***Voskaridou et al. (2003)*** have shown, in 13 patients with TM and osteoporosis, that serum levels of sRANKL were slightly increased, compared with controls, while the OPG serum levels were significantly reduced in this cohort of patients in TM patients and controls, thus the ratio of sRANKL/OPG was increased in TM patients (***Voskaridou et al., 2003***).

In accordance with these results, ***Morabito et al. (2004)*** has shown, in 30 patients with TM and osteoporosis, using the same methods, that sRANKL serum levels were significantly increased in TM patients and controls, while OPG serum levels were reduced, but not significantly compared with controls. Those studies also confirmed that the ratio of sRANKL/OPG is increased in patients with TM and osteoporosis, providing evidence for the role of RANKL/OPG system in the pathogenesis of osteoporosis in thalassaemia. The increase of RANKL, followed by unmodified OPG levels, with the consequent increase of

RANKL/OPG ratio may represent the cause of uncoupling on bone turnover observed in thalassaemia patients.

Morabito et al. (2004) have shown a negative correlation between RANKL and free testosterone in male thalassaemia patients and with 17-b oestradiol in female thalassaemia patients, which suggests that the RANKL/OPG system may be involved in mediating the action of sex steroids on bone. Furthermore, a correlation between the sRANKL/ OPG ratio and erythropoietin levels that has also been reported recently, represents a mechanism through which anaemia, by continuously stimulating the erythropoietin synthesis and determining bone marrow hyperplasia, may increase bone resorption through enhanced RANKL levels ***(Morabito et al., 2004).***

OSTEOPOROSIS

Definition

Ostoporosis is a disease characterized by low bone mass and micro architectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture incidence ***(Melton et al., 1983).***

Pathophysiology of osteoporosis

The pathogenesis of osteoporosis is complex and multifactorial. Alterations in bone density almost certainly represent the final common pathway by which pathologic factors affect risk of future ostoeporotic fracture. The interplay of various physiologic processes, which result in peak bone mass, and maintenance of adult bone mass are the key to understanding the pathogenesis of the disease. Changes in hormonal status, and in particular estradiol, clearly are important factors in both formation and resorption of bone in men and women. Perturbations in growth hormone activity, musculoskeletal function, dietary intake of calcium and vitamin D, and genetic determinants are also important pathogenic factors ***(Rosen et al., 2000).***

Osteoporosis can result from inadequate peak bone mass, excessive bone resorption, or impaired bone formation. These can be affected by genetics, nutrition,

lifestyle, systemic hormones, and local factors. The relative importance of these mechanisms is not fully understood and may differ among patients ***(Raisz et al., 2000).***

Recent research has focused on investigating specific genes that may underlie the development of osteoporosis. Genes that have been examined include those that code for collagen, the estrogen receptor and vitamin D receptor, a swell as for apolipoprotein E ***(Eisman et al., 1999)***.

Risk factors of osteoporosis

Risk factors of osteoporosis can be classified as modifiable or non-modifiable. It is useful to identify modifiable risk factors and to implement change as part of treatment or preventive program ***(WHO, 1994).***

Modifiable risk factors

They include: inadequate exercise, inadequate nutrition (calcium, vitamin D, balanced diet), Medications (e.g., glucocorticoids, excess thyroid hormone, benzodiazepine, anticonvulsants), smoking, and excess alcohol intake ***(Klibanski et al., 2000).***

Non modifiable risk factors

They include: age, gender, early menopause, genetics, race and ethnic background ***(Klibanski et al., 2000).***

Age:

Both men and women experience an age related decline in bone mineral density starting in midlife ***(Anne, 2001).***

Gender:

Peak bone mass is greater in men than women because men have bigger bones. While peak volumetric bone density (that is, the amount of bone matrix in the bone), is the same in men and in women ***(Stenstron et al., 2001).***

As boys pass through puberty, their growth is associated with testosterone mediated increase in periosteal apposition, which enlarges the diameter of the bone, and is responsible for sex difference in bone size ***(Stenstron et al., 2001).***

Race:

The prevalence of osteoporosis and the incidence of fracture vary by race, white postmenopausal women experience almost three quarters of all hip fractures and have the highest age adjusted incidence of fracture ***(Anne, 2001).***

Genetics:

The importance of genetic factors is illustrated by the results of twin and family studies, which have indicated that hereditary accounts for between 50% and 85% of the variance in bone mineral density, depending on the site examined ***(McGuigan et al., 2002).***

Nutrition

David and coworkers (2000) found that dietary calcium is an important determinant of peak bone mas, as increasing daily calcium through diet or supplementation has been found to increase bone mass.

The new dietary reference intake for calcium (mg/d) are 500 for children aged 1 to 3 years, 800 for children aged 4 to 8 years, 1300 for adolescents aged 9 to 18 years, 1000 for adults aged 19 to 50 years, and 1200 for those more than 50 years of age ***(Yates et al., 1998).***

Millar and cowrokers (2001) found that men with calcium intake of less than 600 mg/day had lower BMD than those with intakes above 600 mg/day.

Vitamin D plays a vital role in regulation of calcium and phosphorous metabolism, promoting calcium absorption from the gut and kidney tubules. Supplementation trials have shown that vitamin D improve

calcium absorption, lower PTH levels, and reduce winter time bone loss in postmenopausal women ***(New, 1999).***

Smoking

Smoking has been reported in both cross sectional and longitudinal studies to be a risk factor for vertebral, forearm, and hip fracture and to be associated with lower BMD ***(Aloia et al., 1995).***

The adverse effect of smoking on bone is likely to be mediated through changes in endogenous estrogen metabolism, estrogen production is decreased, and metabolic clearance has been increased ***(Aloia et al., 1995).***

Exercise

Exercise increase peak bone mass, the increased BMD is likely to be the result of both large bone size (periosteal apposition) and greater volumetric density (change in endocortical modelling and remodeling that produce a thicker cortex in the bigger limb) ***(Brandy et al., 1998).***

Exercise before and after puberty produces large change in BMD; but in adulthood the effects of exercise are less than those observed when exercise is undertaken during growth. The effects in adulthood are small, 1% to

3% increases in BMD have been reported, and these may be lost when exercise stops ***(Heinonen et al., 1996).***

Types of osteoporosis

A) Primary osteoporosis

There are three primary kinds of osteoporosis: type I, type II and idiopathic osteoporosis:

Type I or High turnover osteoporosis

Occurs in 5% to 20% of women, most often between the age of 50 to 75 because of the sudden postmenopausal decrease in estrogen levels, which results in a rapid depletion of a calcium from the skeleton. It is associated with fractures that occur when the vertebrae compress together causing collapse of the spine and with fractures of the hip, wrist, or forearm caused by falls or minor accidents. Type I accounts for the significantly grater risk for osteoporosis in women than in men ***(Dempster et al., 1993).***

Type II or low turnover osteoporosis

Also known as age related or senile osteoporosis and results when the process of resorption and formation of bone are no longer coordinated, and bone breakdown overcomes bone building (this occurs with ageing every one to some degree). Type II osteoporosis affects both men and women and is primarily associated with age and spinal fractures. Older women can have both type I and type II

osteoporosis. The determining factors for the actual existence of osteoporosis, whether type I or type actual II, is the amount of calcium left in the skeleton ***(Dempster et al., 1993).***

Idiopathic osteoporosis:

A rare disorder of unknown cause that affects premenopausal women and men who are middle aged or younger ***(Murray et al., 1999).***

Someone has exceptionally dense bones to begin with; probably never lose enough calcium to reach the point where osteoporosis occurs, whereas a person who has low bone density could easily develop osteoporosis despite losing only a relatively small amount of calcium ***(Notelovitz et al., 1993).***

B- Secondary osteoporsis

Osteoporosis that has an identifiable cause, other than menopause or aging, is known as secondary osteoporosis. For example, endocrine disorders such as hyperthyroidism can disrupt the balance between bone formation and resorption. Medical treatments such as long term corticosteroid or heparin therapy may have a similar effect. Alcoholism and pregnancy may also cause osteoporosis ***(Kanis et al., 1997).***

Secondary osteoprosis may be caused by bone disease as a result of paralysis or other conditions, as

hormonal and nutritional disorders including anorexia nervosa, malabosrption syndrome, multiple myeloma, osteomalacia and Paget's disease ***(Klibanski et al., 2000).***

Table (1): Secondary causes of osteoporosis

Genetic (congenital)	Osteogenesis imperfecta Gonadal dysgenesis Turner syndrome Klinefelter syndrome Hypophosphatasia Homocystinuria Mucopolysaccharidosis Gaucher disease Sickle-cell anemia Thalassemia Hemophilia
Endocrine	Hyperthyroidism Hyperparathyroidism Cushing syndrome Acromegaly Estrogen deficiency Hypogonadism Diabetes mellitus Pregnancy
Deficiency states	Scurvy Malnutrition Anorexia nervosa Protein deficiency Alcoholism Liver disease
Neoplastic	Myeloma Leukemia Lymphoma Metastatic disease
Iatrogenic	Heparin-induced Steroid-induced Dilantin induced
Miscellaneous	Amyloidosis Ochronosis Immobility Weightlessness

www.e-medicine.com/osteoporosis/secondary

Diagnosis of osteoporosis

Early diagnosis is the key to the prevention and treatment of osteoporosis. A healthy skeleton has intrinsic properties that confer strength to resist fracture under ordinary stress. Some of the properties that confer strength and fracture resistance include: bone mass or density and bone quality determined by skeletal composition, fine structure and spatial organization, genometric properties, and rate of remodelling. The current approach to early diagnosis of osteoporosis is based on the measurement of bone mass or bone mineral density (BMD) ***(Joanne and Kamal, 2000).***

Low bone mass is the single most accurate predictor of increased fracture risk. BMD accounts for 70% to 80% of the future risk in older white women and is a far better predictor of osteoporosis than hypertension is for stroke or total cholesterol is for cardiovascular events as quantification of bone quality will help refine our ability to identify patients at risk. BMD can be measured at a variety of skeletal sites using several different methods that have been approved by Food and Drug Administration (FDA) ***(Joanne and Kamal, 2000).***

A diagnosis of osteoporosis is usually made by evaluating your medical history and by a physical

examination. Tests may be done to diagnose or to screen (early detection) for osteoporosis:

1. Bone density measurement (bone mineral density tests) for screening or diagnosis the dual energy X-ray absorptiometry (DEXA) is often used.

2. Ultrasound of bone for screen of osteoporosis.

3. X-ray is not done to check for osteoporosis. As much as 25% to 30% of bone is lost before the loss can be seen on an X-ray ***(Nightingale et al., 1998).***

The approach to the diagnosis of osteoporosis has undergone a radical change in recent years. X-ray technology was the best non invasive method for measuring the micro architectural deterioration, which thus came to define the disease. With the advent of new technology and a more logical approach to risk evaluation, this simple approach is no longer appropriate with a bone density measure to achieve a prediction of future fracture probability ***(Prince et al., 2001).***

The dual energy X-ray absorptiometry (DEXA)

DEXA is currently the preferred method for BMD measurement because of its accuracy, precision, and low radiation exposure. DEXA employs 2 x ray beams of different energy levels that are absorbed differently by

mineral and soft tissue. By combining the data, DEXA can accurately measure bone within soft tissue. DEXA and results are reported as a real density in units of g/cm^2. Results can also be expressed in terms of the number of standard deviations (SD) below average young adult bone mass (commonly referred to as T-scores) ***(Kanis et al., 1994).***

DEXA of the spine and hip is currently believed to be the optimal method and has become widely available. According to a 1997 NOF survey, 89% of bone density tests performed in the United states that year used DEXA. The results of DEXA represent a composite measure of both cortical and trabecular bone and are reported as an areal density in g/cm^2 ***(Blake et al., 1999).***

Bone density reports:

Bone density reports can provide considerable information, including printout of the images obtained and guidance for interpreting the results. A thorough report should include basic information about the patient, indication for the study, the type of instrument used; and the sites measured; comments on the validity of the study (e.g., region of interest, positioning, presence of distortions); results, including BMD. T-score and Z- score; interpretations of the data according to WHO criteria and comment about any diagnostic discordance between sites:

comparison with previous values, with comments on the significance of the gain or loss based on the precision of the method; suggestions for evaluation with conventional radiographs if distortions or questionable changes are noted; and recommendations for follow up testing intervals if sufficient clinical information is provided ***(Blake et al., 1999).***

All bone density measurements report an absolute value (that will differ in amount and units depending on the measurement technique used) and a T and Z scores for that value. A T-score represent the number of SDs above or below the mean bone density value for a gender-matched healthy young adult population (20 to 40 years of age). Ideally then, the T-score represents how well an individual's bone density compares to an optimal peak bone density for that particular test. The T-score is used to identify patients at risk and those requiring intervention based on criteria set by the WHO and the national osteoporosis foundation (NOF). A Z-score is used to identify patients at risk and those requiring intervention based on criteria set by the WHO and the National osteoporosis foundation (NOF). A Z-score represents the number of SDs above or below the bone density value for an age, weight, gender, and ethnicity matched adult population. This measure provides a comparison to age matched individuals and the amount of expected loss with

aging. A significantly lower than expected Z-score (Z < -1.0) implied a degree of bone loss exceeding what can be expected on the basis of age and body size and suggests that secondary causes of bone loss should be evaluated. Z-scores are also useful in evaluating children and adolescents in whom the T-score would below as they have not yet reach peak bone mass ***(Blake et al., 1999).***

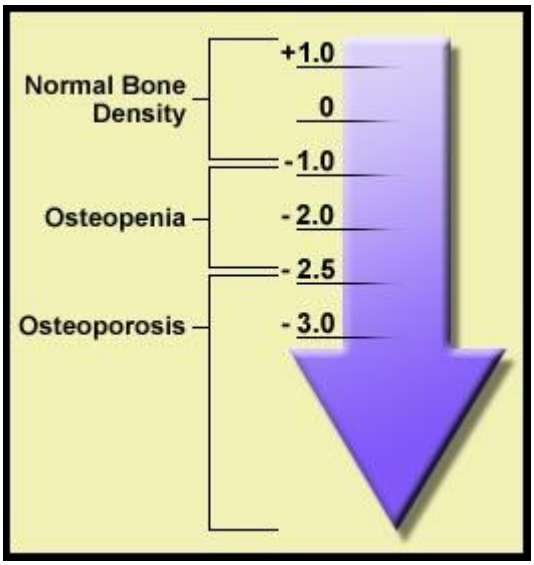
Bone density reports refer to Z-score or T-score. The Z-score (age matched control) compares the patient with a population adjusted for age, sex and weight; the T-score (young normal control) compares the patient with a sex matched population at peak bone mass. Osteopenia is defined as a bone mineral density (BMD) between one standard deviation and 2.5 standard deviations below the young normal control average (0.648-0.833 g/cm^2 for total hip BMD). Osteoporosis is characterized by a BMD lower than 2.5 standard deviations below the young normal control (< 0.648 g/cm^2 for total hip BMD). Established osteoporosis is a bone density lower than 2.5 standard deviations, in the presence of one or more fragility fracture. Based on this definition, clinicians may consider a bone to be osteoporotic if its density has sufficiently decreased as to not be able to withstands the traumas of normal activities, or if there has already been a spontaneous or non traumatic fracture ***(Shechar et al., 1998).***

Table (2): The World Health Organization and National Osteoporosis Foundation criteria for diagnosis and treatment of osteoporosis

Normal	BMC or BMD T-score > -1
Osteopenia	BMC or BMD T-score < -1 but > -2.5
Osteoporosis	BMC or BMD T-score < -2.5
Severe osteoporosis	BMC or BMD T-score < -2.5 + fragility fracture

BMC = Bone mineral content
BMD = Bone mineral density

These criteria are approved in ***The World Congress on Osteoporosis 2000***



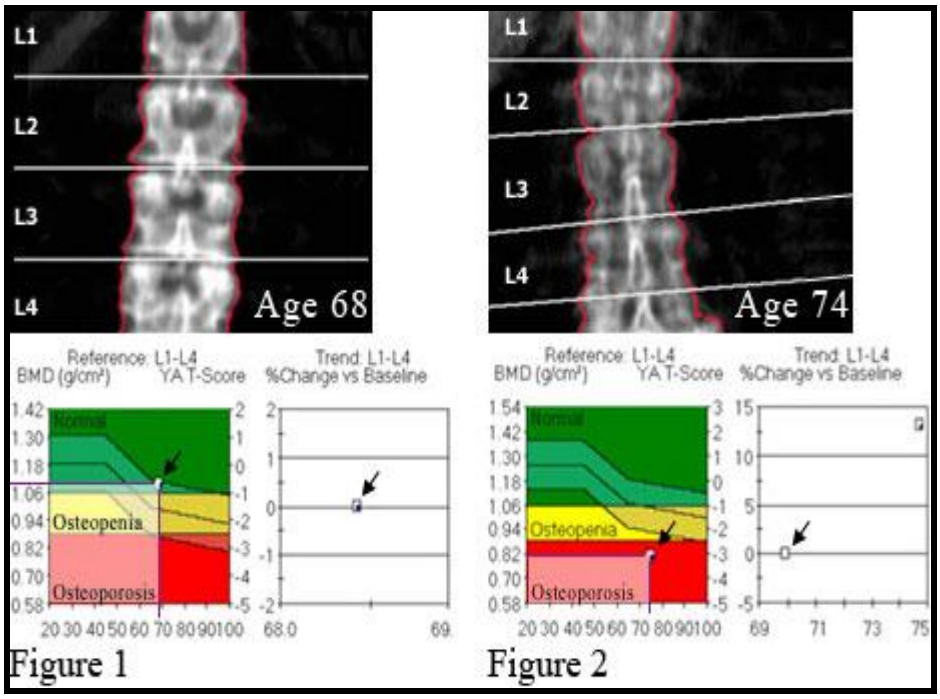


Fig. (**): Shows a bone mineral density (BMD) test of a healthy older woman. The X-ray shows a healthy spine. The BMD is determined by the X-ray. The graph shows the bone mineral density number in the green zone (normal). Figure 2 shows a BMD test of an older woman with osteoporosis. The X-ray shows weaker bones. The graph shows the bone mineral density number in the red zone (osteoporosis), placing her at much greater risk for broken bones (fractures) ***(Carla, 2004).***

