INTRODUCTION

1.1. THALASSEMIA

1.1.1. HISTORY

Thalassemia was first recognized in 1925 by a Detroit physician, Cooley and Lee, who described a series of infants who became profoundly anemic and developed splenomegaly and bone change over the first year of life **(**Cooley and Lee, 1925**).** George and William (1932), described the pathological changes of the condition for the first time, recognized that many of their patients came from the Mediterranean region, and hence invented the word thalassemia from the Greek words (**"**thalassa": meaning sea and ("aima": meaning blood)(Whipple and Bradford, 1932)**.**It was only after 1940 that the true genetic character of this disorder was fully appreciated (Weatherall, 2001)**.**

1.1.2. Definition

Thalassemia are a heterogeneous group of genetic disorder of hemoglobin synthesis characterized by a reduction in the synthesis of one or more of the globins chains leads to imbalanced globin- chain synthesis, defective hemoglobin production causing anemia (Victor et al., 1999).

1.1.3. Classification

The two main types are called Alpha and Beta thalassemia, depending on which part of globin chain is produced in reduced amounts (Victor et al., 1999).

1. 1.3.1. Alpha Thalassemia

Normally, alpha globin chain is made by four genes (two from each parent), two on each strand of chromosome 16.

The alpha thalassemia are caused by a decrease in production of alpha globins chains due to deletion or mutation of one or more of the four alpha globins genes located on chromosome 16 (Hillman and Ault, 2002).

A-Molecular Pathology

Two α thalassemia phenotypes are recognized; one is characterized by thalassemia minor in the heterozygous state and the other is marked by no clinical or hematologic abnormality in the heterozygous state. The former phenotype has been referred to as α -thalassemia 1 and the latter has been labeled α -thalassemia 2. It is now recognized that the α- thalassemia 1 determinants are associated with complete absence of α-globin synthesis and the α thalassemia 2 phenotypes with only a reduction in α-globin synthesis. Accordingly, these two major α thalassemia variants are now designated thalassemia α° and α^{+} Thalassemia (Table1) (Lee et al., 1999).

Alpha (0) thalassemia – More than 20 different genetic mutations that result in the functional deletion of both pair of α-globin genes have been identified. Individuals with this disorder are not able to produce any functional α -globin and thus are unable to make any functional hemoglobin A, F, or A2. This leads to the development of hydrops fetalis, also known as hemoglobin Bart, a condition that is incompatible with extra uterine life.

Alpha (+) thalassemia – There are more than 15 different genetic mutations that result in decreased production of α -globin usually due to the functional deletion of 1 of the 4 alpha globin genes. Based on the number of inherited alpha genes, alpha (+) thalassemia is subclassified into 4 general forms:

- A-Thalassemia (-α/α α) is characterized by inheritance of 3 normal α-genes. These patients are referred to clinically as silent carrier of alpha thalassemia. Other names for this condition are alpha thalassemia minima, alpha thalassemia-2 trait, and heterozygosity for alpha (+) thalassemia minor. The affected individuals exhibit no abnormality clinically and may be hematologically normal or have mild reductions .
- B- Inheritance of 2 normal alpha genes due to either heterozygosity for alpha (+) thalassemia (-α /- α) (one from each of two chromosomes) called a "trans deletion"
- Or homozygosity for alpha (+) thalassemia (α α/--) (two on the same chromosome) called a "cis deletion" results in the development of alpha thalassemia minor or alpha thalassemia-1 trait. When parents are carriers of the cis deletion, there is a one in

four, or 25 percent, chance with each pregnancy, to have a baby with alpha thalassemia major.

- C- Inheritance of one normal alpha gene (-α/--) results in abundant formation of hemoglobin H composed of tetramers of excess beta chains. This condition is known as Hb H disease..
- D- The loss of all four alpha genes produces a condition that is incompatible with life. The gamma chains produced during fetal life associate in groups of four to form an abnormal hemoglobin called "hemoglobin Bart's" (Forget, 2000)..

B-Pathophysiology

The pathophysiology of alpha thalassemia is different to that of beta thalassemia .A deficiency of α chain leads to the production of excess chains or β chains , which form Hb Bart's and Hb H respectively . These soluble tetramers do not precipitate in the bone marrow and hence erythropoiesis is more effective than in β thalassemia. However, Hb H is unstable and precipitates in red cells as they age. The inclusion bodies produced in this way are trapped in the spleen and other parts of the microcirculation leading to shortened red cell survival. Furthermore, both Hb Barts and Hb H have a very high oxygen affinity; because they have no α chains, there is no haem-haem interaction and their oxygen dissociation curves resemble myoglobin (Victor et al., 1999).

There are four subtypes of alpha thalassemia that range from mild to sever in their effect on the body (Cohen et al., 2004).

(1)Silent carrier state

This is the one-gene deletion alpha thalassemia condition. This condition generally causes no symptoms or signs of anemia and will not need treatment because the lack of alpha protein is so small that the hemoglobin functions normally (Hillman and Ault, 2002).

It is called "silent carrier" because it is difficult to identify α thalasemia silent carrier state by standard haematological studies. They are detected only by DNA Studies (Forget, 2000).

(2) Alpha Thalassemia Trait

Also known as mild alpha-thalassemia. These patients have lost two alpha globin genes. Patients with this condition have small red cells and a mild anemia but they do not have clear symptoms. They look and feel normal but may be discovered upon routine testing (Hillman and Ault, 2002).

(3) Alpha Thalassemia Intermedia

Also known as hemoglobin H disease . These patients have lost three alpha globin genes. Patients with this condition have a severe anemia, and often require blood transfusions to survive. Infants born with alpha thalassemia intermedia appear normal at birth but often develop anemia and splenomegaly by the end of their first year. Hepatomegaly is not a common finding and there may be some association

with mental retardation. Due to the hemolytic nature of this anemia, there may be an increase in respiratory infections, leg ulcers and gallstones. Skeletal changes are not commonly seen in hemoglobin H disease (Cohen et al., 2004).

The severe imbalance between the alpha chain production (now powered by one gene, instead of four) and beta chain production (which is normal) causes an accumulation of beta chains inside the red blood cells. Normally, beta chains pair only with alpha chains. With three-gene deletion alpha thalassemia, however, beta chains begin to associate in groups of four, producing abnormal hemoglobin, called "hemoglobin H". The condition is called "hemoglobin H disease".

Hemoglobin H has two problems. First it does not carry oxygen properly, making it functionally useless to the cell. Second, hemoglobin H protein damages the membrane that surrounds the red cell, accelerating cell destruction. The combination of the very low production of alpha chains and destruction of red cells in hemoglobin H disease produces a severe, life-threatening anemia. Untreated, most patients die in childhood or early adolescence (Forget, 2000).