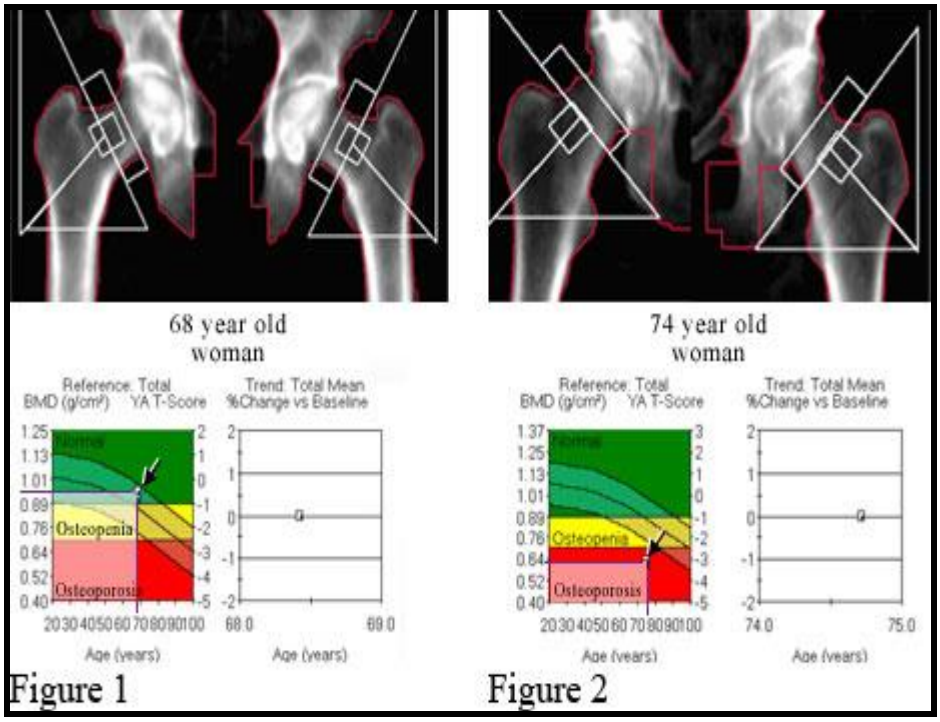


Fig. (**): Shows a bone mineral density (BMD) test of a healthy older woman. The X-ray shows normal hipbones. The graph shows the bone mineral density number in the green zone (normal). Figure 2 shows a BMD test of an older woman with osteoporosis. The X-ray shows weaker hipbones. The graph shows the bone mineral density number in the red zone (osteoporosis), placing her at much greater risk for broken bones (fractures) ***(Carla, 2004)***.

Biochemical markers in diagnosis of osteoporosis

Bone mass or BMD determines approximately 70% of the fracture resistance of bone. The quality of the bone is also important. The amount of remodelling at a given time may be an important factor in bone quality. One of the most important advances in osteoporosis has been the development of biochemical markers to assess the rate of bone remodelling. The markers evaluate a dynamic process of bone remodelling in contrast to the static measure of bone density. Proteins released from osteoblasts can be used to assess bone formation, and products of collagen breakdown can be used to assess bone resorption ***(Lindsay et al., 1998).***

The most widely available markers of resorption are the cross links of type I collagen. When collagen is degraded by osteoclasts, the cross links of collagen and their adjacent peptides are released into the circulation and excreted in the urine (e.g., urinary N-telopeptide cross links of collagen, and C-telopeptide cross links of collagen). The markers of formation include osteocalcin, bone specific alkaline phosphate, and procollagen peptides. Because bone resorption and formation are coupled, the biochemical markers of turnover change in sequence. In response to therapy, changes in bone resorption markers precede those of bone formation. Studies have consistently demonstrated

an inverse correlation between bone turnover as measured by these markers and BMD. In addition, the rate of bone turnover may be independent risk factor for osteoporosis. However, due to the day to day variability of marker assay sand the broad range of normal values, biochemical bone markers individually or in combination are not specific enough to diagnose osteoporosis ***(Lindsay et al., 1998).***

High levels of bone turnover have been associated with more rapid bone loss. Most effective anti-resorptive therapy can reduce pretreatment marker level by 40% to 70% within 3 to 6 months of initiation of treatment. The magnitude of decline of the resorption markers is correlated with the gains in BMD. Thus, bone markers may be useful in monitoring the effect of therapy at an earlier time point as compared with BMD. As research on bone markers develops, we can expect a better understanding of their role in individual patient diagnosis and treatment monitoring ***(Rosen et al., 1996).***

Sex hormones and osteoporosis

Bone mass changes over the life span of an individual. Bone mass increases rapidly from the time of puberty until the mid 20s to mid 30s, at which time peak bone mass is reached ***(Greenspan et al., 1994).***

The imbalance between bone formation and resorption in the remodelling cycle is the morphologic basis

of bone loss. The role of sex steroids in this process is not completely understood, although we do know from animal studies that loss of sex steroids (which occurs with aging, menopause and castration) increases the rate of formation of osteoclasts and osteoblasts in bone marrow by up regulating the biosynthesis and activity of cytokines involved in osteoclastogenesis and osteoblastogenesis ***(The World Congress on Osteoporosis, 2000).***

It has been suggested that estrogen deficiency increases bone resorption partly by causing increased paracrine production of bone resorbing cytokines ***(Horowitz et al., 1993).*** These cytokines appear to increase bone resorption by stimulating the development of osteoclast progenitors and increasing the activity of mature osteoclasts ***(Zheng et al., 1997).***

Estrogen replacement therapy remains an important choice for the treatment and prevention of osteoporosis; estrogen replacement therapy is associated with 30% to 70% reduction in hip fracture incidence. Multiple studies have demonstrated that postmenopausal estrogen use will prevent bone loss at the hip and spine when initiated within 10 years of menopause ***(Lindsay et al., 1996).***

Androgens are important determinants for peak bone mass in men. Bone accretion is closely related to sexual maturity in men who have abnormal puberty or delayed puberty have reduced bone mass. In addition, men with

idiopathic hypogonadotropic hypogonadism have bone mineral density that is more than two standard deviations below the normal mean. Similarly, men had a 7% per year decrease in vertebral BMD in the 2 years following castration ***(Anne and Karen, 2000).***

There is growing evidence that estrogen has an important role in maintaining bone mass in men as in women. Gannari and colleagues studied 240 men classified as osteoporotic, osteopenic, or normal at the proximal femur and revealed that estradiol levels were higher in the normal subjects than in the other 2 groups. Testosterone levels were normal in all groups ***(Gennari et al., 2000).***

OSTEOPOROSIS IN THALASSEMIA

β-thalassemia major is associated with significant bone disease ***(Heaney and Mathkovic, 1992)***. The osteoporosis seen in the thalassemia major is of multifactorial origin. Many factors could be implicated in the mechanism of osteoporosis other than expansion of the marrow cavity caused by bone marrow hyperplasia resulting in thinning of the adjacent bone ***(Giurzio et al., 1991).***

Defective growth affecting both height and weight of thalassemia patient may be due to a significant deficiency of circulating IGF-1 and IGFBP, which play a major role in stimulating linear growth and bone mineralization ***(Saglamer et al., 1992).***

Compromised nutritional status and increased energy expenditure in thalassemic patients can also negatively affect negatively bone mineralization by their important influence on growth hormones GH/IGF-1/IGFBP3; axis which in turn are important in maintenance of normal peak bone mass ***(Fuchs et al., 1996).***

Delayed or lack of pubertal development and decreased sex steroid secretion in thalassemia patients worsen the degree of osteoporosis, as reported that

hypgoandotrophic hypogonadism is the commonest endocrinopathy occurring in thalassemic patients and is responsible for osteopenia in as much as 80% of thalassemic patients ***(Brnadle et al., 1996).***

Development of diabetes mellitus in thalassemic patients also affects bone mineralization. As the reported prevalence of diabetes mellitus in treated β-thalassemic patients is about 16% and the incidence of impaired glucose tolerance is approximately 60%, islet cell destruction secondary to iron overload and or exhaustion of β-cells due to chronic insulin resistance and liver derangement are possible pathogenic factors of DM in thalassemic patients ***(Soliman et al., 1996).***

Chelation therapy leads to copper deficiency in thalassemic patients, and copper is important as an enzyme cofactor in bone formation, and its deficiency results in abnormal collagen formation ***(Rodda, 1994).***

Giardina and coworker (1995) measured bone mineral content of well chelated and transfused thalassemic patients and found a high incidence of osteoporosis of the spine in both sexes. The severity of osteoporosis increased with age and young adult patients attained a spinal bone mineral density far below the age matched controls. There was also a high incidence of vertebral fractures among these patients. ***Jensen and cowrorkers (1998)*** studied 82

patients aged 12-43 years of both sexes with β-thalassemia major receiving optimal treatment, osteoporosis was present in 42%. In male patients both the lumbar vertebrae and femur were involved, whereas in females, osteoporosis affected mainly the spine. Low bone mineral density was found in children as early as 12 years of age, suggesting that the peak bone mass is also adversely affected.

Prevalence of osteoporosis in thalassemia

Wonke et al. (1998) reported a high incidence of osteopenia and osteoporosis in patients with thalassemia major. These bone changes were more severe in males than females, in those with diabetes mellitus and those with hypogonadal hypogonadism.

Ruggero and De Santi (1998) determined the prevalence of fracture in transfusion dependant thalassemic patients in Italy. Many patients showed multiple and recurrent fractures. Children and adolescents sustained fractures more frequently than adults. The majority of observed patients sustained fractures because of moderate or mild trauma. Their results seem to indicate that haemoglobin pretransfusion levels over 9 or 10 gm% and serum ferritin level under 1.000 ng/ml cannot always prevent the risk of fracture.

The study of ***Jensen et al. (1998)*** showed a high prevalence of low bone mass among thalassemic major patients. Bone density scans were performed in patients with transfusion dependant β-thalassemia. Factors known to be associated with low bone mass such as gender, endocrine disorders and lifestyle activities, together with factors specific to the thalassemia and its management, were included.

They found that there were no association between the bone mineral density measurement and the hematological characteristic or treatment details of these patients. They concluded that severely low and low bone mass are common findings in patients with β- thalassemia major despite optimal transfusion and iron chelation.

Presentation of osteoporosis in thalassemic patients

Bone aches

Angastiniotis et al. (1998) reported that an increasing number of adult thalassemics have been complaining of aches and pains of varying degrees of severity. In a minority the pains are debilitating and there is stiffness in movement. Their conclusion was that patients who were late in receiving blood and especially those with thalassemia intermedia had bone mass density (BMD)

below two standard deviations from the mean for the normal population especially in the lumbar spine.).

These patients had a more expanded bone marrow with pressure on cortical bone, which caused pain in several cases. An attempt was made to reduce marrow hyperplasia by using hydroxyurea, results showed relief of pain.

Short stature

With modern treatment and longer survival of patients with homozygous beta-thalassemia, endocrine dysfunction assumed greater importance. Short stature, delayed puberty and hypogonadism are major problems in both adolescent and adult patients. Growth failure had been attributed to GH deficiency (hypothalamic or pituitary), hypothyroidism, delayed sexual maturation, hypogonadism, DM, zinc deficiency, low Hb levels, bone disorders and desferrioxamine toxicity ***(Theodoridis et al., 1998).***

Hypogonaidsm and hypocorticolism form important causes for morbidity in thalassemic children. Thalassemic patients in developing countries may be at risk for endocrine deficiencies at younger ages due to suboptimal iron chelation ***(Gulati et al., 2000).***

Growth retardation in iron overloaded patients is the result of growth hormone deficiency in up to 30% of patients. Height gain can be successfully achieved in these patients with growth hormone treatment ***(Wonke et al., 1998).***

Bone deformities

The ineffective erythropoiesis in thalassemia, increased production of erythropoietin and expansion of the bone marrow to 15-30 times normal, causes distortion and fragility of the bones. The skull of the child is elongated (tower skull) with frontal and posterior bossing, hypertrophy of the maxilla. The study revealed that the spinal deformities in thalassemia represent a distinct type of scoliosis regarding prevalence, curve pattern and etiology ***(Korovessis, 1996).***

Radiological finding

Radiological changes of thalassemia deformities are striking. In the skull there is osteopenia, with widening of the diploic space, thinning or virtual disappearance of the outer table. New bone formation to the inner table (hair on end) appearance, absence of paranasal sinuses and pneumatization with solitary or multiple circumscribed osteolytic areas of the skull ***(Orzincolo et al., 1998).***

There is thinning of the long bones with trabeculation and segmental obliteration of the humeral or femoral epiphysial lines. Extramedullary hemopoiesis may produce bizarre radiological changes. The commonest site for extramedullary hemopoiesis are the spleen, liver chest, less common sites are paravertebral masses and brain lesions.

As the ribs contain hempopoietic marrow at all ages, overactive marrow results in osteoporosis of the ribs, localized lucencies, cortical erosions and rib deformities ***(Lawson et al., 1981).***

Echography could be useful tool as a non invasive follow up technique and it is suggested that the patients should be subjected to this test at least once a year to monitor subsequent liver and spleen damage caused by excess iron and to evaluate the exact role of chelating agents over a longer duration ***(Sheir et al., 1988).***

CONTROL AND PREVENTION OF BETA-THALASSEMIA

Beta thalassemia is a mendilian recessive disorder; the economic and social cost of the disease is high due to patients' life long need for monthly blood transfusions and treatment with iron chelating agent as desferrioxamine. If there is no concomitant reduction in the number of new thalassemia births, there will be accumulative increase in the number requiring treatment. The frequency and severity, and the economic and social costs of thalassemia, support the case for the introduction of carrier screening and counseling program ***(Perera et al., 2000).***

Screening for beta-thalassemia:

The goal of thalassemia screening is the identification, prior to the conception or birth of an affected child, of couples where both parents are thalassemia carriers. When both parents are identified as carriers for beta-thalassemia, the risk of having a fetus who is homozygous or compound heterozygous for the abnormal gene is 25% ***(Yong et al., 1999).***

Inheritance of a single beta thalassemia allele results in mild hypochromic, microcytic anemia. If microcytosis is detected by electronic cell counters, HbA**2** determination is

done, if elevated, and the HbA**2**/HbA**l** ratio is 1:20 instead of the normal 1:40, there is a probably of beta thalassemia trait. If HbA**2** is normal/ consider irondeficiency, α-thalassemia, or rare types of β- thalassemia. RDW (red cell distribution width) which detect the heterogeneity of red size and anisocytosis in the blood smear are more sensitive indicators than MCV to establish the possible origin of microcytic hypochromic anemia. Both should be used together in early diagnosis ***(Romero et al.,1999).***

The RDW is high in iron deficiency, but in most other conditions with microcytosis RDW is normal ***(Romero et al.,1999).***

1) <u>Prenatal diagnosis:</u>

Testing for hemoglobin disorder ranges from detection of globin proteins produced by the affected genes by isoelectric focusing (IEF), electrophoresis, high performance liquid chromatography (HPLC) and mass spectroscopy, to gene analysis by restriction endo-nuclease cleavage, specific oligonucleotide hybridization, and DNA sequencing ***(Larad et al., 2000).***

The most reliable methods for prenatal diagnosis are based on identification of the abnormal gene by direct DNA analysis ***(Laradi et al., 2000),*** which is perpetrated from amniocytes obtained at 15 to 17 weeks of gestation or from chorionic villus sampling at 9 to 11 weeks ***(Rhoads, 1989)***.

TREATMENT OF BETA THALASSEMIA

Treatment of homozygous β-thalassemia with intensive red blood cell transfusion and iron chelation can control its major complications however thalassemia remains a progressive condition that is frequently lethal in the absence of gene therapy. Till now, bone marrow transplantation provides the only available rational treatment for eradication of the β-thalassemic clone. ***(Lucarelli et al., 1999).***

A) Supportive Treatment:

I. Blood transfusion:

The development of better transfusion regimens combined with effective chelation therapy has completely transformed the outlook for patients with severe (β-thalassemia ***(Olivieri and Weatherall, 1998).***

The goals of transfusion include correction of anemia, suppression of erythropoiesis, (which can be measured by the degree of decrement of sTFR concentration reflects bone marrow erythropoietic activity)***(Choi et al., 2000)*** and inhibition of increased gastrointestinal absorption of iron.

The aim of hypertransfusion regimen program is to maintain Hb level above 10 g/dl while the aim of super-transfusion program is to maintain hemoglobin above 12

g/dL which are associated with iron loading, but the amelioration of sever anemia is associated with significant reduction of GIT iron absorption, this offset to some degree the increased transfusional ironload ***(Olivieriand Brittenhan, 1997).***

Neocyte/gerocyte exchange transfusions have been suggested as a method for further reducing iron over load. The method attempts to remove nearly senescent red cells before they are catablolized. Unfortunately, these methods are extraordinarily expensive ***(Forget, 2000).***

II. Iron chelation therapy:

Most patients with thalassemia major die from complications of iron overload. With a total body iron burden of 40gm, organ function begins to fail, and at 60 gm or more, intractable cardiac failure can occur ***(Lukens,1999).***

Iron chelating agents are administered either by SC, IV or oral methods. As a result of programs of desferrioxamine therapy the prognosis for patients in countries able to afford this therapy has greatly improved in contrast to prognosis for patients in developing countries where widespread implementation of this regimen is still problematic ***(Swart et al.,1999).***

A) Parental administration of the iron chelating drugs:

Desferrioxamine (DFO) (Desferal) administration:

Desferrioxamine (DFX is an iron chelation agent widely used in the treatment of transfusional iron overload in patients with thalassemia major and other severe refractory anemias ***(Kyriakou et al., 1998).***

Desferrioxamine mesylate (Desferal, DFO) a siderophore isolated from cultures of streptomyes pilowsus, was introduced in 1960 as a nearly specific iron chelating agent with low toxicity ***(Hoffmann et al., 1988).***

The optimal age for starting desferrioxamine has not been established. Some reports describe success in children as young as 2-4 years ***(Fargion et al., 1982)***, but there may be an adverse effect on growth. Many centers wait until the patient is 5 - 6 years old, when significant iron excretion can be accomplished and patient cooperation is better ***(Forget, 2000).***

The optimal age for starting chelation therapy is either after 10 transfusions if serum ferritin ≥ 1000 ng/dl (***Ragab, 2002).***

These criteria are reached at about 3 years of age ***(Olivieri and Brittenham, 1997)***. When given before 3 years of age/ desferrioxamine must be given at a reduced

dose (20 to 30 mg/kg/day), to prevent the drug's adverse effect on linear growth. Auditary and bone toxicity After 3 years of age, the dose of desferrioxamine is about 40 to 50 mg/kg/day ***(Richardson and Ponka, 1998).***

B) Oral administration of chelating therapy:

(Deferiprone)

Richardson (1999) had reported that the development of an orally effective iron (Fe) chelator for the treatment of iron overload diseases such as beta thalassemia has been a difficult challenge. Even though the drug in current clinical use, desferrioxamine (DFO) is efficient, patients suffer from it not being orally effective and the requirement of long subcutaneous infusion to mobilize sufficient quantities of iron. In addition, DFO is very expensive which precludes its use for the treatment of the world's thalassemic population. Therefore, the development of economical and orally effective iron chelator is of great importance.

In (1987), deferiprone was first reported to be an orally active iron chelating drug in humans ***(Kontoghiorghes et al., 1987).***

Since then, it has under gone numerous clinical trials for the treatment of transfusional iron overload, particularly thalassemia major ***(Hershko and Hoffbrand, 1995).***

Rombos et al. (2000) conducted a study in order to determine the efficacy and safety of deferiprone in Greek thalassemic patients. They found that all patients tolerated the deferiprone well; serum ferritin declined within 4-6 months in most of the patients. The results suggested that deferiprone is a rather safe drug which decreases iron overload without causing considerable side effects in Greek thatassemics. Gastrointestinal disturbances, arthralgia, agranulocytosis and raised levels of transaminases in the liver are some of its known side effects (***Cohen et al., 2000)***.

Despite the introduction of the parenteral iron chelator desferrioxamine more than 30 years ago, 50% of patients of thalassemia major die before the age of 35 years, predominantly from iron induced heart failure. The only alternative treatment is oral deferiprone ***(Anderson et al., 2003).*** Several new drugs are now under trial**.**

III. Splenectomy:

Al-Salem (1999) studied and analyzed the causes, etiology, morbidity, mortality and therapeutic values of splenectomy performed for massive splenomegaly in children. The indications of splenectomy were hypersplenism, and splenic abscess. The transfusion requirements in the patients with beta thalassaemia major decrease markedly post-operatively from 18 transfusion /

year to only 4 transfusion / year and for those with hypersplenism, there was a marked improvement in their blood parameters following splenectomy.

Because of the risk of infection (post-splenectomy syndrome), splenectomy, should usually be delayed till the age of 5 or 6 years ***(Forget, 2000)***.

At least 2-3 weeks prior to splenectomy, polyvalent anti- neumococcal, anti-meningococcal, and anti-hemophilus influenza type B vaccines should be administered ***(Atkinson and Murphy, 1996)***.

Oral penicillin therapy, used as prophylaxis against post- splenectomy infection is now generally given to splenectomized patients with thalassaemia ***(Olivieri and Weatherall, 1998)***.

After splenectomy, striking thrombocytosis may occur. Increased numbers of nucleated red cells appear in the blood, and the presence of many red cell containing inclusion bodies composed of precipitated α-globin chains can be demonstrated ***(Forget, 2000)***.

IV. Vitamin supplementation:

Folic acid:

Folic acid supplements are added frequently to meet the needs of increased erythropoiesis ***(Alter, 2002).***

Vitamin E:

It has long been considered to be a potent antioxidant that protects membrane lipids from attack by free radicals formed when excess iron is present ***(Nathan and* Orkin, *1998).***

B) Curative Treatment:

I. Stem cell transplantation:

Stem cell sources:

1- Bone marrow stem cells:

Bone marrow transplantation in thalassemia represents the only form of radical cure of this disease ***(Giardinic and Lucarelli, 1999).*** Bone marrow transplantation from HLA-identical donors has been successfully performed worldwide in 1000 patients with severe beta thalassemia ***(Giardini, 1997).***

Outcomes after transplantation are greatly influenced by the presence of hepatomegaly, portal fibrosis and ineffective chelating therapy before transplantation ***(Nelson, 2000)***.

Children without any of these risk factors have rates of survival and disease free survival exceeding 90% three years after transplantation (those identified as class 1). By

contrast, in those with all three risk factors, (class 3 patients), the rates are approximately *60%. In* patients with hepatomegaly or portal fibrosis (class 2), the event free survival rate is approximately 80% ***(Olivieri and Weatherall, 1998)***.

2- Peripheral blood stem cell:

During the 1990, peripheral blood stem cells have evolved as the preferred source of hematopoietic stem cell for transplantation ***(Lena et al., 1996).***

Advantages of peripheral blood stem cell transplant include rapid and dural trilineage hemopoietic engraftment and improved tolerance of harvesting procedure ***(Bensinger et al., 2001)***.

One of the main concerns is the risk of increasing acute and chronic GVHD (graft versus host disease) because of the high number of infused peripheral blood lymphocyte. However preliminary results do not show increased incidence or severity of acute GVHD in HLA identical PBSC (peripheral blood stem cell) transplants ***(Bensinger et al., 2001)***.

II. Gene therapy:

Beta-thalassemia is probably the most extensively studied genetic disease ***(Cao and Moip, 2000)***.

Gene therapy aims at replacing a single deficient gene by a functional gene introduced into an autologus and therefore unrejectable tissue. The hemopoietic stem cells are excellent target for gene transfer, since the procurement, exvivo/ manipulation and reimplantation of these cells are easily performed ***(Cohen, 1991)***.

C) New in the Treatment of Beta-Thalassemia:

Therapy of thalassemia has in the past been confined to transfusion and chelation. Recently novel modes of therapy have been developed for thalassemia based on the pathophysiology and molecular pathology of the disease ***(Rund and Rachmilewitz, 2000)***.

Re-activation of the fetal globin genes is the most realistic approach to correct the deranged pathophysiology of the hemoglobinopathies because the presence of gamma-chains can neutralize the toxic effects of the unbound alpha-globin chains in the beta thalassemias and inhibit the polymerization of Hb S in sickle cell syndromes ***(Loukopoulos, 1998)***.

Examples of the first group include the butyric acid and its derivatives and azacytidine. The second group includes erythropoietin and hydroxyurea as the main representative.

A) Butyrate therapy:

Butyrate and related drugs stimulate expression of the gamma-globin genes to adequately balance the excess

alpha-globin chains. ***Reich et al., (2000)*** concluded that butyrate therapy prolongs transfusion intervals.

B) Azacytidine therapy:

Administration of intravenous 5-azacytidine was associated with increase in the hemoglobin concentration in a few patients ***(Lowrey et al., 1982)***. The potential toxicity of the drug later shifted interest to less toxic alternative therapy with hydroxyurea ***(Arruda et al., 1997)***.

C) Hydroxyurea therapy:

Zeng et al. (1995) had reported that the hydroxyurea may have a more general role in augmenting globin synthesis, including beta-globin in some thalassemic patients who maintained the capacity to express normal beta globins, in addition to its known effects in stimulating gamma-globin production. Hydroxyurea (HU) is an antimetabolite drug. It is an effective agent with low toxicity. By activating gamma-globin gene, it has been shown to enhance HbF synthesis in experimental animal and in patients with sickle cell anemia.

Koren et al. (1999) study evaluated the efficacy of hydroxyurea treatment in the prevention of vaso- occlusive crises among children and teen-agers with severe sickle anemia and sickle beta-thalassemia. They found a decrease

in the frequency of vaso-occlusive crises, acute chest syndrome, hemolytic crises, blood transfusions and days spent in the hospital.

Hydroxyurea (HU) enhances fetal hemoglobin production. An increase in total Hb level has been repeatedly reported during HU treatment in several patients with beta- thalassemia major and that eliminate the transfusion requirements in those children's ***(Bradai et al., 2003).***

D) Recombinant erythropoietin trials:

Augmentation of gamma-gene synthesis by using recombinant human erythropoietin represents a new approach to the therapy of transfusion-dependent patients with beta thalassemia. The major limiting factor in designing large scale clinical trials is the relatively high cost of the drug ***(Rachmilewitz and Aker, 1998)***.

Bohl et al. (2000) indicated that continuous delivery of high amount of autologous erythropoietin induced a sustained stimulation of beta-minor globin synthesis and a stable improvement of erythropoiesis in the beta thalassemia mouse model.

E) L-carnitine:

Carnitine is a natural substance, which performs a crucial role in energy supply by controlling the influx of

long chain fatty acids into mitochondria. Carnitine is present in tissues and biological fluids in free and esterified forms. Total carnitine concentration in human tissues is higher in heart and skeletal muscle than in the liver and the brain ***(Ferrari et al., 1992).***

L-propionyl carnitine protects erythrocyte from oxygen reactive species and also stabilizes the damaged membrane probably specific binding with protein and/or phospholipid domains. Low density lipoprotein (LDLs) from human blood was peroxidized by exposure to Cu^{2+} ions in the presence of various L-propionyl carnitine concentrations ***(Bertelli et al., 1994)***.

In a study done by ***Matsumoto et al., (2001)***, it was reported that L-carnitine stabilizes the erythrocyte membrane by improving the uptake of the lipids forming the structure of the membrane. Patients were having a significant increase of the hematocrit level with carnitine administration.

Therapeutic and preventive options for osteoporosis in beta thalassemia

In addition to regular packed RBCs transfusion to maintain hemoglobin level above 10 gm%, intense chelation therapy, proper and aggressive nutritional

intervention, possible new therapeutic interventions would include the following:

1. GH and/or IGF-1 replacement therapy especially for those with GH and/or IGF-1 insufficiency. These measure might increase the circulating IGF-1 level and consequently increase bone formation and prevent osteopenia. Adding IGFBP3 to IGF-1 and /or GH therapy might potentiate their effect on bone growth and mineralization ***(Narusawa et al., 1995).***

2. Treatment with vitamin D at modest doses (800-1.500 IU/ day vitamin D3) may offer a safe and substantial contribution to the prevention of osteoporosis in these patients. As positive correlation of vitamin D levels with BMD of the vertebrae and proximal femur have been found in young and old women with poor vitamin D status ***(Bell, 1995).***

3. Calcium supplementation, which has been shown to increase bone density in normal prepubertal children is anther good potential option ***(Johnston et al., 1992).***

4. Initiation of puberty at an appropriate age through the use of progressively increased doses of androgens for males or estrogen for females. These agents would prevent osteoporosis and increase the BMD

(Margolis et al., 1996). For hypogonadal thalassemic patients with established osteoporosis may be treated with continuous hormone replacement therapy with transdermal estrogen (100 microgram) plus medroxy progesterone for females or HCG for responding males, which best improve the bone density parameters ***(Anapliotou et al., 1995).***

5. Calcitonin has been successful in treating osteopenia. After one year of treatment, bone pain disappeared and radiological signs of osteoporosis have improved significantly ***(Conatan, 1995).***

6. As zinc deficiency secondary to chelation therapy could result in delayed puberty and growth failure, zinc supplementation could be used in treatment or prevention of osteoporosis in β-thalassemic children due its positive effect on linear growth ***(Nakamura et al., 1993).***

7. **Bisphophonates:** non biodegradable compounds characterized by a phosphorous carbon phosphorus bond. They have in general a strong affinity to calcium phosphates and they inhibit bone resoprtion through a cellular mechanism ***(Fleisch, 1993).***

8. **Alendronate:** (alendronic acid; 4-amino-1-hydroxybutylidene bisphosphonate) has demonstrated

effectiveness orally in the treatment and prevention of postmenopausal osteoporosis, corticosteroid induced osteoporosis and Paget's disease of the bone. Its primary mechanism of action involves the inhibition of osteoclastic bone resorption. Alendronate treatment results in early and dose dependent inhibition of skeletal resorption, which can be followed clinically with biochemical markers, and which ultimately reaches a plateau and is slowly reversible upon discontinuation of the drug ***(Porras et al., 1999).***

PATIENT AND METHODS

Study Population:

The study had been conducted on 40 patients 14 males (35%) and 26 females (65%) with β-thalassemia, attending the hematology clinic at Ain Shams University Hospital and the Therapeutic Unit of the Egyptian Company for Blood Transfusion Services in ***VACSERA*** for repeated blood transfusion and follow up.

They were divided into three groups according to bone mineral density of the spine and the left femur.

Group 1: Patients with normal bone mineral density (BMD) (8 patients within normal BMD in the spine and 15 with in normal BMD in the left femur).

Group 2: Patients with osteopenic BMD (12 patients with osteopenic BMD in the spine and 19 with osteopenic BMD in the left femur).

Group 3: Patients with osteoporotic BMD (20 patients with osteoporotic BMD in the spine and 6 with osteoporotic BMD in the left femur).

Patients were subjected to the following:

1 - <u>Full history taking laying stress on:</u>

- Age.
- Age at diagnosis**.**
- Frequency of blood transfusion.
- Administration of iron chelators .
- Compliance to iron chelators.
- History of splenectomy.

2 - <u>Clinical examination with stress on:</u>

- Manifestations of thalassemia and its complication
- Pallor.
- Jaundice.
- Hepato-splenomegaly
- Anthropometric measurements: weight, height and body mass index (B.M.I)

3 -<u>Laboratory investigation including:</u>

- CBC.
- Reticulocyte count.
- ESR.
- Haemoglobin electrophoresis.

- Serum level of Calcium and phosphorus.
- Serum Zinc and Magnesium .
- Iron studies including (S iron, total iron binding capacity and S.ferritin)
- Liver function test (SGOT, SGPT, alkaline phosphatase, total and direct bilirubin, total protein, albumin and gamma GT).
- Kidney function tests (creatinine and urea).
- LDH level.
- Serum uric acid level.

4 -Radiological study:

Measurements of bone mineral density by DEXA were done in 2 sites: lumbar spine (L2 - L4) in the anteroposterior position and proximal femur (neck) on left side using Lunar DPX-MD+ densitometer in Osteoporosis Unit - Ain Shams University Hospitals. All measurements are performed by experienced physicians working in osteoporosis unit.

Principle of action:

The device includes X-ray source that emits two-radiation beam one for penetration of soft tissue and the other for penetration of bone.

The beam travel through the subjects bone and soft tissue, continue upward and enter a detector where the intensity of the incoming beam is registered.

The intensity of the incoming beam alter attenuation reflects the bone mineral density that is a measurement of bone mineral found in the region of interest. BMD is measured in grams per centimeters squared (gm/cm^2). BMD is derived using BMC (bone mineral content) divided by area, where BMC is measured in grams and area is measured in centimeters squared.

This BMD is taken by computer connected to the device that compares the bone density of the subject with reference bone density of an age, sex, weight, height, and ethnic matched subjects and give results in the form of T-score and Z-score.

T-score is the difference between the patient's BMD and the mean value of the reference population.

Z-score is the difference between the patient's BMD and the mean age-matched value of the reference population.

Only spine Z score is available for patients lower than 20 years of age but femur T, Z and spine T- scores in

addition to spine Z score are available for patients more than 20 years.

Statistical analysis:

SPSS statistical software package (V. 9.02, Echosoft Corp., USA, 1998) was used for data analysis. The methods used for statistical analysis were the following:

I. Comparison between 2 independent mean groups for parametric data using Student t test.

2. Comparison between 2 independent groups for non-parametric data using Wilcoxon Rank Sum test.

3. Comparison between more than 2 patient groups for non-parametric data using Kruskall Wallis test.

4. Comparison between more than 2 patient groups for parametric data using Analysis of variance (ANOVA).

5. Ranked Sperman correlation test to study the possible association between each 2 variables among each of the studied groups.

6. Comparison between patient groups for categorized data using Chi-square test.

The probability (p) value was then obtained from all these tests. P -value less than 0.05 was considered significant, while P-value less than 0.01 or 0.001 were considered highly significant.

RESULTS

Forty patients (14 males (35%) and 26 (65%) females) with β-thalassemia have been recruited in this study. They were divided into three groups according to bone mineral density of the spine and the left femur.

Group 1: Patients with normal bone mineral density (BMD) (8 patients with in normal BMD in the spine and 15 with in normal BMD in the left femur).

Group 2: Patients with osteopenic BMD (12 patients with osteopenic BMD in the spine and 19 with osteopenic BMD in the left femur).

Group 3: Patients with osteoporotic BMD (20 patients with osteoporotic BMD in the spine and 6 with osteoporotic BMD in the left femur).

Six of our patient (15%) were of normal BMD, 14 of them (35%) were of ostepenic BMD, the other 20 (50%) were of osteoporotic BMD.