(4)Alpha Thalassemia Major

Also known as hydrops fetalis. In this condition, there are no alpha genes in the individual's DNA, which causes four gamma globins produced by the fetus to form abnormal hemoglobin called hemoglobin Bart's. Most individuals with this condition die before or shortly after birth (Cohen et al., 2004).

Rarely, four gene deletion alpha thalassemia has been detected in utero, usually in a family where the disorder occurred in an earlier child. In utero blood transfusions have saved some of these children (Forget, 2000).

1.1.3.2. Beta Thalassemia

There are more than 200 of mutation within the beta globin gene found worldwide to produce beta thalassemia .Unlike the deletion that constitute most of the alpha thalassemia syndromes ,beta thalassemia are caused by mutation on chromosome 11 that affect all aspect of beta globin production : transcription ,translation , and the stability of the beta globin production (Howard et al.,1996).

A-Molecular Pathology

There are two types of β thalassemia, ß$^{+}$ and ß° thalassemia, in which there is , respectively a reduction in and total absence of beta chain production(Table1) .Beta thalassemia major usually results from the homozygous state for either ß$^{+}$ or ß° thalassemia, or occasionally from the compound heterozygous state for both ß$^{+}$ and ß° thalassemia. Homozygous ß° thalassemia is associated with a predominance of Hb F, no Hb A ,and variable amounts of Hb A2.In individuals with homozygous ß$^{+}$ thalassemia ,the amounts of Hb A are variable,Hb F is increased and is distributed heterogeneously among red cells, and Hb A2 is normal, decreased , or elevated (Lee et al.,1999).

B-Pathophysiology

The molecular defects in β thalassemia result in absent or reduced β chain production .Alpha chain synthesis is unaffected and hence there is imbalanced globin chain production leading to an excess of α chains. In the absence of their partners, they are unstable and precipitate in the red cell precursors, giving rise to large intracellular inclusions, which interfere with red cell maturation. Hence, there is a variable degree of intramedullary destruction of red cell precursors (i.e. ineffective erythropoiesis). Those red cells that mature and enter the circulation contain α chain inclusion, which interfere with their passage through the microcirculation, particularly in the spleen. These cells, which show a variety of abnormalities of membrane structure and permeability, are prematurely destroyed and thus the anemia of β thalassemia results from both ineffective erythropoiesis and a shortened cell survival. The anemia acts as a stimulus to erythropoietin production and this causes expansion of the bone marrow, which may lead to serious deformities of the skull and long bones. Because the spleen is being constantly bombarded with abnormal red cells, it hypertrophies (Victor et al., 1999).

Table1: Thalassemia Genotypes and Syndromes

Alpha Thalassemia	**α genes**	**Globin Chains**	**Hemoglobin**	**Anemia**
Normal	αα/αα	$\alpha_2\beta_2$	A	None
Silent Carrier	αα/α-	$\alpha_2\beta_2$	A	None
Trait	α-/α- --/αα	$\alpha_2\beta_2$	A	Mild
Hb H disease	--/-α	$\alpha_2\beta_2$, β_4	A, H	Intermediate
Hydrops fetalis	--/--	γ_4, $\zeta_2\gamma_2$	Barts Portland	Lethal
Beta Thalassemia	**β genes**	**Globin Chains**	**Hemoglobin**	**Anemia**
Normal	β/β	$\alpha_2\beta_2$	A	None
Thalassemia minor	β^+/β β°/β	$\alpha_2\beta_2$, $\alpha_2\delta_2$, $\alpha_2\gamma_2$	A, A_2, F	Mild
Thalassemia major	β^+/β^+ β°/β°	$\alpha_2\beta_2$, $\alpha_2\delta_2$, $\alpha_2\gamma_2$ $\alpha_2\gamma_2$, $\alpha_2\delta_2$	A, A_2, F F, A_2	Severe Severe
HPFH*	γ/γ	$\alpha_2\gamma_2$	F	Mild

There are three general categories of beta thalassemia that also range from mild to severe in their effect on the body (Rund and Rachmilewitz, 1995).

(1)Beta thalassemia Minor

Also known as thalassemia Trait. In this condition, one of the two beta globin genes is abnormal but the lack of beta protein is not great enough to cause problems in the normal functioning of the hemoglobin (Rund and Rachmilewitz, 1995).

Alpha chain production continues at a near normal rate. The alpha chains combine with the available beta chains resulting in decreased levels of hemoglobin A here still remains excess alpha chains and this stimulates the increased production of delta chains. The alpha and delta chains combine to form increased amounts of hemoglobin A2.

This if there is still an excess of alpha chains the normal mechanism which switches off gamma chain production does not function correctly and the rate of gamma chain Production is greater than in a normal adult. results in the formation of increased amounts of hemoglobin F (Weatherall, 2001).

A person with this condition simply carries the genetic trait for thalassemia and have a 50/50 chance to pass the gene to their offspring, who would also have thalassemia minor and will usually experience no health problems other than possible mild anemia (Lee et al.,1999).

(2) Beta thalassemia Intermedia

In this condition, an affected person has two abnormal genes but is still producing some beta globin. In this condition the lack of beta protein in the hemoglobin is great enough to cause a moderately severe anemia and significant health problems, including fatigue or shortness of breath, bone deformities, mild jaundice and enlargement of the spleen.(Forget, 2000) There is a wide range in the clinical severity of this condition, and the borderline between thalassemia intermedia and the most severe form, thalassemia major, can be confusing. The deciding factor seems to be the degree of anemia and the amount of blood transfusions required by the patient. The more dependent the patient is on blood transfusions, the more likely he or she is to be classified as thalassemia major(Rund and Rachmilewitz, 1995).

(3) Beta thalassemia Major

It is also called Cooley's anemia , named after the doctor who first described it in 1925 (Cooley and Lee, 1925).

Beta thalassemia Major is the most severe form of beta thalassemia in which the complete lack of beta globin production, preventing the production of significant amounts of Hb A. The severe imbalance of globin chain synthesis (alpha >> beta) results in ineffective erythropoiesis and severe microcytic hypochromic anemia. The excess unpaired alpha-globin chains aggregate to form precipitates that damage red cell membranes, resulting in intravascular hemolysis. Premature destruction of erythroid precursors results in intramedullary death and ineffective erythropoiesis.

The profound anemia typically is associated with erythroid hyperplasia and extramedullary hematopoiesis (Cunningham et al., 2004).

At birth the baby with thalassemia major seems entirely normal. This is because the predominant hemoglobin at birth is still fetal hemoglobin (Hb F). Hb F has two alpha chains (like Hb A) and two gamma chains (unlike Hb A). It has no beta chains so the baby is protected at birth from the effects of thalassemia major.

Anemia begins to develop within the first year after birth. It becomes progressively more and more severe. The infant fails to thrive (to grow normally) and often has problems feeding (due to easy fatigue from lack of oxygen, with the profound anemia), bouts of fever (due to infections) to which the severe anemia predisposes the child and diarrhea and other intestinal problems. Without treatment, the spleen, liver, and heart become enlarged, and bones can become thin and brittle ,the result is death before age twenty (Rund and Rachmilewitz, 1995).