Descriptive data of the clinical parameters, hematological parameters, BMD and trace element and iron profile of the patients are discussed in tables 3,4,5,6 respectively:

Table (3): Descriptive data of the clinical parameters of the patients

Parameter	Range	Mean ±SD	Median
Age (years)	22 – 45	31.02 ±6.23	30
Height (cm)	151 - 181	161.33±7.6	159
Weight (kg)	46 - 89	63.1±11.61	62
Age at diagnosis (years)	1 - 30	18.9±9.52	21.5
Age of 1st blood transfusion (years)	1 - 30	18.63±9.77	22
Interval between transfusion (weeks)	0 - 12	2.94±2.35	3

Table (4): Descriptive data of the haematological parameters of the patients

Parameter	Range	Mean ±SD	Median
Hb (g/dl)	5.3 -11.2	7.48 ± 1.22	7.2
R.B.C ($10^3/mm^3$)	1.59- 5.48	3.73 ± 0.93	3.545
HCT %	20.3- 40.1	25.11 ± 4.35	24.6
MCV fL	51- 120	72.37 ±16.88	67
MCH pg	15.8 -33.3	21.50 ± 4.52	20.8
Hb A %	15 – 89	66.09 ± 19.24	73
Hb A2 %	2.2 - 9.7	5.53 ± 2.28	5
Hb F %	6 - 80	29 ± 19.47	20
Platelet ($10^3/mm^3$)	34.4 - 982	568.85 ± 269.95	547

Table (5): Descriptive data of the Bone Mineral Density of the patients

Parameter	Range	Mean ±SD	Median
BMD Spine	-4.1 – 0.1	-2.16 ±1.38	-2.15
BMD Femur	-2.5 – 1.4	-0.95 ±1.21	-1.2

BMD = Bone Mineral Density
SD = Standard deviation

Table (6): Descriptive data of the trace element and iron profile of the patients

Parameter	Range	Mean ±SD	Median	Lab references range
Ca (mg/dL)	8.6 - 10.2	9.58 ± 0.54	9.7	8.4 - 10.5
PO4 (mg/dL)	2.4 - 5.00	3.61 ± 0.62	3.6	2.4 - 4.6
Mg (mg/dL)	1.3 - 2.8	2.13 ± 0.34	2.1	1.9 – 2.5
Zn (µg/dL	46 - 78	61.70 ±9.57	61	1.6 – 151.4
ALK P	86 - 340	186.18 ± 78.94	161	64 - 306
Iron (ug/dL)	72 - 318	168.64 ± 72.71	158	89 - 158
S. ferritin (ng/dL)	128 -2989	1260.73±796.74	1402.5	18 - 370
T.I.B.C (mg/dL)	147 -364	250.52 ± 64.26	247	259 - 388

Table (7): Correlation between normal, osteopenic and osteoporotic BMD in both spine and femur of the patient from one side and trace element, minerals and iron profile from the other side.

Param.	Group	Spine			Femur		
		Mean ± SD	P value	Sig.	Mean ± SD	P value	Sig.
Ca	Normal BMD Oteopenic Osteoporotic	9.65 ± 0.38 9.58 ± 0.56 9.54 ± 0.62	>0.05	NS	9.76 ±0.48 9.67 ± 0.45 9.40 ± 0.57	>0.05	NS
PO4	Normal BMD Oteopenic Osteoporotic	3.95 ± 0.27 3.48 ± 0.28 3.47 ± 0.79	>0.05	NS	3.91 ±0.43 3.80 ±1.03 3.31 ± 47	<0.05	S
Mg	Normal BMD Oteopenic Osteoporotic	1.26 ± 0.04 2.01 ± 0.4 2.61 ± 0.19	<0.01	HS	2.03 ±0.43 2.16 ±0.05 2.23 ± 0.24	>0.05	NS
Zn	Normal BMD Oteopenic Osteoporotic	65.6 ± 8.21 62.5 ± 12.42 58.25 ± 8.19	< 0.05	Sig.	67.33 ±5.95 63 ± 9.4 58.89 ± 9.95	<0.05	S
ALK P	Normal BMD Oteopenic Osteoporotic	131 ± 31.39 173 ± 101.51 224.5± 68.81	<0.01	HS	164.8±74.91 197.1± 87.24 205 ±51.89	>0.05	NS
Iron	Normal BMD Oteopenic Osteoporotic	132 ±62.01 151.5 ±56.72 197.49 ±74.79	<0.05	S	126.8± 38.9 179.6±110.8 198.2±66,63	<0.05	S
TIBC	Normal BMD Oteopenic Osteoporotic	263.16 ±42.2 260.53±39.39 260.5 ±76.49	>0.05	NS	271.15±77.44 235.65±99.48 230.35±62.94	>0.05	NS
S. ferritin	Normal BMD Oteopenic Osteoporotic	802.33±42.14 855.25±587.5 1697.95±743.7	<0.01	HS	915.06±642.801 299.84±779.4 2001±765.32	>0.05	NS

We used Kruskall Wallis Test nonparametric for data and Analysis of Variance (ANOVA) for parametric data.

On comparing between the three studied groups according to the spine BMD, there was a statistically high significant difference as regard serum magnesium which was higher in osteoporotic group (P value < 0.01 i.e highly significance F ratio 10.108.)

On comparing between the three studied groups according to the spine BMD, there was statistically high significant difference as regard serum alkaline phosphatase which was higher in osteoporotic group (P value < 0.01 i.e highly significance)

On comparing between the three studied groups according to the spine BMD, there was statistically high significant difference as regard serum ferritin which was higher in osteoporotic group (P value < 0.01 i.e highly significance)

On comparing between the three studied groups according to the spine BMD, there was statistically significant difference as regard serum zinc which was lower in osteoporotic group (P value < 0.05 i.e significance F ratio 6.39)

On comparing between the three studied groups according to the spine BMD, there was statistically significant difference as regard serum iron which was higher in osteoporotic group (P value < 0.05 i.e significance).

Comparing between the three studied groups according to the spine BMD, there is statistically non-significant difference as regard serum phosphorus (P value > 0.05 i.e non significance F ratio 2.739)

On comparing between the three studied groups according to the spine BMD, there was statistically non-significant difference as regard the total iron binding capacity (TIBC) (P value > 0.05 i.e non significance F ratio 2.538)

On comparing between the three studied groups according to the spine BMD, there was statistically non-significant difference as regard serum calcium (P value > 0.05 i.e non significance F ratio 0.164)

On comparing between the three studied groups according to the left femur BMD, there was statistically significant difference as regard serum phosphorus (P value < 0.05 i.e significance F ratio 5.038).

On comparing between the three studied groups according to the left femur BMD, there was statistically significant difference as regard serum zinc which was lower in osteoporotic group (P value < 0.05 i.e significance F ratio 5.27).

On comparing between the three studied groups according to the left femur BMD, there was statistically significant difference as regard serum iron which was higher in osteoprotoic group (P value < 0.05 i.e significance F ratio 7.056).

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard serum calcium (P value > 0.05 i.e non significance F ratio 2.161)

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard serum magnesium (P value > 0.05 i.e. non significance F ratio 1.485).

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard serum alkaline phosphatase (P value > 0.05 i.e. non significance).

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard the total iron binding capacity (TIBC) (P value > 0.05 i.e non significance).

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard serum ferritin (P value > 0.05 i.e non significance).

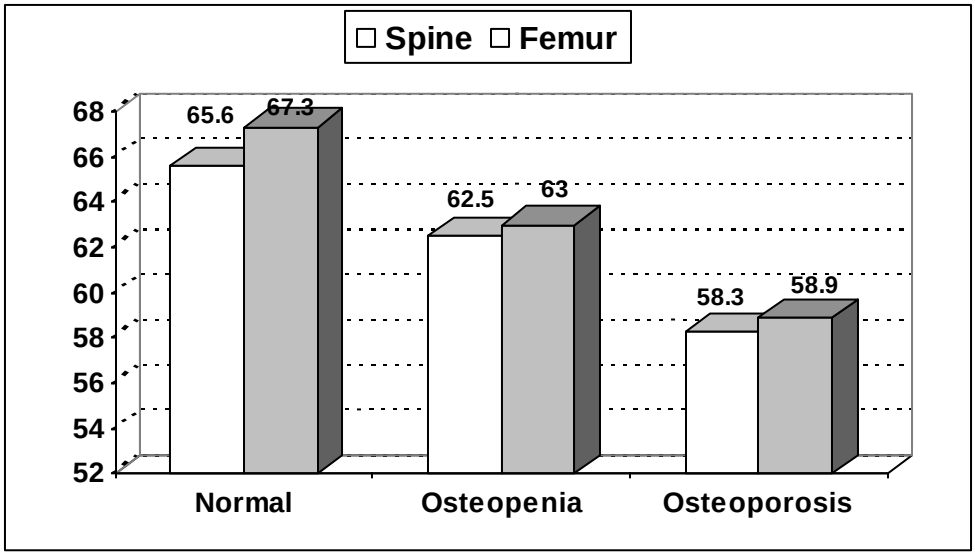


Fig. (1): Comparison between normal, osteoporosis and osteopenia as regards mean value of serum Zinc

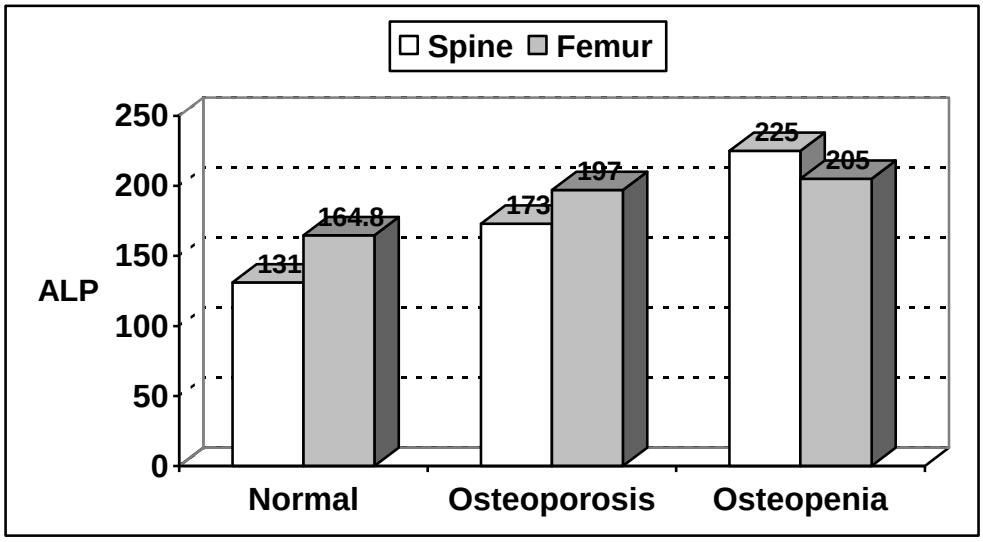


Fig. (2): Comparison between normal, osteoporosis and osteopenia as regards mean value of ALP

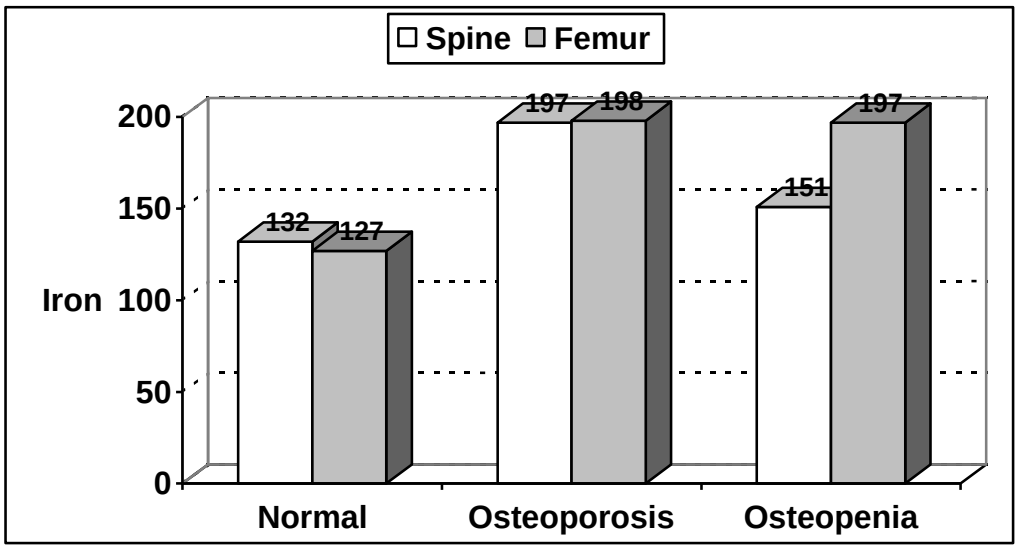


Fig. (3): Comparison between normal, osteoporosis and osteopenia as regards mean value of serum iron

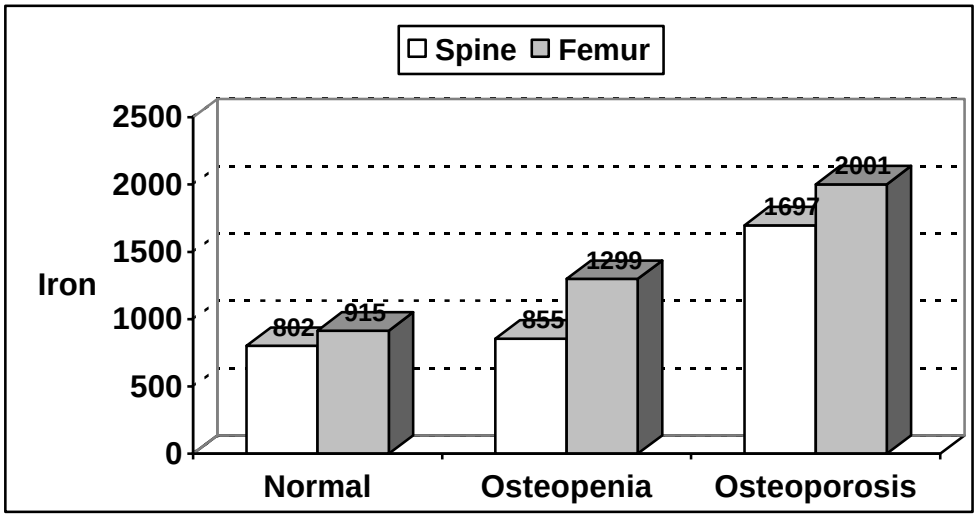


Fig. (4): Comparison between normal, osteoporosis and osteopenia as regards mean value of serum ferritin

Table (8): Correlation between normal, osteopenic and osteoporotic BMD in both spine and femur of the patient as regard the clinical parameters

Param.	Group	Spine			Femur		
		Mean ± SD	P v	Sig.	Mean ± SD	P v	Sig.
Age	Normal BMD Oteopenic Osteoporotic	27 ± 0.76 29.6 ± 4.5 33.5 ± 7.3	<0.05	S	28 ± 4.9 30.5 ± 4.2 41.3 ± 3.6	<0.01	HS
Height	Normal BMD Oteopenic Osteoporotic	169.7 ± 2.8 162 ± 10.2 157.5 ± 3.2	<0.01	HS	167.3 ±6.9 158.4 ± 6.1 155.7 ±1.9	<0.01	HS
Weight	Normal BMD Oteopenic Osteoporotic	69.2 ± 11.7 68.8 ± 11 57.2 ± 8.9	<0.01	HS	68.1 ± 9.8 65.3 ±8.8 58.4 ±12.3	<0.05	S
BMI	Normal BMD Oteopenic Osteoporotic	23.92 ±4.11 26.45 ± 4.56 23.7 ± 3.72	>0.05	NS	24.32 ± 3.15 23.36 ± 4.93 26.94 ± 3.47	>0.05	NS
Age at diag.	Normal BMD Oteopenic Osteoporotic	26.5 ± 2.8 17 ± 8.2 15± 10.2	<0.01	HS	22.3 ±6.3 19.3±10.2 17.5± 9.9	>0.05	NS
Age of 1st blood transf.	Normal BMD Oteopenic Osteoporotic	26.68 ±2.5 17.3 ±7.9 14.4±10.4	<0.01	HS	22.3±6.3 19.7± 9.8 16.5±10.5	>0.05	NS
The interval bet. Blood Transf.	Normal BMD Oteopenic Osteoporotic	4.4 ±4.1 2.7 ±0.7 1.3 ±0.5	<0.01	HS	3.4 ±3.2 3 ±0.00 2.18 ±0.8	>0.05	NS

We used Kruskall Wallis Test for nonparametric data and we Analysis Of Variance (ANOVA) for parametric data.

On comparing between the three studied groups according to the spine BMD, there was statistically highly significant difference as regard the height of patients which was lower in osteoporotic group (P value < 0.01 i.e highly significance F ratio 13.387)

On comparing between the three studied groups according to the spine BMD, there was statistically highly significant difference as regard the weight which was lower in osteoporotic group (P value < 0.01 i.e highly significance F ratio 6.666)

On comparing between the three studied groups according to the spine BMD, there was statistically highly significant difference as regard the age at diagnosis which was lower in osteoporotic group (P value < 0.01 i.e highly significance).

On comparing between the three studied groups according to the spine BMD, there was statistically highly significant difference as regard the age of first blood transfusion which was lower in osteoporotic group (P value < 0.01 i.e. highly significance).

On comparing between the three studied groups according to the spine BMD, there was statistically highly significant difference as regard the interval between blood transfusion which was shorter in osteoporotic group (P value < 0.01 i.e highly significance).

On comparing between the three studied groups according to the spine BMD, there was statistically significant difference as regard the age of patients (P value < 0.01 i.e significance F ratio 4.001).

On comparing between the three studied groups according to the spine BMD, there was statistically non-significant difference as regard the body mass index (BMI) (P value > 0.05 i.e non significance F ratio 13.387).

On comparing between the three studied groups according to the left femur BMD, there was statistically highly significant difference as regard the age of the patient which was higher in osteoporosis (P value < 0.01 i.e. Highly significance F ratio 20.112).

On comparing between the three studied groups according to the left femur BMD, there was statistically highly significant difference as regard the height which was lower in ostoeporotic group (P value < 0.01 i.e. Highly significance F ratio 12.301).

On comparing between the three studied groups according to the left femur BMD, there was statistically significant difference as regard the weight which was higher in osteoporotic group (P value < 0.01 i.e. Significance F ratio 3.446).

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard the body mass index (BMI) (P value > 0.05 i.e. non significance F ratio 1.700)

On comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard the age at diagnosis (P value > 0.05 i.e. Non significance).