This anemia requires lifelong regular blood transfusions and considerable ongoing medical care. Over time, these frequent transfusions lead to excessive amounts of iron in the body. Left untreated, this excess iron can deposit into the liver, heart and other organs and can lead to a premature death from organ failure(Cunningham et al.,2004).

1.2. EPIDEMIOLOGY

1.2.1. Geographic Distribution

Thalassemia is considered the most common genetic disorder world wide (Illeyman et al ., 2000). Thalassemia is found in some 60 countries with the highest prevalence in the Mediterranean region, parts of North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia, especially Thailand and southern China together composing the so-called thalassemia belt (Hoffman et al., 2005). In Asia, the highest incidence of thalassemia found in Maldives with a carrier rate of 18% of the population (Furuumi et al., 2006) . The estimated prevalence is 16% in people from Cyprus(Yaish, 2007**)** , in Thailand 1% are affected and more than 20 million were thalassemia carriers (Chonnanit et al. ,2005) and 3-8% in populations from Bangladesh , China , India , Malaysia and Pakistan (Hoffman et al., 2005).

In Europe, the highest concentrations of the disease are found in Greece, including the Greek islands; in parts of Italy, lower Po valley (Peres et al., 1996); in southern Italy; and in the Italian islands Sicily, Sardinia (Guiso et al., 1996) , and Malta Corsica (French island) and Crete (Greek islands)(Hoffman et al., 2005). A very low prevalence has been reported from people in northern Europe (0.1%) and Africa (0.9%) (Ballas et al., 1997).

The highest frequency of the alpha thalassemia genes is found in Southeast Asia (Ko and Xu , 1998),Africa (Ballas et al., 1997) and in Mediterranean region including Portugal with incidence of α -thalassemia carriers is (10%)(Peres et al., 1996), (18%) in Sardinians(Guiso et al., 1996), and 7% in Greece(Hoffman et al., 2005).

The population of northern Thailand, with a prevalence of about 5% to 10%, Harbors one of the highest incidences of α-thalassemia in the world (Figure1) (Chonnanit et al. ,2005). About 150 million people worldwide carry ß-thalassemia genes. Beta thalassemias are distributed widely in Many Mediterranean islands, including Cyprus (Yaish, 2007), Sardinia (11-34%)(Hoffman et al., 2005).), and Sicily (10%), have a significantly high incidence of severe beta thalassemia, constituting a major public health problem (Hoffman et al., 2005). For instance, in Cyprus, 1 in 7 individuals carries the gene, which translates into 1 in 49 marriages between carriers and 1 in 158 newborns expected to have β thalassemia major (Yaish, 2007).

The genes are particularly prevalent in Italy and Spain. Other regions with the high gene frequency are Greece (5-15%) (Hoffman et al., 2005).) , Iran (4-10%) (Zlotogora, 1995), and Thailand (1-9%) (Chonnanit et al. ,2005). Many of ß-thalassemia gene mutations detected in Turkish (Hoffman et al., 2005), Kurdish(Cohen and Filon, 1991), Bulgarian (Hoffman et al., 2005), Asian Indian origin (Vaz et al., 2000) and Pakistan (Vaz et al., 2000).

The β thalassemias are rare in Africa, except for some isolated pockets in West Africa, notably Liberia, and in parts of North Africa (Ballas et al., 1997).

High prevalence of both α - and ß-thalassemia is also present in southern China (Ko and Xu, 1998) and Taiwan (Hoffman et al., 2005).

Alpha- and beta-thalassemia are endemic in almost all countries of the Arab world probably due to the historical presence of malaria in the region , and the high level of consanguinity (Zlotogora, 1997).

Many studies indicate that these diseases are common in the Gulf region such as Bahrain (18% in α thalassemia and 11% in β Thalassemia), Oman (6% in α thalassemia and in β thalassemia5,3%), UAE (3% in α thalassemia and in β thalassemia 2,40%) and in Qataris(28% in α thalassemia and 17% in β thalassemia) (Zahed, 2001).

These diseases are also common in the other Arab countries such as Libya (5% in α thalassemia and in β Thalassemia 4%), Tunisia (4,8 in α thalassemia and in β Thalassemia 4,4%), Algeria (9 in α thalassemia and in β Thalassemia 3%) and Jordan (3,3 in α thalassemia and in β Thalassemia 3,5%) (Zahed, 2001).

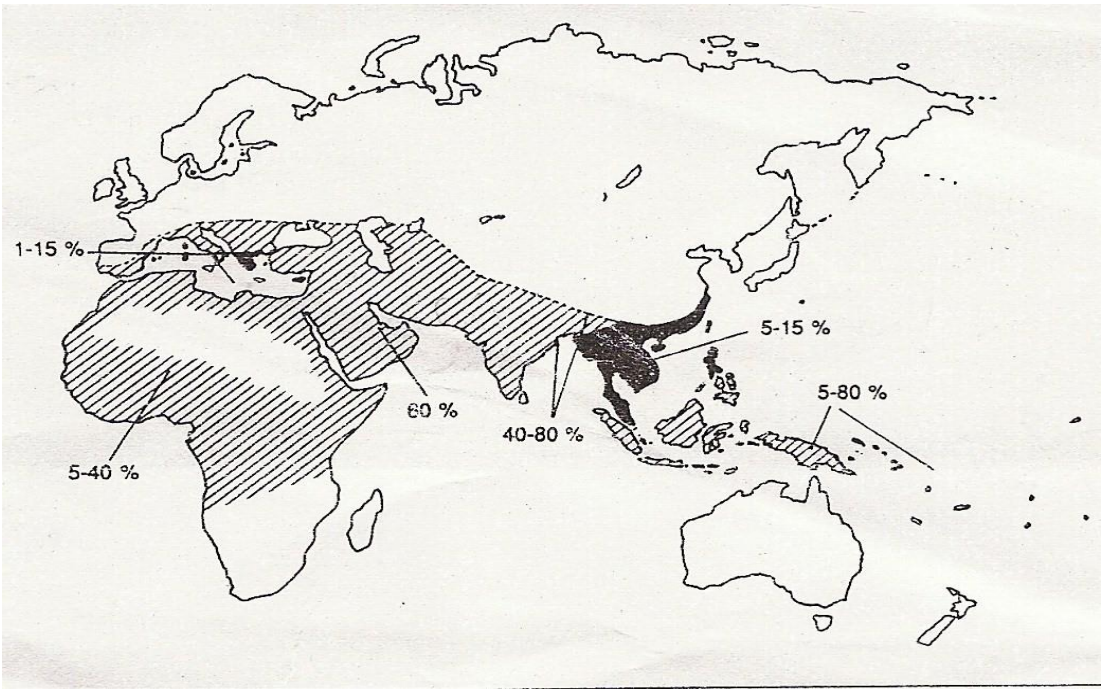

Figure1: The world distribution of α thalassemia

1.2.2.Thalassemia In Saudi Arabia

A)Alpha Thalassemia

The occurrence of α- thalassemia in Saudi Arabia has been recorded since 1969, and the distribution of the genes in the Kingdom has since been studied extensively (El-Hazmi, 1992).