On Comparing between the three studied groups according to the left femur BMD, there was statistically non-significant difference as regard the age of first blood transfusion (P value > 0.05 i.e. Non significance).

On comparing between the three studied groups according to the left femur BMD, there is statistically non-significant difference as regard the interval between blood transfusion (P value > 0.05 i.e non significance).

Table (9): Comparison between users and non users of iron chelating agents as regard to minerals, trace elements and iron profile

Parameter	Users of iron chelator	Non users of iron chelator	P value	Sig.
	Mean ± SD	Mean ± SD		
Ca	9.58±0.59	9.57±0.45	>0.05	NS
PO4	3.55±0.73	3.73±0.35	>0.05	NS
Mg	2.05±0.33	2.27±0.31	<0.05	S
Zn	59.43± 6.97	62.92±3.70	<0.05	S
Iron	145.71±67.15	180.99±73.83	<0.05	S
T.I.B.C	257.87±69.73	236.86±52.23	>0.05	NS
S.ferritin	1066.14±540.05	1365.50±897.78	<0.05	S

We used Wilcoxon Rank Sum test to compare between the two groups for nonparametric data while, we used Student t test to compare between the two groups for parametric data.

Comparing between users and non users of iron chelating agents as regard minerals, trace elements and iron profile revealed:

Comparing between serum magnesium in both groups showed statistically significant differences which was lower in iron chelator users with T value 2.0097 and P value <0.05.

Comparing between serum zinc in both groups showed statistically significant difference which was lower in iron chelator users with T value -1.852 and P value <0.05.

Comparing between serum iron in both groups showed statistically significant difference which was lower in iron chelator users with Z value 1.9766 and P value <0.05.

Comparing between serum ferritin in both groups showed statistically significant difference which was lower in iron chelator users with Z value 1.4767 and P value <0.05.

Comparing between serum calcium in both groups showed no statistically significant difference with T value -0.517 and P value >0.05.

Comparing between serum phosphorus in both groups showed no statistically significant difference with T value 0.8616 and P value >0.05.

Comparing between total iron binding capacity (TIBC) in both groups showed no statistically significant difference with T value -0.9863 and P value >0.05.

Table (10): Comparison between non compliant and compliant patients to iron chelating agents as regard to minerals, trace elements and iron profile

Parameter	-ve compliance	+ve Compliance	P value	Sig.
	Mean ± SD	Mean ± SD		
Ca	10.5 ± 0.12	9.37 ± 0.59	<0.01	HS
PO4	3.45 ± 0.61	3.59 ± 0.78	>0.05	NS
Mg	2.13 ± 0.14	2.02 ±0.39	>0.05	NS
Zn	62 ± 11.48	36.33 ±7.51	<0.01	HS
Iron	197.75 ±90.23	173.54± 66.88	>0.05	NS
T.I.B.C	236.81± 65.86	302.25 ± 55.93	<0.01	HS
S.ferritin	1406.61±753.96	1273± 1218.16	>0.05	NS

We used Wilcoxon Rank Sum test to compare between the two groups for nonparametric data while, we used Student t test to compare between the two groups for parametric data.

Comparing between non compliant and compliant patients iron chelating agents as regard to minerals, trace elements and iron profile revealed:

Comparing between serum calcium in both groups showed statistically highly significant difference which was lower in compliant patients with T value 3.1546 and P value <0.01.

Comparing between serum zinc in both groups showed statistically highly significant difference which was lower in compliant patients with T value 1.7331 and P value <0.05.

Comparing between total iron binding capacity (T.I.B.C) in both groups showed statistically highly significant difference which was lower in compliant patients with T value 2.5514 and P value <0.01.

Comparing between serum phosphorus in both groups showed no statistically significant difference with T value -0.4592 and P value >0.05.

Comparing between serum magnesium in both groups showed no statistically significant difference with T value 0.7177 and P value <0.05.

Comparing between serum iron in both groups showed no statistically significant difference with Z value -0.111 and P value >0.05.

Comparing between serum ferritin in both groups showed no statistically significant with Z value 1.000 and P value >0.05.

Table (11): Comparison between non splenectomized and splenectomized patients as regard minerals, trace elements and iron profile

Parameter	-ve Splecectomy	+ve Splecectomy	P value	Sig.
	Mean ± SD	Mean ± SD		
Ca	9.64 ± 0.46	9.52 ± 0.61	>0.05	NS
PO4	3.52 ± 0.79	3.71 ± 0.37	>0.05	NS
Mg	2.21 ± 0.28	2.05 ±0.38	>0.05	NS
Zn	60.50 ± 11.21	62.90 ±7.68	>0.05	NS
Iron	138.80 ±57.66	198.49± 75.19	<0.01	HS
T.I.B.C	252.45± 72.84	248.50± 56.22	>0.05	NS
S.ferritin	831.6± 600.04	1689.85±745.17	<0.01	HS

We used Wilcoxon Rank Sum test to compare between the two groups for nonparametric data while, we used Student t test to compare between the two groups for parametric data.

Comparing between serum iron in both groups showed statistically high significant difference which was higher in splenectomized patients with Z value -2.489 and P value <0.01.

Comparing between serum ferritin in both groups showed statistically highly significant difference which was higher in splenectomized patients with Z value -2.813 and P value <0.01.

Comparing between serum calcium in both groups showed no statistically significant difference with T value 0.7296 and P value >0.05.

Comparing between serum phosphorus in both groups showed no statistically significant difference with T value -0.9893 and P value >0.05.

Comparing between serum magnesium in both groups showed no statistically significant difference with T value 1.5169 and P value >0.05.

Comparing between serum zinc in both groups showed no statistically significant difference with T value -0.111 and P value >0.05.

Comparing between total iron binding capacity (T.I.B.C) in both groups showed no statistically significant with T value -0.1961 and P value >0.05.

Table (12): Comparison between BMD of both spine and femur and using of iron chelating agents

Parameter	BMD SPINE		BMD FEMUR	
Group	-ve iron chelators	+ve iron chelators	-ve iron chelators	+ve iron chelators
	n14/40 (%)	n26/40 (%)	n14/40 (%)	n 26/40 (%)
Normal BMD	6/40 (15%)	2/40 (5 %)	8 /40 (20 %)	7/40 (17.5%)
Osteopenic BMD	8/40 (20%)	4 /40 (10 %)	6/40 (15 %)	13/40 (32.5%)
Osteoporotic BMD	0 /40 (0 %)	20/40 (50 %)	0/40 (0 %)	6/40 (15%)
Total	14 /40 (35%)	26/40 (65%)	14/40 (35%)	26/40 (65%)
Chi-square	21.685		5.545	
P value	P < 0.01		P < 0.01	
Significance	H S		H S	

We used Chi-square test to compare between patient groups for categorized data.

As regard spine, among the 14 patient not using iron chelator, we found 6 patients(42.9%)were of normal BMD,8 patients (57.14%) were osteopenic and non of them (0%) were osteoporotic while among the 26 patient using iron chelator, 2 patients (7.69%) were of normal BMD,4 patients(15.38%) were osteopenic and 20 of them (76.22)% were osteoporotic (P value < 0.01).

As regard femur, among the 14 patient not using iron chelator, we found 8 patients (57.14%) were of normal

BMD, 13 patients (42.86%) were osteopenic and non of them (0%) were osteoporotic, while among the 26 patients using iron chelator, 7 patients (26.92%) were of normal BMD, 13 (50%) were osteopenic and 6 (23.1%) were osteoporotic (P value < 0.01)

Table (13): Comparison between BMD and compliance to iron chelators

Parameter	BMD SPINE		BMD FEMUR	
Group	-ve compliance to chelators	+ve compliance to chelators	-ve compliance to chelators	+ve compliance to chelators
	n 8/40 (%)	n18/40 (%)	n8/40 (%)	n18/40 (%)
Normal BMD	0/40 (0%)	2/40 (7.7%)	2/40 (7.7%)	5/40 (19.3%)
Osteopenic BMD	2/40 (7.69%)	2/40 (7.7%)	4/40 15.4%)	9/40 (34.6%)
Osteoporotic BMD	6/40 (23.1%)	14/40 (53.9%)	2/40 (7.7%)	4/40 (15.4%)
Total	8/40 (30.77%)	18/40 (69.23%)	8/40 (30.7%)	18/40 (69.2%)
Chi-square	1.589		0.043	
P value	P < 0.05		P > 0.05	
Significance	S		N S	

We used Chi-square test to compare between patient groups for categorized data.

As regard spine, among the 8 non compliant patients to iron chelator, we found that non of them (0%) were of normal BMD, two of them (25%) were osteopenic and 6 of

them (75%) were osteoporotic while among the 18 compliant patients to iron chelators, 2 of them (11.1% were of normal BMD, and 2 of them (11.1) were osteopenic and 14 (77.8%) were osteoporotic (P value < 0.05).

As regard femur, among the 8 non compliant patients to iron chelators, 2 patients (25%) were of normal BMD,4 patients (50%) were osteopenic and 2 patients (25%) were osteoporotic while among the 18 compliant patients to iron chelators,5 of them (27.7%) were of normal BMD, 9 (50%) were osteopenic and 4 of them (22.2%) were osteoporotic (P value > 0.05).

DISCUSSION

Investigation of the mechanisms that underlie the bone pathology in thalassaemia major has started rather late, in spite of the fact that the prominent facial and skull deformities, as well as the frequent fractures, were all noted in the original report of this disease by ***Cooley and Lee (1925).*** However, in recent years, the increased survival of patients brought about by more efficient therapy and the recognition of an enlarging number of patients with thalassaemia intermedia made bone metabolism studies absolutely necessary because the related morbidity varies widely across the patient population and often imposes a customized treatment. Quantification of bone disease in thalassaemia is best performed using dual X-ray absorptiometry ***(Vichinsky, 1998)***

Calcium plays a major role in bone mineralization at all ages 99% of the body's calcium is in the bones so its deficiency results in poor mineralization of bone and teeth, osteomalacia, osteoporosis, tetany, rickets and impairment of growth

In correlation to our finding the recent study done by ***Soliman et al. (1998)*** in the university of Alexandria to evaluate the bone mineral density by assessing calcium-phosphorus balance in 30 pre pubertal children with β

thalassemia major showed that although a significant decrease of BMD was found in all thalassemic patients however hypocalcaemia was detected in 5 of the 30 patients.

In another study ***Chao (1996)***, the level of Calcium, Phosphorus, and Ca-P related hormones were not significantly changed in the patients than controls, but he showed marked decrease of BMD in all the thalassemic patients. Also ***Moulas, (1997)*** showed that serum calcium, inorganic phosphorus and alkaline phosphatase level in the thalassemic patients were not significantly different from those in the controls. In the older group of thalassemic patients serum calcium was lower than in the controls.

The result of our study revealed that serum calcium showed non scientifically difference between the three groups regard spine and femur, on the other hand, serum phosphorus was significantly lower in osteoporotic patient regard to the left femur. Also serum alkaline phosphatase in our study showed highly significant difference between the 3 groups regard to the spine witch was lower in osteoporosis.

Iron overload, a common finding in thalassemic patients, has been suggested as another contributing factor for the development of reduced bone mass ***(Singer et al.,1999, Bordat et al., 1993).***

In a study done by ***Somnuek et al.,*** showe that the deposition of iron in trabecular bone had negative correlation with the trabecular bone volume and BMD, suggesting the contribution of iron toxicity to the reduction of bone mass in thalassemia.

Rioja et al. (1990) studied iliac crest biopsies from 17 thalassemic children with severe skeletal changes; they detected histochemically iron deposits at various sites and mostly severe cortical bone changes including fissures and focal mineralization defects.

In our study we found that there was highly significant difference between the three group the spine BMD regard to that serum ferritin with more prominent increasing in osteoporosis while regard serum iron ,it was significantly difference regard to both spine and femur BMD between the three group which was more in osteoporotic group.

Johnson (1999), explain this by excess iron deposition in bone may influence osteoblast number and activity and interfere with mineralization, and consequently

lead to osteoporosis. Elevated serum ferritin level, especially in transfusion-dependant patient, indicate iron deposit in organs including bone.

Zinc deficiency in patients with thalassemia major is an issue under debate. Although some studies have reported zinc deficiency in thalassemic patients (***Kajanachumpol, et al., 1997; Modarresi, et al., 2000),*** there are reports indicating higher levels of serum zinc in these patients ***(Bashir, 1995).***

In the study by ***Kwan et al.,*** only 3 out of 68 thalassemic patients had zinc deficiency, although the possible marginal status of zinc nutrition in the patients was not ruled out.

The mechanisms responsible for decreased serum zinc levels in thalassemics are not well understood yet. Some investigators suggested that hyperzincuria might lead to zinc deficiency in these patients ***(Dogru et al., 1979)***. It is not exactly known whether urinary zinc excretion, which is already increased in thalassemic patients, is further increased by DFO ***(Uysal et al., 1993).***

A few studies have indicated that deferiprone may, in addition to iron, chelate zinc as well. This is an important issue since zinc plays an important role in several physiological functions; protein synthesis, gene expression,

immunity, wound healing amd maintenance of integrity of intra-cellular organelles***(Hipolito et al .,1980).***

In addition, it has been recently noted that deferiprone therapy is associated with zinc depletion in thymocytes ***(Maclean et al., 2001).***

In our study, we found that the level of serum zinc in patients receiving iron chelator was statistically significant lower than non receiving iron chelators***. Rea et al.*** suggested hypertransfusional treatment to prevent zinc deficiency in thalassemics.

Other factors that affect serum zinc levels in normal population, such as dietary habits, geographical factors, and ethnicity*),* may also have an impact in thalassemics.

Plasma zinc is metabolically active and fluctuates in response to low dietary intake ***(John and Anderson, 2000).***

The essential role of several trace minerals, especially zinc, manganese, and copper for organic bone matrix synthesis, has been shown for at least three decades ***(Strause et al., 1994).***

Zinc deficiency has been reported in osteoporotic patients ***(Ilich and Kerstetter, 2000; De Sanctis and Wonke, 1994)***.

Zinc regulates the secretion of calcitonin and influences bone turnover.

A high prevalence of osteoporosis is found among with transfusion-dependent β-thalassemic adults ***(Jensen, et al., 1998).***

We also found a considerably significant correlation between serum zinc and BMD among the patients.

Hormone deficiency, bone marrow expansion, nutritional deficiency, and desferrioxamine toxicity are important factors ***(Vichinsky, 1998).***

De Sanctis and Wonke (1994) have suggested that chelation of trace elements (zinc, copper) might play an important role in bone growth.

When facing extremely low zinc intakes, tissue and cellular redistribution of zinc from selected tissues such as bone occurs as a secondary adjustment event and zinc homeostasis is maintained primarily by other mechanisms. Physiologic signs of zinc depletion and changes in tissue zinc concentrations are not evident until a drop in plasma zinc concentration occurs ***(King, et al.,).***

Considering the role of zinc in mineral matrix formation and because zinc is a component of alkaline phosphatase ***(Ilich and Kerstetter, 2000., Honda, 1997)***.

bone zinc depletion might be an explanation for the existing association between low serum zinc and BMD our study.

In our study, DEXA scan done to our patients showed that the majority (85%) of them had reduced BMD with 20 patients (50%) showing osteoporosis and 14 patients (35%) showing osteopenia.

Several studies reported obvious decrease in bone mineral density in thalassemic patients ***Voskaridou et al. (2000)*** found that osteoporosis or osteopenia (Z - score lower than -2.5 and -1.0 respectively)were constant feature of all patients .

Jensen et al. (1998) studied 82 patients who were well transfused and who received regular desferrioxamine. The overall prevalence of patients with severely low bone mass was 51% and those with low bone mass 45% .Similar results from 50 patients were also reported by ***Giardina et al. (1995)*** and ***Molyvda-Athanassopouloy et al. (1999)***.

In addition, ***Angastiniotis et al. (1998)*** reported that 75% of a large group of patients with mild thalassemia who started transfusion therapy late in life had a bone mass density below 2 SD. Using radiography, ***Katz et al. (1994)*** found evidence of osteoporosis of in patients and pathological fractures in 4 out of 28 well transfused

thalassemic patients, aged (10 - 25) years. Also patients with β-tha1assemia major were more effected than β-thalassemia intermedia .This may be due to the late start of desferrioxamine in the patients of β-thaiassemia intermedia allowed a relatively good number of osteoblasts to remain functional while the early administration of the drug in thalassemia major inhibit this function, desferrioxamine inhibits DNA synthesis ***(Wonke, 1998).***

Also, more significant bone disease in β-thalassemia major may be explained by earlier and more severe exposure to anemia since early life.

Our results revealed highly significant difference in BMD regard age of the patients with more prominent osteoporsis in the older patients regarding to the left femur also a significant difference was found regard to spine. Moreover, a highly significant difference was found between T- score of the spine and the age of the first blood transfusion and also with the transfusion frequency.

In support of our findings, ***Voskaridou et al., (2000)*** found that pronounced decrease in BMD in patients with β-thalassemia major is likely to reflect the erythroid bone marrow early in life

The decrease of bone mineral value was more prominent in the lumber spine in our study than that of the

left femur ,.the lumber spine (which consist primarily of trabeculer bone and wide bone marrow space ,is more affected .The explanation of this finding is that increased erythropoiesis occurs mainly in the axial skeleton.

Moreover, axial BMD increases more rapidly than appendicular BMD during puberty (***Rubin et al., 1993***) and thus making this site particularly interesting for such studies

The effect of iron chelation treatment on trace metal in patient with iron overload depend on the affinity of the chelator to these metal.

In our study we found that there is highly significant difference in the three group in BMD of the spine regard to the interval of blood transfusion with more prominent osteoporosis with the short interval, this could be due to the severity of the disease (thalassemia major has more frequent in blood transfusion).

Mahachoklertwattana et al. (2003) confirm our result by studying BMD of 48 suboptimally treated patient with β- thalassemia disease 16 of them(33.3%) were transfusion dependant (TD) and 32 (66.6%) were transfusion in dependant (TI) and found that patient with undertransfused β-thalassemia major had greater bone

marrow expansion compared with patients with untransfused moderately sever β-thalassemia disease

As regard effect of splenectomy on thalassemic patient, in our study we found that there is highly significant deference in serum iron and ferritin with increasing in patients underwent splenectomy. A lot of studies support our finding.