Several studies have shown a high frequency of alpha-thalassemia genes in different region of Saudi Arabia but it is more frequent in the Eastern province than in the Northwestern Province of the country (Qadri, and Islam, 2000). Al-Qatif region in the Eastern Province of Saudi Arabia has the nation's highest frequencies of both Alpha Thalassemia Major and Alpha Thalassemia Trait, hemoglobin H disease(Hb H)also been found (George et al., 2001).

Between September 2000 and November 2001, 20% of patients who were sent to the Hematology Department at Qatif Central Hospital for evaluation had thalassemia comprising 24 females (61.5%) and 15 males (38.5%**), (**89.4 %) patients were thus Classified as mild and (10,6%) as severe Thalassemia (George et al., 2001).

In other study El-Hazmi et al (1992) investigate the molecular basis of deletion type of alpha-thalassemia in 226 subjects from the eastern and 61 subjects from the northwestern regions of the country.

The frequencies of alpha thalassemia trait in Eastern Province of Saudi Arabia including Al-Hafouf (19%(-α/-α) and 2,2% (--/αα)), and in northwestern regions including Kibber the alpha thalassemia trait was (4.9%(-α/-α) and 1%(--/αα)) ,Al-

Ula (0,5%(-α/-α) and 1%(--/αα)). The frequencies of silent carrier alpha thalassemia (-α/ αα) in Al-Hafouf (36.7%), Al-ola (12,1%), and Khaiber (14.7%).

The frequency of HbH disease is low and absence of hydrops fetalis in both regions (El-Hazmi, 1992).

B) Beta Thalassemia

Beta thalassemia is common in the kingdom of Saudi Arabia along the coastal strip of the Red Sea and in the Eastern Province around Jubail , Qateef , Dammam and Hofuf , (Figure 2)(AL-Awamy et al., 2002).

Due to a high prevalence of thalassemia in Saudi Arabia the Saudi Ministry Of Health designed a protocol for premarital testing after the royal decree in December 2003, which was implemented by a February 2004 (Al-Suliman, 2006).

As part of the Saudi Premarital Screening Program, King Fahd Hospital, Hofuf, Saudi Arabia determines the prevalence of beta-thalassemia trait among subjects coming for premarital screening in the Al-Hassa area.

All Saudi participants (n=8918), including 4218 (47.3%) males and 4700 (52.7%) females were screened. The prevalence of beta-thassemia trait with high hemoglobin A2 and microcytic hypochromic anemia was 3.4% (307/8918) (AL-Suliman, 2006).

A total of 488,315 individuals screened, 3.22% had thalassemia trait, and 0.07% had thalassemia disease. Both the diseases were focused mainly in the eastern, western, and southwestern parts of the country. Among the 207,333 couples who were issued certificates for matching, 2.14% were declared high risk (Nasser et al, 2007).

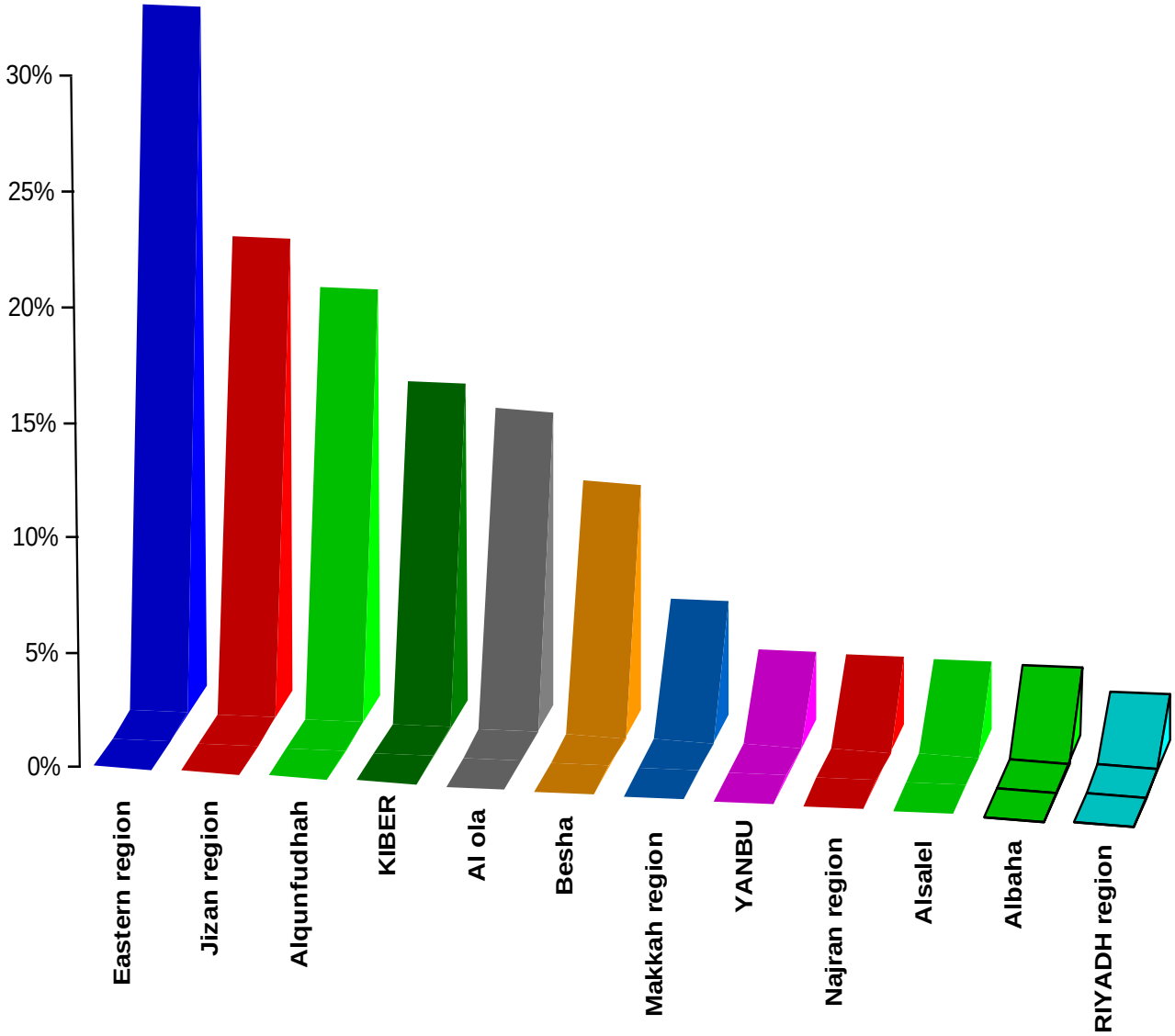

Figure2: Distribution of β thalassemia in different parts of Saudi Arabia

1.3. Clinical features

A-Signs and Symptoms

Alpha thalassemia silent carriers generally have no signs or symptoms of the disorder. People who have alpha or beta thalassemia trait can have mild anemia. However, many people with this type of thalassemia may be asymptomatic or experience very few symptoms. Symptoms may be worse in individuals that are pregnant, under stress, or malnourished. Symptoms may include: Fatigue. This may be the only symptom that an individual with beta thalassemia minor exhibits. Fatigue is caused by the decreased oxygen carrying capacity of the red blood cells, resulting in lowered oxygenation for cells and tissues and the skin color of may be pale (pallor) due to oxygen deprivation in blood (Satwani et al., 2005).

People with beta thalassemia intermedia have mild to sever moderate anemia .In the beta thalassemia intermedia the patients with Hb of much below 7 or 8 gm/dl excess energy consumption due to the profound hemolysis can produce small stature, poor weight gain, poor energy levels, susceptibility to infection and a yellow discoloration (jaundice) of the skin, eyes, and mucous membranes caused by increased amounts of bilirubin in the blood. Further, the extreme activity of the bone marrow produces bone deformities of the face and other areas. People with hemoglobin H disease have severe thalassemia. Signs and symptoms occur within the first 2 years of life. They may include severe anemia and other serious health problems, such as: Pale and

listless appearance, Poor appetite, Dark urine. Beta thalassemias major (also called Cooley's anemia) has severe thalassemia .Affected infants fail to thrive and gain weigh normally and become progressively pale. Feeding problems, diarrhea, irritability, fever and progressive enlargement of the abdomen due to splenomegaly and prominence of the cheek bones tends to obscure the base of the nose and to expose the upper teeth, puffiness of the eyelid and a tendency to a Mongoloid slant of the eyes are common presenting symptoms (Cunningham et al., 2004).

B-Complications

Over the past three decades, regular blood transfusions have dramatically eliminated the complications of thalassemia and compensatory bone marrow expansion, improved the quality of life permit normal development throughout Childhood and extend survival. But, transfusion results in a complication due to iron overload (Satwani et al., 2005).

Heart disease is the most important complication and the main determinant of survival. It is responsible for more than half of the deaths. It may take the form of hypertension and heart failure (Borgne et al., 2005). Endocrine complications include diabetes Mellitus, hypothyroidism, hyperparathyroidism, hypogonadism and delayed puberty (Shamshirsaz et al., 2003).

Endocrine complications along with trace elements deficiency and other metabolic disturbances also lead to growth failure and short stature (Low Louis, 2005).

Less significant complications include hepatic Involvement (Perifanis et al., 2005) and neurological complications (Zafeiriou et al., 2006).

In untransfused thalassemic patients, the spleen, liver, heart, and bone marrow become greatly enlarged. Expansion of marrow cavities and thinning of cortices produce a variety of bone abnormalities in patients who are not optimally transfused (Satwani et al., 2005). Result of bone biopsies from untransfused thalassemic patients show osteoporosis with increased bone resorption, decreased mineralization, and a decreased number of bone forming sites (Low Louis, 2005).

1.4. Diagnosis

The step in the diagnosis of the different forms of thalassemia include the initial recognition of the disease as thalassaemic disorder its further definition appropriate hematological and hemoglobin analytical procedures and its differentiation from other congential and acquired disorder of hemoglobin synthesis which can mimic the thalassemia syndromes (Satwani et al., 2005).

1.4.1. Hematological and Hemoglobin analysis

A) Alpha thalassemia

In silent carrier state Patients are essentially asymptomatic and the CBC, hemoglobin electrophoresis, and peripheral smear are usually normal. Slight hypochromia and

microcytosis may be evident by microscopic evaluation. The hydrops fetalis syndrome is recognized by the finding of a hydropic infant with a severe anemia , a thalassemic blood picture, and the presence of 80% or more Hb Barts on hemoglobin electrophoresis (Borgne et al., 2005) Hemoglobin H disease is identified by the finding of a typical thalassaemic blood picture with elevated reticulocyte count , multiple inclusion bodies in the red cells after incubation with brilliant crysyl blue, and the finding of variable amount of Hb H on hemoglobin electrophoresis. In alpha thalassemia minor the red cell is abnormal with microcytosis, hypochromia ,and elevated amount of Hb barts noted (3%-8%) (Satwani et al., 2005).

B) Beta thalassemia

The homozygous for the sever form of beta thalassemia are easily recognized by the hematological change with very high level of Hb F;Hb A2 values.The heterozygous states are recognized by microcytic hypochromic red cells and elevated level of Hb A2 (Cunningham et al., 2004).

1.5. Prognosis

When thalassemia was first described, the prognosis for patients was poor because the condition was incompatible with life. However, as research into this debilitating disease progressed, it was discovered that the 'prognosis' of Thalassemia usually refers to the likely outcome of Thalassemia. The prognosis of Thalassemia may include the duration of Thalassemia, chances of complications of Thalassemia, probable outcomes, prospects for recovery, recovery period for Thalassemia, survival rates,

death rates, and other outcome possibilities in the overall prognosis of Thalassemia (Musallam et al., 2008**)**.The prognosis and survival of thalassemia depend on the severity of the disease, for people with β-thalassemia minor or α-thalassemia minor life expectancy is normal and for people with Hb H disease is varies, the outlook is dependent on the clinical course and the state of the liver and spleen (Borgne et al.,1998) . Life expectancy of patients with thalassemia major has significantly increased in recent years, as reported by several groups in different countries. However, complications are still frequent and affect the patients' quality of life. In a recent study from the United Kingdom, it was found that 50% of the patients are died before age 35. At that age, 65% of the patients from an Italian long-term study were still alive. Heart disease is responsible for more than half of the deaths. The prevalence of complications in Italian patients born after 1970 includes heart failure in 7%, hypogonadism in 55%, hypothyroidism in 11%, and diabetes in 6%. Similar data were reported in patients from the United States (Borgne et al., 2005).