Pootrakul et al. (1981) Found that there was a significantly higher serum ferritin levels in splenectomized patients with beta-thalassemia disease than in the nonsplenectomized ones. The observation is compatible with previous observations that splenectomy in thalassemia is associated with increased iron deposition and increased transferrin iron saturation. ***(Al-Salem and Nasserulla, 2002).***

CONCLUSION

As the longevity of patient with thalssemia increases, osteoporosis will become an increasingly prominent problem.

Osteoporosis is a progressive disease, supervesion and early diagnosis is important, as well as treatment of established disease.

In conclusion, children and adult with thalssemia major display continues bone resorption, the severity of which appears not to depend on their age, however bone formation is still active ,thus giving a hope that adequate therapy may be of benefit.

RECOMMENDATION

1- Routine periodic evaluation of BMD by DEXA scans in patient with β-thalassemia especially in highly-chelated long standing cases.

2- Routine supplementation of thalassemic patient with zinc ,calcium , vitamin D use of antiresoptive agent e.g. Bisphosphonates, especially in those with advanced bone disease and with prophylactic dose in asymptomatic patients to avoid worsening of their bone situation.

3- Proper chelation therapy to avoid iron overload and its impact on bone disease and other complication in thalassemia.

SUMMARY

Osteoporosis has been described extensively in adult thalassemic patient. In this study, we measured the bone mineral density (BMD) of 40 thalassemic patients (14 males (40%) and 26 females (60%) with an age of 22-45 years (mean 31.02±6.23 years) attending in the hematology clinic at Ain Shams University Hospital and the Therapeutic Unit of the Egyptian Company for Blood Transfusion Services in ***VACSERA*** for repeated blood transfusion and follow up.

The aim of our study was to correlate the biochemical and mineral changes with the bone density changes of thalassemic patients through:

(1) Full history taking stressing on age at diagnosis, frequency of blood transfusion, administration of iron chelators, patient compliance and history of splenectomy.

(2) Estimation of serum zinc, calcium, phosphorus, magnesium, total alkaline phosphatase, serum iron and serum ferritin

They were divided into three groups according to bone mineral density of the spine and the left femur.

Our study confirmed that patient with thalassemia have a lower bone mineral density parameters. Regarding to DEXA results, most of the patients' BMD were affected (85%) with 50% of them having osteoporosis and (35%) having osteopenia.

Also there was a highly significant difference between the degree of BMD affection in the left femur and the level of serum zinc in plasma being lower significantly in osteoporotic patients.

As regard to total alkaline phosphatase there were a highly significant difference between the three groups but it was higher significantly in the osteoporotic patients.

Serum iron showed a highly significant difference between the three groups and it was significantly high in the osteoporitic patients and this support the data that iron has toxic effect on the bone. Thus, with the use of iron chelators iron and ferritin in the plasma can be lowered; thus decreasing their effect on the bone and this fact was confirmed in our study which revealed a significant lower level of serum iron and serum ferritin in patients on iron chelation therapy compared with those not using iron chelators.

Although the effect of iron chelators on the serum iron is favorable for thalassemic patient, it is not the same to serum zinc. Iron chelators also chelate zinc and other trace elements, and this was shown in our study which showed a significantly lower level of serum zinc and magnesium in patient using iron chelating agent with highly significant decrease in serum zinc in patients showing good compliance for chelation therapy. This result may suggest that treatment of thalassemic patients with chelation therapy may share in lowering the BMD as zinc play an important functional role in bone metabolism and bone turn over.

We conclude that significant bone disease is detected in the majority of thalassemic patients together with significant association with low serum zinc. Thus, bone disease in thalassemia is multifactorial and as treatment methods are contributing, patients should be screened regularly for serum zinc and be given prophylactic supplementation if necessary.

REFERENCES

Abdollah Shamshirsaz, A., Bekheirnia, M.R., Kamgar, M., Pourzahedgilani, N., Bouzari, N., Habibzadeh, M., Hashemi, R., Abdollah Shamshirsaz, A.H., Aghakhani, S., Homayoun, H. & Larijani, B. (2003):

Metabolic and endocrinologic complications in beta-thalassemia major: A multicenter study in Tehran. BMC Endocrine Disorders, 3, 1–6.

Aloia JF, Vaswani A, Ma R, Flaster E (1995):

To what extent is bone mass determined by fat free or fat mass? Am J Clin Nutr 61 (5): 110-4.

Anapliotou, M.L., Kastanias, I.T., Psara, P., Evangelou, E.A., Liparaki, M. & Dimitriou, P. (1995):

The contribution of hypogonadism to the development of osteoporosis in thalassaemia major: new therapeutic approaches. Clinical Endocrinology, 42, 279–287.

Angastiniotis M, Parlides N, Aristidou K, Kanakas A, Yerakaris M, Eracleous E, Posporis T (1998):

Bone pain in thalassaemia: assessment of DEXA and MRI findings. J Pediatr Indocrinol Metab; 11 supple. 3: 779-84.

Anne K (2001):

NIH consensus development panel on osteoporosis prevention, diagnosis, and therapy. JAMA, February 14, Vol. 285, No. 6.

Anne MK and Karen MP (2000):

Osteoporosis pathogenesis, diagnosis, and treatment in older adults. Rheumatic disease clinics of north America, 26-3.

Arcasoy, A., Canata, D., Sinav, B., Kutlay, L., Oguz, N. & Sen, M. (2001):

Serum zinc levels and zinc binding capacity in thalassemia. Journal of Trace Elements in Medicine and Biology, 15, 85–87.

Bekheirnia, M.R., Abllah Shahirsaz, A., Kamgar, M., Bouzari, N., Erfanzadeh, G., Pourzahedgilani, N., Tataie, S.M., Abllah Shahirsaz, A., Kimiagar, M., Ezzati, F. & Larijani, B. (2004):

Serum zinc and its relation to bone mineral density in beta-thalassemic adolescents. Biological Trace Element Research, 97, 215–224.

Bell NH (1995):

Vitamin D metabolism aging and bone loss. J Clin Endocrinol. Metab., 80: 1051

Berkovitch, M., Bistritzer, T., Milone, S.D., Perlman, K., Kucharczyk, W. & Olivieri, N.F. (2000):

Iron deposition in the anterior pituitary in homozygous beta-thalassemia: MRI evaluation and correlation with gonadal function. Journal of Pediatric Endocrinology and Metabolism, 13, 179–184.

Binkley, N.C., Schmeer, P., Wasnich, R.D. & Lenchik, L. (2002):

What are the criteria by which a densitometric diagnosis of osteoporosis can be made in males and non-Caucasians? Journal of Clinical Densitometry, 5(Suppl.), 19–27.

Blake G, Wahner HW, Fogeiman I, (1999):

The evaluation of osteoporosis: Dual energy X-ray absorptiometry and Ultrasound in Clinical practice. 2nd ed., London, UK: Martin Dunitz Ltd; pp 312-350

Bonjour, J.P., Ammann, P. & Rizzoli, R. (1999):

Importance of preclinical studies in the development of drugs for treatment of osteoporosis: a review related to the 1998 WHO guidelines. Osteoporosis International, 9, 379–393.

Bradney M, Pearce G, Naughton G, Sulivan C, Bass S, Beck T, Carlson J, Seeman E (1998):

Moderate exercise during growth in prepubertal bodys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. J Bone Miner Res., 13: (12): 1814-21.

Brandle M, Galeazzi RL, Morant R and Oesterle M (1996):

Ostepenia in beta thalassemia major. Schweiz. Med. Wochenscher, Nov., 2: 126 (44): 1867-1874.

Brill, P.W., Winchester, P., Giardina, P.J. & Cunningham-Rundles, S.(1991):

Deferoxamine-induced bone dysplasia in patients with thalassemia major. American Journal of Roentgenology, 156, 561–565.

Camaschella C, Cappellini MD. 1995:

Thalassemia intermedia. Haematologica 1995;80:58-68.

Canatan D, Akar N, and Arcasoy A (1995):

Effects of calcitonin therapy on osteoporosis in patients with thalassemia Acta Haematol., 93: 20-23

Carmina, E., Di Fede, G., Napoli, N., Renda, G., Vitale, G., Lo Pinto, C., Bruno, D., Malizia, R. & Rini, G.B. (2004):

Hypogonadism and hormone replacement therapy on bone mass of adult women with thalassemia major. Calcified Tissue International, 74, 68–71.

Cefalu, C.A. (2004):

Is bone mineral density predictive of fracture risk reduction? Current Medical Research and Opinion, 20, 341–349.

Chan, Y.L., Pang, L.M., Chik, K.W., Cheng, J.C. & Li, C.K. (2002):

Patterns of bone diseases in transfusion-dependent homozygous thalassaemia major: predominance of osteoporosis and desferrioxamine- induced bone dysplasia. Pediatric Radiology, 32,492– 497.

Christenson, R.H. (1997):

Biochemical markers of bone metabolism: an overview. Clinical Biochemistry, 30, 573–593.

Cohen, A.J. & Roe, F.J. (2000):

Review of risk factors for osteoporosis with particular reference to a possible aetiological role of dietary salt. Food and Chemical Toxicology, 38, 237–253.

David LN, Syndey LB and Chalotte FS (2000):

Bone health and osteoporosis. Clinics in Sports Medicine Vol., 19, No. 2 April.

De Sanctis, V., Pinamonti, A., Di Palma, A., Sprocati, M., Atti, G., Gamberini, M.R. & Vullo, C. (1996):

Growth and development in thalassaemia major patients with severe bone lesions due to desferrioxamine. European Journal of Pediatrics, 155, 368–372.

De Sanctis, V., Stea, S., Savarino, L., Scialpi, V., Traina, G.C., Chiarelli, G.M., Sprocati, M., Govoni, R., Pezzoli, D., Gamberini, R. &Rigolin, F. (1998):

Growth hormone secretion and bone histomorphometric study in thalassaemic patients with acquired skeletal dysplasia secondary to desferrioxamine. Journal of Pediatric Endocrinology and Metabolism, 11(Suppl. 3), 827–833.

De Vernejoul, M.C., Girot, R., Gueris, J., Cancela, L., Bang, S., Bielakoff, J., Mautalen, C., Goldberg, D. & Miravet, L. (1982):

Calcium phosphate metabolism and bone disease in patients with homozygous thalassemia. Journal of Clinical Endocrinology and Metabolism, 54, 276–281.

De Virgiliis, S., Congia, M., Turco, M.P., Frau, F., Dessi, C., Argiolu, F., Sorcinelli, R., Sitzia, A. & Cao, A. (1988):

Depletion of trace elements and acute ocular toxicity induced by desferrioxamine in patients with thalassaemia. Archives of Disease in Childhood, 63, 250–255.

Dempster DW, Lindsay R (1993):

Pathogenesis of osteoporosis. Lancet, 341: 797-801

Di Stefano, M., Chiabotto, P., Roggia, C., Garofalo, F., Lala, R., Piga, A. & Isaia, G.C. (2004):

Bone mass and metabolism in thalassemic children and adolescents treated with different iron-chelating drugs. Journal of Bone and Mineral Metabolism, 22, 53–57.

Diamond, T., Stiel, D. & Posen, S. (1989):

Osteoporosis in hemochromatosis: iron excess, gonadal deficiency, or other factors? Annals of Internal Medicine, 110, 430–436.

Dresner Pollack, R., Rachmilewitz, E., Blumenfeld, A., Idelson, M. & Goldfarb, A.W. (2000):

Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. British Journal of Haematology, 111, 902–907.

Eisman JA (1999):

Genetics of osteoporosis. Endocrine Reviews; 20 (6): 788-504.

Fleisch H (1993):

New bisphosphonates in osteoporosis. Osteoporos. Int., 3 (suppl. 2): S15-22.

Fuchs, GJ, Tienboon P, Linpisarn SL et al. (1996):

Nutritional factors and thalassemia major. Arch Dis Child 74: 244-277.

Garofalo, F., Piga, A., Lala, R., Chiabotto, S., Di Stefano, M. & Isaia, G.C. (1998):

Bone metabolism in thalassemia. Annals of New York. Academy of Sciences, 850, 475–478.

Geusens, P.P. & Boonen, S. (2002):

Osteoporosis and the growth hormone-insulin-like growth factor axis. Hormone Research, 58(Suppl. 3), 49–55.

Giardina, P.J., Schneider, R., Lesser, M., Simmons, B., Rodriguez, A., Gertner, J., New, M. & Hilgartner, M.W. (1995):

Abnormal bone metabolism in thalassaemia. In: Endocrine Disorders in Thalassaemia (ed. by S. Ando et al.), pp. 39–46. Springer, Berlin.

Giurzio E, Bria M, Bisconte MG, Caracciolo M, Qualtieri A, Misasi, M, Grillo G and Brancati C (1991):

Osteoporosis in patients affected with thalassemia major. Chirugi Degli Organi di movimento, 69-374.

Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J, Charles P, ERiksen EF (2000):

Commonly recommended. Daily intake of vitamin D is not sufficient if sunlight exposure is limited. J Intern Med., 247 (2): 260-268

Grampp, S., Genant, H.K., Mathur, A., Lang, P., Jergas, M., Takada, M., Gluer, C.C., Lu, Y. & Chavez, M. (1997):

Comparisons of non-invasive bone mineral measurements in assessing age-related loss, fracture discrimination, and diagnostic classification. Journal of Bone and Mineral Research, 12, 697–711.

Gratwick GM, Bullough PG, Bohne WHO, Markenson AL, Peterson CM. (1978):

Thalassemia osteoarthropathy. Ann Intern Med 1978;88:494-501

Greenspan SL, Maitland LA, Myers ER et al. (1994):

Femoral bone loss progresses with age: A longitudinal study in women over age 65. Jbone Miner Res., 9: 1959-1965

Grinberg LN, Rachmilewitz EA.(1995):

Oxidative stress in ß-thalassemic red blood cells and potential use of antioxidants. In: Beuzard Y, Lubin B, Rosa J, eds. Sickle cell disease and thalassaemia: new trends in therapy. London: Colloque INSERM/John Libby Eurotext, 1995:519-24

Grosveld F, Dillon N, Higgs D.(1993):

The regulation of human globin gene expression. Clin Haematol 1993;6:31-55.

Gulati R, Bhata V, Agawal SS (2000):

Department of genetics, Sanjay Gandhi postgraduate insitutte of medical science,s luck now, India Early onset of endocrine abnormalities in beta thalasaemia major in developing country. J Pediatr Endocrinol Metab., 13 (6): 561-6.

Hajjar, R.R. & Kamel, H.K. (2004):

Osteoporosis for the home care physician. Part 1: etiology and current diagnostic strategies. Journal of the American Medical Directors Association, 5, 192–196.

Hatori, M., Sparkman, J., Teixeira, C.C., Grynpas, M., Nervina, J., Olivieri, N. & Shapiro, I.M. (1995):

Effects of deferoxamine on chondrocyte alkaline phosphatase activity: proxidant role of deferoxamine in thalassemia. Calcified Tissue International, 57, 229

Heinonen A, Kannus P, Sievanen H, Oja P, Pasanen M, Rinne M, Uusi Rasi K, Vuori I (1996):

Randomized controlled trial of effect of high impact exercise on skeletal risk factors for osteoporotic fractures. Lancet 348: 1343-1347.

Ho PJ, Hall GW, Luo LY, Weatherall DJ, Thein SL.(1998):

Beta thalassaemia intermedia: is it possible consistently to predict phenotype from genotype? Br J Haematol 1998;100:70-78.

Horowitz MC (1993):

Cytokines and estrogen in bone: anti-osteoporotic effects. Science. 19: 202-239.

Jensen, C.E., Tuck, S.M., Agnew, J.E., Koneru, S., Morris, R.W., Yardumian, A., Prescott, E., Hoffbrand, A.V. & Wonke, B. (1998):

High prevalence of low bone mass in thalassaemia major. British Journal of Haematology, 103, 911–915.

Jensen, C.E., Tuck, S.M., Old, J., Morris, R.W., Yardumian, A., De Sanctis, V., Hoffbrand, A.V. & Wonke, B. (1997):

Incidence of endocrine complications and clinical disease severity related to genotype analysis and iron overload in patients with beta-thalassaemia. European Journal of Haematology, 59, 76–81.

Joanne S, Kamal S (2000):

Diagnosis of osteoporosis. Center, Farmington, Connitcut, Clincial Cornerstone 1 (6): 1-2

Johanson, N.A. (1990):

Musculoskeletal problems in hemoglobinopathy. The Orthopedic Clinics of North America, 21, 191–198.

Johnston, C.C. Jr., Miller, J.Z., Slemenda, C.W., Reister, T.K., Hui, S., Christian, J.C. & Peacock, M. (1992):

Calcium supplementation and increases in bone mineral density in children. New England Journal of Medicine, 327, 82–87.

Kanis JA, Delmas P, Burchkardt P et al. (1997):

Guidelines for diagnosis and management of osteoporosis. Osteoprosis International; 7: 390-406.

Kanis JA, Melton LJ, Christiansen C et al. (1994):

Prospective: the diagnosis of osteoporosis. J Bone Miner Res., 9: 1137-1141.

Kazazian HH (1985):

The nature of mutation hosp Pract 20: 55-8.

Klibanski A, Adams L, Bassford T et al. (2000):

Osteoporosis prevention, diagnosis, and therapy. Am NIH consens statement. March 27-29; 17 (1): 1-36.

Korovessis PG, papanastasiou D, Deratis NG (1996):

Prevalence of scolisos in beta thalassemia. J Spinal discord 9 (2): 170-3.

Lala, R., Chiabotto, P., Di Stefano, M., Isaia, G.C., Garofalo, F. & Piga, A. (1998):

Bone density and metabolism in thalassaemia. Journal of Pediatric Endocrinology and Metabolism, 11(Suppl. 3), 785–790.