1.6. Biochemical changes

A variety of biochemical changes have been observed. There have been conflicting reports about the levels of certain trace metals in the blood of thalassaemic patients (Shamshirsaz et al., 2003). Several workers have observed increased serum cupper (Fuchs et al., 1996) and decreased of serum zinc levels (Arcasoy et al., 2001; Bekheirnia et al., 2004).Studies also demonstrated decreased serum magnesium levels (Fuchs et al., 1996; Joiner, 1993) in the blood of thalassemic patients. Furthermore, some studies have reported Phosphorus and Calcium were within normal level (Chao and Hwang, 1996;Eshghi et al., 2007) but this result are

disagrees with other studies that found low serum Ca levels(Aleem et al., 2000;Zafeiriou et al., 2006) and high Phosphorus levels (Pratico et al., 1998) in the serum of thalassemic patients . In the study of Al-Samarrai et al. (2008), one hundred and five thalasemic blood transfusion, level of some essential elements in thalassemic patients in Mosul, Iraq were measured including Zinc(Zn), Magnesium(Mg), Cupper (Cu), Calcium(Ca) and Phosphorus(P). In this study, the thalassemic patients showed low levels of serum Zn and high levels of Cu in comparison with the control group. The etiologic factor of Zn efficiency in thalassemic patients is reduced Zn and chelation therapy. Urinary loss of Zn is another factor that may contribute to the Zn deficiency, and an increase of glomerular filtration rate of Zn can also be responsible for hyperzincuria resulting from the release of Zn from hemolyzed red cells.

Hypercupremia in thalassemic patients remains unclear. Iron absorption needs Cu containing enzymes and cofactor, and it affects the release of iron in Hb synthesis. Hypercupremia occurs in acut and chronic infection and hemochromatosis, which is a principle complication of thalassemia (Beutler et al.,2001). Low serum Ca levels and high Phosphorus levels were found in 20.9% of the patients, whereas, in 79.1% of patients the mean values of the two element were within normal limits .These result are in agreement with the finding of other studies (Aleem et al., 2000;Zafeiriou et al., 2006). Hypocalcemia and hyperphosphatemia in these patients seem to be related to hypoparathyrodism (HPT) which is syndrome associated with thalassemia major .The cause of HPT is assumed to be due to iron deposition in parathyroid glands. Serum Mg was low in thalassemic patients than control. Hypomagnesemia may occur due to HPT resulting from iron overload (Al-Samarrai et al., 2008).

1.7. Osteoporosis

To appreciate the pathogenesis of osteoporosis, one must understand normal bone structure and physiology.

1.7.1. Structure of Bone

Bone is a hard connective tissue consisting of cell embedded in a matrix of mineralized ground substance and collagen fibers (Bryan and Gerard , 2005).
Bone is an extremely complex tissue that regulates its mass and architecture to meet two critical and competing responsibilities: one structural and the other metabolic .In the first case, the skeleton provides a sophisticated framework for the body; it protects vital organs and facilitates locomotion. Second, it serves as a mineral reservoir that contains 99 percent of the body total calcium, 85 percent of its phosphorus, and 66 percent of its magnesium (Kelley et al., 1997).
The 206 bones in the body serve several other purposes. The bone marrow is responsible for blood cell production also the bone support the soft tissue of the body and gives it form and shape (Bryan and Gerard , 2005).

1.7.2. Macroscopic Structure of Bone

Macroscopically the skeleton is composed of two different types of bone.
A-Compact bone (cortical bone)
Cortical bone comprises 80% of adult skeletal mass. Cortical bone forms a covering over all the bones, but is especially developed to form the cortices of the shafts of the long bones (Bryan and Gerard , 2005). Cortical bone has a similar chemical

composition to trabecular bones, but usually differs in a systematic way with regard to its relative abundance of other minor elements (Kelley et al., 1997).

B-Spongy bone (trabecular bone, cancellous bone)

Trabecular bone form about 20% of adult skeletal mass .It is spongy in appearance and provides strength and elasticity. It comprises the trabecular lining of the marrow cavities and constitutes a major portion of the axial skeleton and ends of long bones (Kelley et al., 1997).

1.7.3. Bone Remodeling Process

Bone is not at all dead material but is metabolically very active indeed. All adult bone is tightly controlled via a dynamic and continuous process called bone remodeling cycle (Garnero, and Delmas, 2002).

The bone metabolism always occurs on the surface of bone at focused sites, each of which is termed a bone multicellular unit (BMU) .Bone remodeling Process described many years ago by Frost and others in 1963as illustrated in (Figure 3 -6) (Lawrence, 2005). The purpose of remodeling is to regulate calcium homeostasis, repair micro-damaged bones (from everyday stress) but also to shape and sculpture the skeleton during growth (Garnero, and Delmas, 2002).

They can occur either on the surface of trabecular bone as irregular howship lacunae or in cortical bone as relatively uniform cylindrical haversian systems (Lawrence, 2005). Bone remodeling consists of two distinct stages – resorption and formation. It

starts with the removed old bone from the skeleton and then continues with the formation of new bone.

Bone remodeling process involves the activity of special cells called Osteoclasts and Osteoblasts (Figure 3-6) (Lips, 2001).

1-Osteoblasts

Osteoblasts are typically oval, with a large eccentric nucleus, and the cytoplasm element of the osteoblast include abundant endoplasmic reticulum, Golgi bodies and numerous free ribosome. The Osteoblasts principal function is to form bone, first with secretion of the osteoid and then with subsequent nucleation and mineralization of the osteoid matrix to become bone (Bryan and Gerard , 2005). During this process the osteoblast seems to incarcerate itself within this newly formed bone to become an osteocyte .The function of osteocyte are liberating some of the bone salt from the calcified matrix by what has been called osteocytic osteolysis (Kelley et al., 1997). Osteoblasts have a high intracellular concentration of the zinc containing enzyme alkaline phosphatase, which play a major role in the formation of the mineral deposits in the matrix, 90% of which is type I collagen .The collagen fibers are synthesized and secreted by the osteoblast (Resnick, 1996).

2-Osteoclasts

Osteoclasts are the largest of the bone cells and are multinuclear (with up to50 nuclei). Osteoclasts are possess large numbers of mitochondria, vacuoles, and lysosomes and have well- developed Golgi bodies (Bryan and Gerard , 2005).

They are located on bone surfaces in what are called Howships lacunae or resorption pits. These lacunae, or resorption pits, are left behind after the breakdown of bone and often present as scalloped surfaces (Bryan and Gerard , 2005). Because the Osteoclasts are derived from a monocyte stem cell lineage, they are equipped with engulfment strategies similar to circulating macrophage (Resnick, 1996).
Osteoclast function is specialized for bone resorption. The highly polarized nature of the cell allows secretion of protons from the proton pump into the resorption bay. The protons accumulate within this confined subosteoclastic space, lowering the PH of this micro milieu to a level sufficient to dissolve the mineral phase of the matrix and activate osteoclastic hydrolytic enzymes. The organic matrix that remains is subsequently dissolved by lysosomal enzymes released across the ruffled border. Leaving the Osteoclasts signature scalloped resorption cavities (Bryan and Gerard , 2005). Absence of Osteoclast leads to osteoporosis which can be fatal in childhood (Resnick, 1996).
In normal bone both processes are in balance during the second to approximately the fourth decade of life (Hubert et al., 2004).
An excess of resorption over formation leads to the bone loss and increased propensity to fracture that is characteristic of osteoporosis (Stephen, 2005).

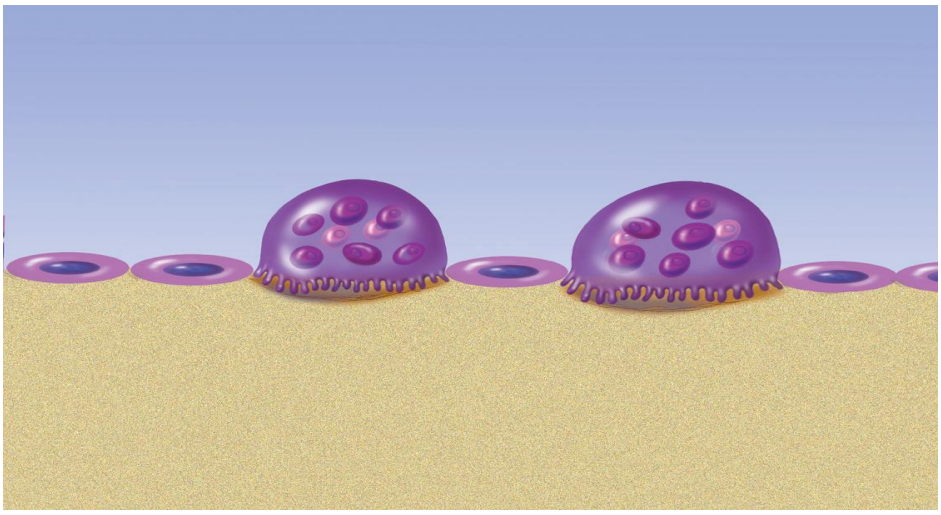

Figure 3: Bone Resorption

In this phase, cells that break down bone – called osteoclasts – act on the trabecular bone surface to erode the mineral and matrix.

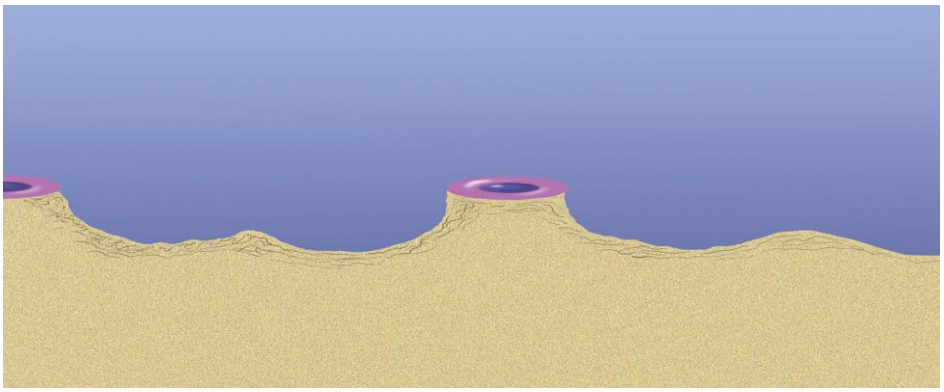

Figure 4: Bone Resorption Complete

This phase is complete when small cavities are created in the surface of the trabecular bone (bone has been removed).

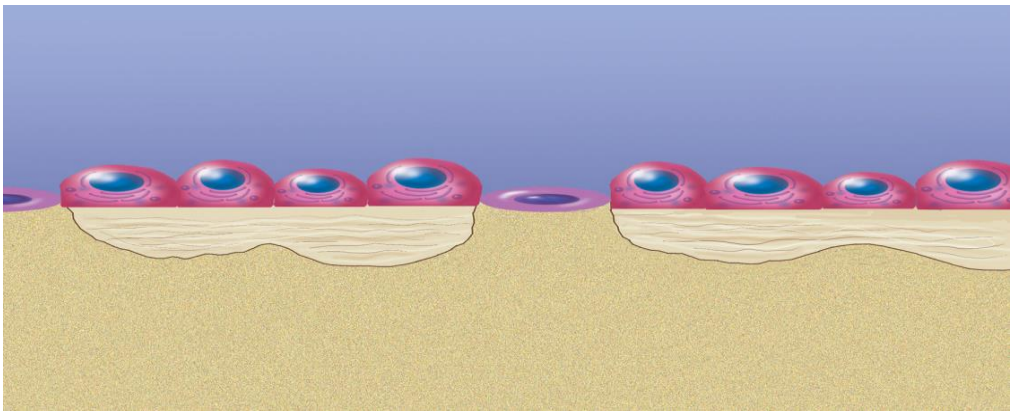

Figure 5: Bone Formation

Cells that form new bone – called osteoblasts – work to repair the surface and fill the eroded cavities with new bone that then has to be mineralized (calcified).

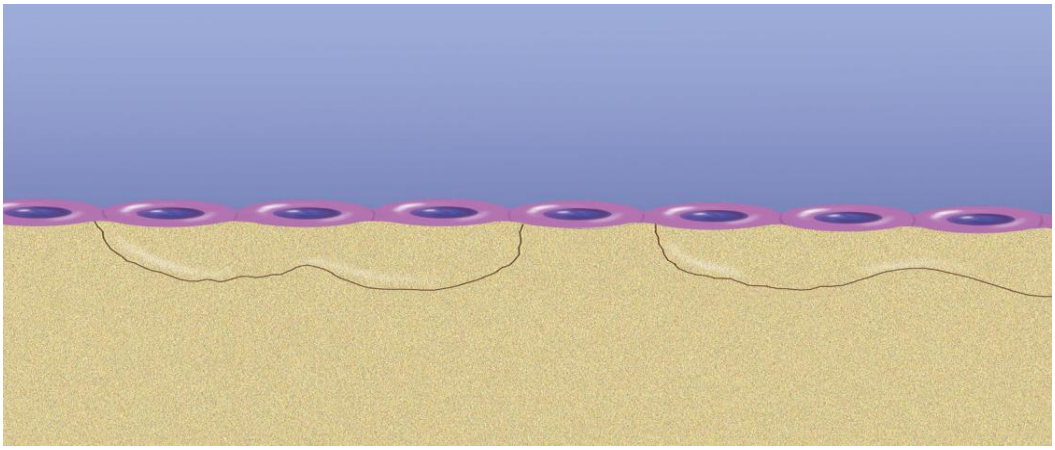

Figure 6: Completion

The bone surface is restored and covered by a layer of protective bone cells called lining cells. The new bone is calcified and the remodeling process is completed

1.7.4. Biochemical markers of bone Remodeling

Biochemical markers of bone turnover are substances found in blood and urine that reflect rates of bone resorption or bone formation (Markus, 2005).

A-Markers of Bone Formation

Bone formation markers are products of active osteoblasts expressed during different phases of osteoblast development. They are considered to reflect different aspects of osteoblast function and of bone formation. All markers of bone formation are measured in serum or plasma (Markus, 2005).