Lane, N.E., Bloch, D.A., Jones, H.H., Marshall, W.H. Jr., Wood, P.D. & Fries, J.F. (1986):

Long-distance running, bone density, and osteoarthritis. JAMA, 255, 1147–1151.

Lasco, A., Morabito, N., Gaudio, A., Buemi, M., Wasniewska, M. & Frisina, N. (2001) :

Effects of hormonal replacement therapy on bone metabolism in young adults with beta-thalassemia major. Osteoporosis International, 12, 570–575.

Lasco, A., Morabito, N., Gaudio, A., Crisafulli, A., Meo, A., Denuzzo, G. & Frisina, N. (2002):

Osteoporosis and beta-thalassemia major: role of the IGF-I/IGFBP-III axis. Journal of Endocrinology Investigation, 25, 338–344.

Lawson JP, Albow RC and Pearson A (1981):

The ribs in thalassaemia. Pediatric radiology; 140: 663-672

Lindsay R, Bush TL, Grady D et al. (1996):

Therapeutic controversy: Estrogen replacement in menopause. J Clin Endocrinol Metab., 81: 3829-3838

Lindsay R, Meunier PJ (1998):

Osteoporosis: review of evidence for prevention, diagnosis and treatment and cost effective analysis. Osteoporosis Int. 8 (Suppl 4): S1-S88.

Ma, E.S., Lam, K.K., Chan, A.Y., Ha, S.Y., Au, W.Y. & Chan, L.C. (2003):

Transferrin receptor-2 polymorphisms and iron overload in transfusion independent b-thalassemia intermedia. Haematologica, 88, 345–346 .

Mahachoklertwattana, P., Chuansumrit, A., Sirisriro, R., Choubtum, L., Sriphrapradang, A. & Rajatanavin, R. (2003a):

Bone mineral density, biochemical and hormonal profiles in suboptimally treated children and adolescents with beta-halassaemia disease. Clinical Endocrinology, 58, 273–279.

Mahachoklertwattana, P., Sirikulchayanonta, V., Chuansumrit, A., Karnsombat, P., Choubtum, L., Sriphrapradang, A., Domrongkitchaiporn, S., Sirisriro, R. & Rajatanavin, R. (2003b):

Bone histomorphometry in children and adolescents with beta- thalassemia disease: iron-associated focal osteomalacia. Journal of Clinical Endocrinology and Metabolism, 88, 3966–3972.

Margolis RN, Canalis E and Partrige NL (1996):

Anabolic hormones in bone: basic research therapeutic potential J Clin Endocrinol. Metab., 81: 72-877

McGuigan FE, Murray L, Gallagher A, Davey Smith G, Neville CE, Vanhof R, Boreham C, Ralston SH (2002):

Genetic and environmental determinants of peak bone mass is young men and women. J Bone Miner Res 17 (7): 1273-9

Michelson, J. & Cohen, A. (1988):

Incidence and treatment of fractures in thalassemia. Journal of Orthopaedic Trauma, 2, 29–32.

Miller DR, Krall EA, Anderson JJ et al. (2001):

Dietary mineral intake and low bone mass in men. J Bone Miner REs 16 (suppl. 1): S395.

Monegal A, Navasa M, Guanabens N, Peris P, Pon sF, Martinez de Osaba Rimola A, Rodes J and Munoz Gomwz J (1997):

Osteoporosis and bone mineral metabolism disorders in cirrhotic patients referred for orthotopic liver trnasplantation. Calcie. Tissue int., 60 (2): 148-154

Morabito, N., Gaudio, A., Lasco, A., Atteritano, M., Pizzoleo, M.A., Cincotta, M., La Rosa, M., Guarino, R., Meo, A. & Frisina, N. (2004):

Osteoprotegerin and RANKL in the pathogenesis of thalassemia induced osteoporosis: new pieces of the puzzle. Journal of Bone and Mineral Research, 19, 722–727.

Mundy, G.R. (1999):

Cellular and molecular regulation of bone turnover. Bone, 24 (5 Suppl.), 35S–38S.

Murray MT, Pizzorno JE Jr (1999):

Osteoporosis. In textbook of natural medicine, 2+nd (ed): 1999; Vol. 2, pp. 1453-1461. Edinburgh: Chruchill Livingstone.

Nakarmura T, Nishiyama S, Futagoshi Suginohara Y, Matsuda I and Higasi A (1993):

A mild moderate zinc deficiency in short children: Effect of zinc supplementation on linear growth velocity. J Pediatr., 123: 65-69.

Narusawa K, Nakmamura T and Suzaki K (1995):

The effect of recombinant human insulin like growth factor (rhIGF)-and rhIGF-1/IGF brinding protein -3 administration on rat osteopenia induced by ovariectomy with concomitant bilateral sciatic neurectomy. J Bone Miner. Res., 10: 1853-1864

Nathan DG, Gunn RB.(1966):

Thalassemia: the consequences of unbalanced hemoglobin synthesis. Am J Med 1966;41:815-830.

New SA (1999):

Bone health; The role of micronutrients. Br Med Bull 55: 619-633

Nguyen, T.V., Pocock, N. & Eisman, J.A. (2000):

Interpretation of bone mineral density measurement and its change. Journal of Clinical Densitometry, 3, 107–119.

Nightingale SL (1998):

Ultrasound device for evaluating bone strength approved. JAMA., 279 (17): 1339.

Noelovitz M (1993):

Osteoporosis: screening, prevention and management. Fertile Sterile., 59: 707-725.

Olivieri, N.F. (1999) :

The beta-thalassemias. New England Journal of Medicine, 341, 99–109.

Olivieri, N.F., Koren, G., Harris, J., Khattak, S., Freedman, M.H., Templeton, D.M., Bailey, J.D. & Reilly, B.J. (1992):

Growthfailure and bony changes induced by deferoxamine. AmericanJournal of Pediatric Hematology and Oncology, 14, 48–56.

Olszynski, W.P., Shawn Davison, K., Adachi, J.D., Brown, J.P., Cummings, S.R., Hanley, D.A., Harris, S.P., Hodsman, A.B., Kendler, D., McClung, M.R., Miller, P.D. & Yuen, C.K. (2004):

Osteoporosis in men: epidemiology, diagnosis, prevention, and treatment. Clinical Therapeutics, 26, 15–28.

Orkin SH.(1995):

Regulation of globin gene expression in erythroid cells. Eur J Biochem 1995;231:271-281.

Orvieto R, Leichter I, Rachmilewitz EA, Margulies JY.(1992):

Bone density, mineral content, and cortical index in patients with thalassemia major and the correlation to their bone fractures, blood transfusions, and treatment with desferrioxamine. Calcif Tissue Int 1992 ; 50:397-399.

Orzincolo C, Castaldi G, Gariani L, Franceschini F, Corcione S and Scutellari PN (1998):

Circumscribed lytic lesions of the thalassemic skull. Skeletal radiology, 7: 345-347

Perifanis V, Garipidou V, = Balans I, Zarotiades G (2001):

The pattern of bone disease in thalassemia major patients; endocrinal growth and bone; the 8th International Conference on thalassemia, 2001, p 248.

Piga, A., Luzzatto, L., Capalbo, P., Gambotto, S., Tricta, F. & Gabutti, V. (1988) :

High-dose desferrioxamine as a cause of growth failure in thalassemic patients. European Journal of Haematology, 40, 380–381 .

Pootrakul, P., Hungsprenges, S., Fucharoen, S., Baylink, D., Thompson, E., English, E., Lee, M., Burnell, J. & Finch, C. (1981):

Relation between erythropoiesis and bone metabolism in thalassemia. New England Journal of Medicine, 304, 1470–1473.

Porras AG, Holland SD, Gertz BJ (1999):

Pharmacokinitics of alendronate. Clinic Phrmacokinitics; May 36 (5): 315-28

Prince RL (2001):

How to diagnose the presence of osteoporosis and assess the risk of fracture. Best Pract Res Clin Rheumatol Jul., 15 (3): 345-58.

Raiola, G., Galati, M.C., De Sanctis, V., Caruso Nicoletti, M., Pintor, C., De Simone, M., Arcuri, V.M. & Anastasi, S. (2003) :

Growth and puberty in thalassemia major. Journal of Pediatric Endocrinology and Metabolism, 16(Suppl. 2), 259–266.

Rasiz LG, Prestwood KM (2000):

Epidemiology and pathogenesis of osteoporosis. Clin Cornerstone; 2 (6): 1-10

Riggs, B.L. (2000) :

The mechanisms of estrogen regulation of bone resorption. Journal of Clinical Investigation, 106, 1203–1204.

Riggs, B.L., Khosla, S. & Melton, L.J. III (1998):

A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. Journal of Bone and Mineral Research, 13, 763–773.

Rioja L, Girot R, Garabedian M, Cournot-Witmer G.(1990):

Bone disease in children with homozygous beta-thalassemia. Bone Miner 1990;8:69-86

Rodda CP (1994):

Body properties in patients with homozygous β-thalassemia. Acta Pediatr, Suppl., 406: 107-108.

Rodda, C.P., Reid, E.D., Johnson, S., Doery, J., Matthews, R. & Bowden, D.K. (1995):

Short stature in homozygous beta-thalassaemia is due to disproportionate truncal shortening. Clinical Endocrinology, 42, 587–592.

Roseen C (1996):

Osteoporosis: diagnostic and therapeutic principles. Totowa, New Jersey: Humana Press, pp 123-130

Rubin, k., Schirduan, V., Gendreu, P., Sarfarazi, M., Mendola, R & Dalsky,G.(1993):

Predictors of axial and peripheral bone mineral density in healthy children and adolescent, with special attention to the role of puberty. Journal of Paediatrics , 111, 158-159.

Rucker, D., Ezzat, S., Diamandi, A., Khosravi, J. & Hanley, D.A. (2004):

IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clinical Endocrinology, 60, 491– 499.

Ruggero L and De Sanctis V (1998):

Multicentral study on prevalence of fractures in transfusion dependent thalassemia patients. J Pediatr Endocrinol Metab., 11 Spp 3, 773-8.

Rund D and Rachmilewitz E (2000):

New trends in the treatment of beta-thalassemia. Crit Rev. Oncol Hematol., 3 (2): 105-18

Rund D, Oron-Karni V, Filon D, Goldfarb A, Rachmilewitz E, Oppenheim A. (1997):

Genetic analysis of ß-thalassemia intermedia in Israel: diversity of mechanisms and unpredictability of phenotype. Am J Hematol 1997;54:16-22.

Saglamer L, Ulukutlu L, Ercan O et al. (1992):

Evaluation of growth hormone and insulin like growth factor-1 in prepubertal children with thalassemia. Turk. J Pediatr., 34: 63-69.

Schrier SL.(1997):

Pathobiology of thalassemic erythrocytes. Curr Opin Hematol 1997;4:75-78.

Shalev O, Repka T, Goldfarb A, et al.(1995):

Deferiprone (L1) chelates pathologic iron deposits from membranes of intact thalassemic and sickle RBC both in vitro and in vivo. Blood 1995;86:2008-2013

Sheir S, Khalifa AS, Mokhtar MG, El Tayeb H and Aly A (1988):

Hepatic changes in β-thalassemia major by ultrasonogrpahy. The Egyptian Journal of Pediatrics vol. 5 NO. 1-2, 1-12

Shinar E, Rachmilewitz EA.(1993):

Haemoglobinopathies and red cell membrane function. Baillieres Clin Haematol 1993;6:357-369.

Soliman AT, El-Banna N, Salimi I et al. (1996):

Insulin and glucagons responses to provocation with glucose and arginine in prepubertal children with thalassemia major before and after long-term blood transfusion. J Trop Pediatr., 42: 291-296.

Soliman, A.T., El Banna, N., Abdel Fattah, M., El Zalabani, M.M. & Ansari, B.M. (1998) :

Bone mineral density in prepubertal children with beta-thalassemia: correlation with growth and hormonal data. Metabolism, 47, 541–548.

Sorensen S, Rubin E, Polster H, Mohandas N, Schrier S.(1990):

The role of membrane skeletal-associated alpha-globin in the pathophysiology of beta-thalassemia. Blood 1990;75:1333-1336.

Stenstron MG, Norjavaara E, Hulten L et al. (2001):

Late normal puberty in men is associated with ISDs lower bone mass in spine and hip at 21 years of age. A longitudinal study among young healthy men between 16 and 21 years of age. J Bone Miner Res 16 (Suppl. 1): S185.

The World Congress on Osteoporosis (2000):

An overview. Ego Seeman, MD. June 15, 2000.

Thein SL(1993):

ß-Thalassaemia. Baillieres Clin Haematol 1993;6:151-175.

Theodoridis C, Ladis V, Papatheodorou A, Berdenz H, Palamidou F, Evagelopoulou C, Athanassak K, Konstantoura O, Kattamic C (1998):

Growth and management of short stature in thalassaemia major. J Pediatr Endocrino (Metal 11 supp. 3: 835-44).

Todd, J.A. & Robinson, R.J. (2003):

Osteoporosis and exercise. Postgraduate Medical Journal, 79, 320–323.

Vichinsky, E.P. (1998) :

The morbidity of bone disease in thalassemia. Annals of New York Academy of Sciences, 850, 344–348.

Voskaridou, E., Kyrtsonis, M.C., Terpos, E., Skordili, M., Theodoropoulos, I., Bergele, A., Diamanti, E., Kalovidouris, A., Loutradi, A. & Loukopoulos, D. (2001):

Bone resorption is increased in young adults with thalassaemia major. British Journal of Haematology, 112, 36–41.

Voskaridou, E., Terpos, E., Spina, G., Palermos, J., Rahemtulla, A., Loutradi, A. & Loukopoulos, D. (2003):

Pamidronate is an effective treatment for osteoporosis in patients with beta-thalassaemia. British Journal of Haematology, 123, 730–737.

Weatherall DJ, Clegg JB. (2001):

The thalassaemia syndromes. 4th ed. Oxford, England: Blackwell Scientific (in press).

Whedon, G.D. (1984):

Disuse osteoporosis: physiological aspects. Calcified Tissue International, 36(Suppl. 1), 146–150.

Wickramasinghe SN. (1976):

The morphology and kinetics of erythropoiesis in homozygous ß-thalassaemia. In: Congenital disorders of erythropoiesis. Amsterdam: Elsevier, 1976:221-37.

Wonke, B. (2001):

Clinical management of beta-thalassemia major. Seminars in Hematology, 38, 350–359.

Wonke, B., Jensen, C., Hanslip, J.J., Prescott, E., Lalloz, M., Layton, M., Erten, S., Tuck, S., Agnew, J.E., Raja, K., Davies, K. & Hoffbrand, A.V. (1998) :

Genetic and acquired predisposing factors and treatment of osteoporosis in thalassaemia major. Journal of Pediatric Endocrinology & Metabolism, 11(Suppl. 3), 795–801.

Wood WG, Weatherall DJ.(1983):

Developmental genetics of the human haemoglobins. Biochem J 1983;215:1-10.

Wood WG.(1993):

Increased HbF in adult life. Baillieres Clin Haematol 1993;6:177-213.

World Health Organization (1994):

Assessment of Fracture Risks and Its Application to Screening for Post-Menopausal Osteoporosis. Technical Report Series 843. WHO, Geneva. Writing Group for the ISCD Position Development Conference (2004) Diagnosis of osteoporosis in men, premenopausal women, and children. Journal of Clinical Densitometry, 7, 17–26.

Yates AA, Schlicker SA, Suitor CW (1998):

Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins and choline. J Am diet Assoc 98 (6): 699-706.

Yuan J, Angelucci E, Lucarelli G, et al.(1993):

Accelerated programmed cell death (apoptosis) in erythroid precursors of patients with severe beta-thalassemia. Blood 1993;82:374-377.

Zheng SX, Vrindts Y, Lopez M et al. (1997):

Increase in cytokine production IL-1 beta, IL-6, TNF-alpha but not IFN-gamma, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas. 26: 63-71.

Perera DM, Gunasekara D, Wijekoon A, Petrou M.

Related Articles, Links Thalassaemia control in developing countries--the Sri Lankan perspective. Ceylon Med J. 2000 Mar;45(1):12-6. Review.

Yong KN, Wadsworth D, Langlois S, Yong SL, Wilson RD.

Thalassemia carrier screening and prenatal diagnosis among the British Columbia (Canada) population of Chinese descent. Clin Genet. 1999 Jan;55(1):20-5.

Laradi S, Haj Khelil A, Omri H, Chaieb A, Mahjoub T, Benlimam H, Amri F, Saad A, Miled A, Leturcq F, Ben Chibani J, Beldjord C.

[Molecular analysis and prenatal diagnosis of beta-thalassemia: about our experience in central Tunisia] Ann Biol Clin (Paris). 2000 Jul-Aug;58(4):453-60. French.

Romero Artaza J, Carbia CD, Ceballo MF, Diaz NB.

[Red cell distribution width (RDW): its use in the characterization of microcytic and hypochromic anemias] Medicina (B Aires). 1999;59(1):17-22. Spanish.

Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D, Polchi P, Andreani M, Gaziev D, Erer B, Ciaroni A, D'Adamo F, Albertini F, Muretto P.

Bone marrow transplantation in adult thalassemic patients. Blood. 1999 Feb 15;93(4):1164-7.

Weatherall D.

Related Articles, Links From genotype to phenotype: genetics and medical practice in the new millennium. Philos Trans R Soc Lond B Biol Sci. 1999 Dec 29;354(1392):1995-2010.

Choi JW, Kim CS, Pai SH.

Erythropoietic activity and soluble transferrin receptor level in neonates and maternal blood. Acta Paediatr. 2000 Jun;89(6):675-9.

Olivieri NF, Rees DC, Ginder GD, Thein SL, Brittenham GM, Waye JS, Weatherall DJ.

Treatment of thalassaemia major with phenylbutyrate and hydroxyurea. Lancet. 1997 Aug 16;350(9076):491-2. No abstract available.

Fargion S, Taddei MT, Gabutti V, Piga A, Di Palma A, Capra L, Fontanelli G, Avanzini A.

Early iron overload in beta-thalassaemia major: when to start chelation therapy? Arch Dis Child. 1982 Dec;57(12):929-33.

Molyvda-Athanasopoulou E, Sioundas A, Karatzas N, Aggellaki M, Pazaitou K, Vainas I.

Bone mineral density of patients with thalassemia major: four-year follow-up.Calcif Tissue Int. 1999 Jun;64(6):481-4. PMID: 10341019 [PubMed - indexed for MEDLINE]

Olivieri NF, Brittenham GM.

Iron-chelating therapy and the treatment of thalassemia. Blood. 1997 Feb 1;89(3):739-61. Review. Erratum in: Blood 1997 Apr 1;89(7):2621.

Richardson DR, Ponka P.

Development of iron chelators to treat iron overload disease and their use as experimental tools to probe intracellular iron metabolism. Am J Hematol. 1998 Aug;58(4):299-305. Review.

Richardson DR.

The therapeutic potential of iron chelators. Expert Opin Investig Drugs. 1999 Dec;8(12):2141-2158.

Kontoghiorghes GJ, Aldouri MA, Hoffbrand AV, Barr J, Wonke B, Kourouclaris T, Sheppard L.

Effective chelation of iron in beta thalassaemia with the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one. Br Med J (Clin Res Ed). 1987 Dec 12;295(6612):1509-12.

Rombos Y, Tzanetea R, Konstantopoulos K, Simitzis S, Zervas C, Kyriaki P, Kavouklis M, Aessopos A, Sakellaropoulos N, Karagiorga M, Kalotychou V, Loukopoulos D.

Chelation therapy in patients with thalassemia using the orally active iron chelator deferiprone (L1). Haematologica. 2000 Feb;85(2):115-7.

Al-Salem AH.

Is splenectomy for massive splenomegaly safe in children? Am J Surg. 1999 Jul;178(1):42-5.

Alter BP.

Bone marrow failure syndromes in children. Pediatr Clin North Am. 2002 Oct;49(5):973-88.

Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D, Polchi P, Andreani M, Gaziev D, Erer B, Ciaroni A, D'Adamo F, Albertini F, Muretto P.

Bone marrow transplantation in adult thalassemic patients. Blood. 1999 Feb 15;93(4):1164-7.

Giardini C.

Treatment of beta-thalassemia. Curr Opin Hematol. 1997 Mar;4(2):79-87.

Lena D,Orsini A, Perrimond H, Vovan L, Badens C.

[Genetic abnormality of unrecognized hemoglobin: alpha-thalassemia] Arch Pediatr. 1996 Mar;3(3):210-2. French.

Bensinger WI, Storb R.

Allogeneic peripheral blood stem cell transplantation. Rev Clin Exp Hematol. 2001 Jun; 5(2):67-86.

Cao A, Moi P.

Genetic modifying factors in beta-thalassemia. Clin Chem Lab Med. 2000 Feb;38(2):123-32.

Rund D, Rachmilewitz E.

New trends in the treatment of beta-thalassemia. Crit Rev Oncol Hematol. 2000 Feb;33(2):105-18. Review

Loukopoulos D, Voskaridou E, Stamoulakatou A, Papassotiriou Y, Kalotychou V, Loutradi A, Cozma G, Tsiarta H, Pavlides N.

Hydroxyurea therapy in thalassemia. Ann N Y Acad Sci. 1998 Jun 30;850:120-8.

Reich S, Buhrer C, Henze G, Ohlendorf D, Mesche M, Sinha P, Kage A, Muller C, Vetter B, Kulozik AE. Oral isobutyramide reduces transfusion requirements in some patients with homozygous beta-thalassemia. Blood. 2000 Nov 15;96(10):3357-63.

Arruda VR, Lima CS, Saad ST, Costa FF.

Successful use of hydroxyurea in beta-thalassemia major. N Engl J Med. 1997 Mar 27;336(13):964. No abstract available.

Zeng YT, Huang SZ, Ren ZR, Lu ZH, Zeng FY, Schechter AN, Rodgers GP.

Hydroxyurea therapy in beta-thalassaemia intermedia: improvement in haematological parameters due to enhanced beta-globin synthesis. Br J Haematol. 1995 Jul; 90(3):557-63.

Lowrey CH, Horwitz AF.

Effect of inhibitors of cholesterol synthesis on muscle differentiation. Biochim Biophys Acta. 1982 Aug 18;712(2):430-2.

Koren A, Segal-Kupershmit D, Zalman L, Levin C, Abu Hana M, Palmor H, Luder A, Attias D.

Effect of hydroxyurea in sickle cell anemia: a clinical trial in children and teenagers with severe sickle cell anemia and sickle cell beta-thalassemia. Pediatr Hematol Oncol. 1999 May-Jun;16(3):221-32.

Bradai M, Abad MT, Pissard S, Lamraoui F, Skopinski L, de Montalembert M.

Hydroxyurea can eliminate transfusion requirements in children with severe beta-thalassemia. Blood. 2003 Aug 15;102(4):1529-30. Epub 2003 Apr 17.

Rachmilewitz EA, Aker M.

The role of recombinant human erythropoietin in the treatment of thalassemia. Ann N Y Acad Sci. 1998 Jun 30;850:129-38. Review.

Bohl D, Bosch A, Cardona A, Salvetti A, Heard JM.

Improvement of erythropoiesis in beta-thalassemic mice by continuous erythropoietin delivery from muscle. Blood. 2000 May 1;95(9):2793-8.

Ferrari R, Di Lisa F, de Jong JW, Ceconi C, Pasini E, Barbato R, Menabo R, Barbieri M, Cerbai E, Mugelli A.

Prolonged propionyl-L-carnitine pre-treatment of rabbit: biochemical, hemodynamic and electrophysiological effects on myocardium. J Mol Cell Cardiol. 1992 Mar;24(3):219-32.

Bertelli A, Conte A, Ronca G.

L-propionyl carnitine protects erythrocytes and low density lipoproteins against peroxidation. Drugs Exp Clin Res. 1994;20(5):191-7.

Matsumoto Y, Amano I, Hirose S, Tsuruta Y, Hara S, Murata M, Imai T.

Effects of L-carnitine supplementation on renal anemia in poor responders to erythropoietin. Blood Purif. 2001;19(1):24-32.

Alter,B.P. (2002):

The Hemoglobinopathies In Hematology of infancy and childhood. Nathan DG ,Orkin SH, Ginsburg D and Look T.(eds),6th edition.Philadelphia,Harcourt Health Science p.385-398.

Olivieri, N.F. and Weatherall, D.J. (1999)

Thalassemia In Pediatric hematology text book. 2nd edition London Edinburg New York Philadilphia Sydney Torento, 307-327.

Giardini C and Lucarelli G (1999):

Bone Marrow Transplantation for beta Thalassemia. Hematol Oncol Clin North Am;13(5):1059-46

Thein SL, Winichagoon P, Hesketh C, Best S, Fucharoen S, Wasi P, Weatherall DJ.

The molecular basis of beta-thalassemia in Thailand: application to prenatal diagnosis. Am J Hum Genet. 1990 Sep;47(3):369-75.

Gennari C.

World congress on osteoporosis 2000. IDrugs. 2000 Oct;3(10):1154-6.

Gennari C,Reginster JY, Bruyere O, Audran M, Avouac B, Body JJ, Bouvenot G, Brandi ML, Kaufman JM, Lemmel EM, Vanhaelst L, Weryha G, Devogelaer JP.

Do estrogens effectively prevent osteoporosis-related fractures? The Group for the Respect of Ethics and Excellence in Science. Calcif Tissue Int. 2000 Sep;67(3):191-4.

Melton LJ 3rd, Seeman E, O'Fallon WM, Riggs BL.

Risk factors for spinal osteoporosis in men. Am J Med. 1983 Dec;75(6):977-83.

Rosen C,Wallach S, Cohen S, Reid DM, Hughes RA, Hosking DJ, Laan RF, Doherty SM, Maricic M, Brown J, Barton I, Chines AA.

Effects of risedronate treatment on bone density and vertebral fracture in patients on corticosteroid therapy. Calcif Tissue Int. 2000 Oct;67(4):277-85.

Matkovic V, Heaney RP.

Calcium balance during human growth: evidence for threshold behavior. Am J Clin Nutr. 1992 May;55(5):992-6.

Perera DM, Gunasekara D, Wijekoon A, Petrou M.

Thalassaemia control in developing countries--the Sri Lankan perspective. Ceylon Med J. 2000 Mar;45(1):12-6.

Rhoads GG, Jackson LG, Schlesselman SE, de la Cruz FF, Desnick RJ, Golbus MS, Ledbetter DH, Lubs HA, Mahoney MJ, Pergament E, et al.

The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. N Engl J Med. 1989 Mar 9;320(10):609-17.

Olivieri NF, Weatherall DJ.

The therapeutic reactivation of fetal haemoglobin. Hum Mol Genet. 1998;7(10):1655-8.

Lukens JN., Pearson HA.

Ferrokinetics in the syndrome of familial hypoferremic microcytic anemia with iron malabsorption. J Pediatr Hematol Oncol. 1999 Sep-Oct;21(5):412-7.

Swart PJ., Kuipers ME, de Vries HG, Eikelboom MC, Meijer DK

Synergistic fungistatic effects of lactoferrin in combination with antifungal drugs against clinical Candida isolates. Antimicrob Agents Chemother. 1999 Nov;43 (11):2635-41.

Hoffmann W,Angelika L, Graf N.

[Cardiac dysfunction in children with thalassemia major] Klin Padiatr. 1988 Mar-Apr;200(2):102-7. German.

Ragab L, Ibrahim HA, Eid AS, Kotb T, Konsowa MF.

Suitability of soluble transferrin receptor for the clinical diagnosis of different types of anaemia in children. East Mediterr Health J. 2002 Mar-May;8(2-3):298-307.

Al-Refaie FN, Hershko C, Hoffbrand AV, et al.

Results of long-term deferiprone (L1) therapy: a report by the International Study Group on Oral Iron Chelators. Br J Haematol. 1995;91:224–9.[Medline]

Hershko C, Hoffbrand AV,al-Refaie FN, Kosaryan M, Olivieri NF, Tondury P, Wonke B.

Results of long-term deferiprone (L1) therapy: a report by the International Study Group on Oral Iron Chelators. Br J Haematol. 1995 Sep;91(1):224-9.

Anderson LJ, Westwood M, Pennell DJ.

Treatment of cardiac iron overload in thalassemia major. Haematologica. 2003 May;88(5):481-2.

Alter BP.

Bone marrow failure syndromes in children. Pediatr Clin North Am. 2002 Oct;49(5):973-88.

David G. Nathan (Editor), Frank A. Oski (Editor), Stuart H. Orkin (Editor) Nathan and Oski's

Hematology of Infancy and Childhood, Vol. Volume #2 (5th edition)

Lewis BA, Orkin SH.

A functional initiator element in the human beta-globin promoter. J Biol Chem. 1995 Nov 24;270(47):28139-44.

Giardina P,Hayward A, Ambruso D, Battaglia F, Donlon T, Eddelman K, Giller R, Hobbins J, Hsia YE, Quinones R, Shpall E, Trachtenberg E.

Microchimerism and tolerance following intrauterine transplantation and transfusion for alpha-thallassemia-1. Fetal Diagn Ther 1998;13:8-14.

Alan R Liss, 1988.

Thalassemia: pathophysiology and management, part A. New York: [review].

Shechar Dworski, BSc, (1998).

Geriatrics & Aging Article. Osteopenia--Fracture Risk Doubles Every Decade Past Age 50.Volume 1, Number 3, NovemberDecember 1998, Pages 2,24

Sir John V. Dacie and SM Lewis (1996):

Investigation of abnormal haemoglobins and thalassemia. Practical haematology New York and Tokyo P., 249-86.

Thein SW (1993):

β-thalassemia. In the hemogbloinopathies. Baillier's clinical haematology, Eds DR. Higgs and DJ Weatherall, Vol. 6: 151.

Bunn HF, and Forget BG (1986):

Hemogbloin: molecular, genetic and clinical aspects Saunders, Philadelphia.

Weatherall DJ et al. (1981):

Molcular basis for mild forms of homozxygous β-thalassemia lancet 1: 527.

Cohen A et al. (1980): Transfusion requirements and splenectomy in thalassemia major. J Pedi., 97: 100.

Weatherall DJ, Clegg JB, Higs et al. (1995):

The hemoglobinopathies. In: the metabolic basis of inherited disease (C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Valle, eds.), pp 3417-3484. New York: McGraw-hill.

Weatherall DJ and Clegg JB (1981):

The thalassemia syndromes, 3rd ed., Blackwell scientific publications, Oxford.

Kyriakou D, Eliopoulos AG, Papadakis Absent, Alexandriakis M, Eliopoulos GD (1998):

Decreased expression of cmyoncprotein by peripheral blood mononuclear cells in thalassemia patients receiving desferrioxamine. Eur J Haematol., 60 (1): 21-7.

Perere DM, Dunasekara D, Wifekoon A, Petrou M (2000):

Thalassemia control in developing countries. The srelankan perspective Cylon Med J 45 (1): 16-16.

Bank A (2005):

Understanding globin regulation in β -thalassemia: It's as simple as α,β,γ,δ. The Journal of Clinical Investigations. Volume 115, No. 6. p. 1470-73.

List of Contents

List of Tables

List of Tables (Cont..)

List of Figures

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INTRODUCTION

AIM OF THE WORK

MOLECULAR PATHOLOGY, PATHOPHYSIOLOGY AND CLINICAL FORMS OF THALASSEMIA

PATHOPHYSIOLOGY OF BONE DISEASE IN THALASSSEMIA

OSTEOPOROSIS

OSTEOPOROSIS IN THALASSEMIA

CONTROL AND PREVENTION OF BETA-THALASSEMIA

PATIENTS AND METHODS

RESULTS

DISCUSSION

CONCLUSION

RECOMMENDATIONS

SUMMARY

REFERENCES

ARABIC SUMMARY

تقييم الكثافة المعدنية العظيمة وربطها بالتغيرات الكيميائية فى مرضى أنيميا البحر الأبيض المتوسط من نوع بيتا

بروتوكول رسالة توطئة للحصول على درجة الماجستير

فى أمراض الدم

مقدم من

الطبيب/ إبراهيم محمد عثمان

بكالوريوس الطب والجراحة

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٢٠٠٥

الملخص العربي

تعتبر هشاشة العظام من الأمراض التي لوحظ ارتباطها بمرض أنيميا البحر المتوسط بصورة متكررة وقد ساعدت الأساليب الحديثة في العلاج – والعقاقير المستخدمة في إزالة الحديد الزائد من الجسم . على الزيادة في العمر الافتراضي لدي هؤلاء المرضي ونشأ نتيجة لهذه الزيادة ظهور بعض المضاعفات الناتجة إما بسبب المرض أو بسبب العلاج.

وقد قمنا في هذا العمل بتقييم الكثافة العظمية لدي ٤٠ مريضا مصابون بمرض الثلاسيميا –٦٠% منهم من الإناث و ٤٠% من الذكور – والمترددين على مستشفات جامعة عين شمس وعلى الوحدة العلاجية بالشركة المصرية لخدمات نقل الدم التابعة للشركة القابضة للمستحضرات الحيوية واللقاحات (***فاكسيرا***).

كذلك قمنا في هذا العمل بمقارنة تلك الهشاشة العظمية بالتغيرات الكيميائية وكذلك بنسب العناصر النادرة – خاصة عنصر الزنك – لديهم.

قد خضع المرضي للتحاليل والفحوصات التالية:–

(١) تم أخذ تاريخ تفصيلي للمرضي متضمنا التاريخ المرضي ومعدلات نقل الدم ومدي طواعية المريض للعقاقير المستخدمة لإزالة الحديد من الجسم.

(٢) تم إجراء التحاليل المعملية مع تحديد نسب الكالسيوم والفوسفور والماغنسيوم والخارصين ومستوي الفريتين في الدم.

(٣) تم تقييم الكثافة العظيم لديهم باستخدام جهاز الأشعة DEXA.

وقد قمنا بتقسيم المرضي بناء على مدي تأثر الكثافة العظمية لديهم في عظام العمود الفقري وفي عظام الفخذ إلى ثلاث مجموعات.

وقد أكدت نتائج هذا البحث أن مرضي الثلاسيميا تنتشر بينهم معدلات الإصابة بأمراض هشاشة العظام بنسبة عالية، فقد وجدنا أن ٨٥% من المرضي قد تأثرت الكثافة العظيمة لديهم بهذا المرض.

أيضا أكدت النتائج أن مستوي الخارصين يقل بصورة ملحوظة في المرضي المصابين بهشاشة العظام. ومن المعروف أن عنصر الخارصين يلعب دوراً رئيسياً في تكوين الأنسجة الضامة كما أنه يعتبر عاملاً محفزاً للعديد من الأنزيمات التي تستخدم في بناء أنسجة العظم.

كذلك أظهرت النتائج أن نسبة الحديد في الدم كانت مرتفعة في المرضي المصابين بهشاشة العظام وهذا قد يُفسر بتأثير الحديد السلبي علي العظام.

وعندما قارنا بين استخدام العقاقير المزيلة للحديد من الجسم وبين مستوي الخارصين والحديد في المرضي و جدنا أنه بالرغم من الأثر الإيجابي لهذه العقاقير – والمتمثل في تقليل نسبة الحديد الزائدة في الجسم – إلا أن النتائج أظهرت التأثير السلبي لتلك العقاقير على مستوي الخارصين في الدم بل وجد أن المرضي الذين يتعاطون تلك العقاقير بصورة منتظمة هم أكثر عرضة للإصابة بهشاشة العظام مما يتبين لنا أن العلاج أيضا قد يلعب دور في زيادة نسبة هشاشة العظام في مرض الثلاسيميا.