1-Serum Total Alkaline Phosphatase (ALP)

ALP is a ubiquitous, membrane-bound tetrameric enzyme attached to glycosyl-phosphatidylinositol moieties located on the outer cell surface. The precise function of the enzyme is yet unknown, but it obviously plays an important role in osteoid formation and mineralization. AP reflects Osteoblast activity in bone, so any increase in ALP is reflects increased release from Osteoblasts reflecting cellular activity of Osteoblasts (Langlois et al., 1994). ALP measured in serum, but lacks sensitivity and specificity for osteoporosis, because it can be elevated or decreased with many diseases. The total AP (TAP) consists of several dimeric isoforms, which originate from various tissues: liver, bone, intestine, spleen, kidney, and placenta (Magnusson et al., 1999).

2-Osteocalcin (OC)

OC is also known as bone gamma- carboxyglutamic acid. It is a major non-collagen protein found in bone and dentin. OC is a 5.8 kDa, hydroxyapatite-binding, protein exclusively synthesized by osteoblast, and one of the major characteristics of OC is 3 vitamin-K dependent, gamma-carboxyglutamic acid (Gla) residues, which are responsible for the calcium binding properties of the protein (Gundberg and Nishimoto, 1999). At its carboxy-terminus amino acid protein rich in (glutamic acid), OC can also interact with other proteins, including cell surface receptors. Earlier research has suggested that OC is involved in the process of osteoid mineralization, as the protein is expressed mainly during this phase of bone formation (Markus, 2005).

OC is considered a specific marker of osteoblast function. It is estimated that, directly after its release from osteoblasts, the largest part of the newly synthesized protein is incorporated into the extracellular bone matrix where it constitutes approximately 15% of the non-collagenous protein fraction. A smaller fraction is released into the circulation where it can be detected by immunoassays (Baumgrass et al., 1997)**.**

B-Markers of Bone Resorption

The majority of bone resorption markers are degradation products of bone collagen. Collagen type 1 represents over 90% of the protein in bone, and it is natural that many bone markers are derived from released collagen fragments (Markus, 2005).

1-**Hydroxypyridinium Crosslinks of Collagen Pyridinoline (PYD) and Deoxypyridinoline (DPD)**

Hydroxypyridinium cross-links of collagen, PYD and DPD are formed during the extracellular maturation of fibrillar collagens during bone resorption, crosslinked collagens are proteolytically broken down and the crosslink components are released into the circulation and the urine (Lips, 2001). The two hydroxypyridinium components show a high specificity for skeletal tissues. While PYD is found in cartilage, bone, ligaments and vessels, DPD is almost exclusively found in bone and dentin. Thus, the pyridinium crosslinks are currently viewed the best indices for assessing bone resorption (Markus, 2005).

2-Collagen Cross-links (NTX, CTX)

CTX and NTX are a specific breakdown product of the type-I collagen released as amino and carboxy-terminal fragment of collagen during bone resorption with cross links attached (Markus, 2005). NTX and CTX are measured in the urine or serum. They are used as a marker of bone turnover which considered a specific sensitive marker of Osteoclast function.

Levels of CTX and NTX are high in children, who have a great deal of bone resorption associated with growth and modeling of the ends of the long bones. NTX or CTX tests help to assess the activity of other diseases such as Paget's disease or metastatic bone but these tests should not be used for routine screening for osteoporosis (Woitge et al., 1999).

1.7.5. Definition of osteoporosis

Osteoporosis is defined as a systemic skeletal disease characterized by: Low bone mass, Micro architectural deterioration of bone tissue,increased bone fragility and susceptibility to fracture (Raisz, 2005). Osteoporosis occurs when there is a loss of bone mineral density (BMD),increase bone resorption or as a result of imbalance between formation and resorption (Kanis, 1997).

1.7.6. Classification of Osteoporosis

Osteoporosis is classified into subsets based on clinical features, presence of other conditions, and the relationship of disease to age (Kanis, 1997).

A-Primary Osteoporosis

In those cases no leading risk factor or causal factor is evident (Table 2). Primary Osteoporosis occurs in 80% of cases.

B-Secondary osteoporosis

Secondary osteoporosis is approximately 20%of the cases. They are occurs as a result of well-defined feature of another disease, or medical therapy .Both lead to significantly accelerated bone loss (Kanis, 1997).

Secondary osteoporosis, also known as high-turnover osteoporosis, is a condition of an increased rate of bone remodeling-or an increase in the amount of bone remodeled. This condition causes an overall increase in the rate of bone loss (Raisz, 2005). Secondary osteoporosis can also have four hormonal causes (Poole and Compston, 2006):

- Hyperparathyroidism
- Hyperthyroidism
- Hypercortisolism
- Diabetes

Secondary osteoporosis can also be the result of disorders where the bone marrow cavity expands at the expense of trabecular bone for example (Poole and Compston, 2006):

- Thalassemia
- Multiple myeloma
- Leukemia

Table2: Classification of osteoporosis

Classification of osteoporosis
A-primary osteoporosis
– Postmenopausal type I
(trabecular bone: spine fracture)
– Senile type II
(Compact bone: hip fracture)
B-Idiopathic(both sexes)
Juvenile
Adult

1.7.7. Diagnosis of Osteoporosis

Diagnosis of osteoporosis is made with BMD (bone mineral density) testing. BMD(also called bone densitometry) is the measurement of a bones mineral content and bone mineral density (Poole and Compston , 2006). Several types of bone densitometry are used today to detect bone loss but the gold standard for diagnosis of osteoporosis is Dual energy X-ray absorptiometry (DXA) because it is fast , non-invasive, accurate precise, short scan time, low radiation dose (less than a chest radiograph), reasonable cost and requires no injections ,special diet, sedation or any other advance preparation (Kanis, 2002). DXA scans are primarily used to evaluate bone mineral density, measure total body composition and fat content, detect osteoprosis before a fracture occurs ,confirm a diagnosis of osteoprosis, predict the chances of developing a fracture in the future , and monitor the effects of treatment .

It is the most widely used method both in research studies and in clinical practice (Dennison and Cole, 2005).

1.8 OBJECTIVES

The aim of this work is to Study the morbidity of bone disease (osteopenia and osteoporosis) in thalassemia patients by assess bone mineral density (BMD) at L2 , L4 and total BMD, T-score at L2 , L4 and total T-score in patients with thalassemia by using dual X-ray absorptiometry (DXA) and to determine some biochemical changes on the serum of these patients that may affect BMD such as Calcium , Magnesium , Phosphorus and Parathyroid Hormone.

Bone turnover in patients with thalassemia will be studied by assessment of bone formation marker (Osteocalcin (OST) and Bone-specific Alkaline Phosphatase (BAP)) and bone resorption marker (C-terminal telopeptides of type I collagen (CTX) , N-terminal telopeptide of type I collagen (NTX) , Pyridinoline crosslinks (PYD) and Total Deoxypyridinoline crosslinks (tDPD)) .