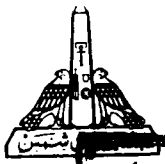

Study Of The Effect Of Seasonal Variation On Bone Growth And Osteocalcin Level In Children-Egypt

Thesis

Submitted for Fulfillment of Ph.D Degree in Childhood Studies-Medical Department

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بسم الله الرحمن الرحيم

"قالوا سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم"

صدق الله العظيم

(سورة البقرة - الآية ٣٢)

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Manal Fahmy Gohar

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List of Abbreviations

1 α (OH) D	: 1 α –hydroxy vitamin D
1,24,25 $(OH)_3$ D	: 1,24,25 trihydroxy vitamin D
1,25 $(OH)_2$ D	: 1,25 dihydroxy vitamin D
1,25 DHCC	: 1,25 dihydroxy cholecalciferol
24,25 $(OH)_2$ D	: 24,25 dihydroxy vitamin D
25 HCC	: 25- hydroxycholecalciferol
25 OHD	: 25 hydroxy vitamin D
ARVDD	: Autosomal recessive vitamin D dependant rickets
BAP	: Bone alkaline phosphatase
BGP	: Bone gamma carboxy glutamic acid containing protein
BSP	: Bone Sialoprotein
Da	: Dalton
G.H	: Growth hormone
G.H.D	: Growth hormone deficiency
GAGs	: Glycosaminoglycans
GFR	: Glomerular filtration rate
Gla	: Gamma carboxyglutamic acid
HHRH	: Hereditary hypophosphatemic rickets with hypercalciuria
I α-HCC	: I alpha hydroxycholecalciferol
I.m	: Intra muscular
IDDM	: Insulin dependant diabetes mellitus
GF-I	: Growth factor I

I.U	: International Unit
LHRH	: Lutenizing hormone releasing hormone
MGP	: Matrix Gla protein
ml	: milliliter
ng	: nanogram
O.C	: Osteocalcin
PG	: Proteoglycans
PTH	: Parathyroid hormone
RIA	: Radioimmunoassay
TAP	: Total alkaline phosphatase
TSH	: Thyroid stimulating hormone
XLH	: X-linked hypophosphatemic rickets
γ- CGlu	: Gamma carboxyglutamic acid

INTRODUCTION
&
AIM OF THE WORK

Introduction & Aim Of Work

Osteocalcin is the most abundant non-collagenous protein in the bone matrix and is partly released in blood ***(Tarallo et al., 1990).***

Osteocalcin or bone gamma carboxy glutamic acid containing protein (BGP) is a protein synthesized by osteoblasts and incorporated in the bone matrix. It is a sensitive marker of bone formation and it parallels the growth velocity curve during childhood and adolescence ***(Lippincott and Wilkens, 2000).***

Osteocalcin accounts for approximately 25% of non collagenous protien found in adult bone and is therefore the most abundant protein in the human skeleton (about 15 g/kg body weight of man). Mineralized bone and dentin matrices contain high concentration of osteocalcin , whereas osteoid and predentin do not ***(Hochberg and karger 2002).***

The synthesis of osteocalcin is under the effect of 1,25(OH)2 D , the active metabolite of vitamin D ***(Long et al. ,1999).***

The carboxylated from of osteocalcin (a vitamin K dependent process) favour the deposition of hydroxyapatite in the organic bone matrix and hence the regulation of bone mineralization ***(Fewtrell et al., 2000).***

The aim of this study is to investigate possible effect of seasons on the level of serum osteocalcin in infants to determine the effect of seasons on bone growth.

REVIEW OF LITERATURE

Osteocalcin

A.Structure:

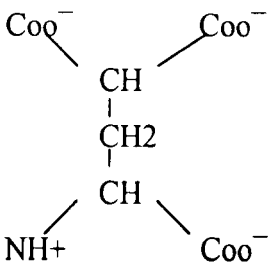


Fig.(1): Structure of Osteocalcin

(Peterson et al., 1992).

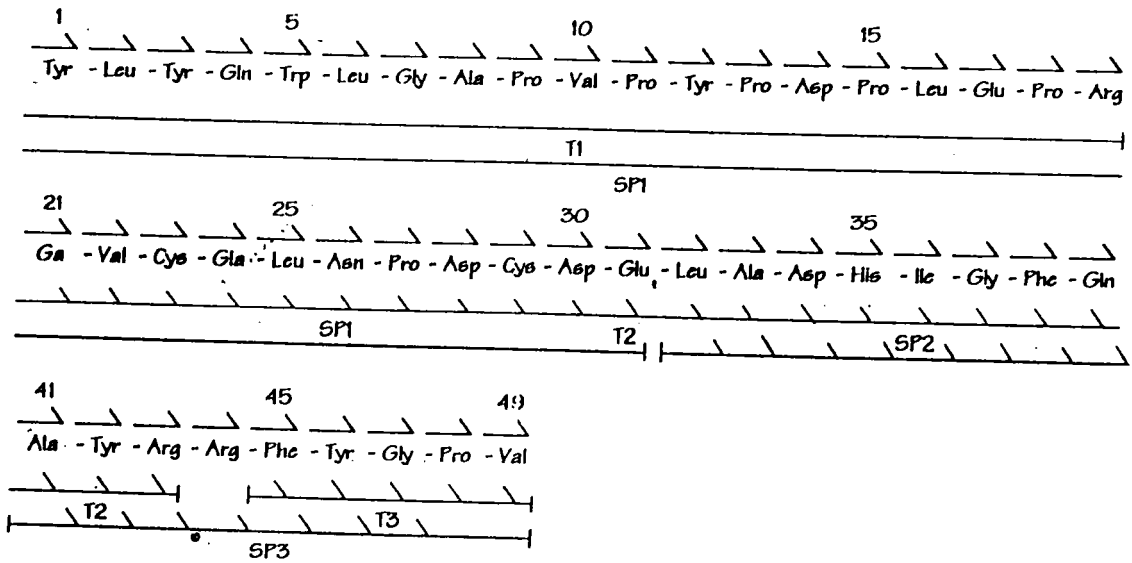
Osteocalcin is a low molecular weight protein (5800 Daltons). This is a 49 single main protein which contains three residues of gamma carboxy glutamic acid (Gla) in positions 17, 21 and 24 (the glutamic acid residue at position 17 was found to be only 9% gamma carboxylated, while positions 21 and 24 are fully carboxylated). It has an isoelectric pH of 4.0 ***(Long et al., 1999).*** It accounts for approximately 25% of the non-collagenous protein found in adult bone and is therefore the most abundant protein in the human skeleton (about 15g / kg body weight of man ***(Cortes et al., 1999).***

The name osteocalcin (osteo: Greek for bone; Calc: Latein for lime salts; in: protein) is derived from:

1) The Ca++ affinity of this protein.

2) The abundance of this protein in bone tissues .

(Hauschka et al., 1989).



Fig(2): Amino acid sequence of the human bone GLa protein

(Poser et al., 1980).

B.Properties of osteocalcin:

This protien contains 46-50 amino acid residues, depending on the species, with three residues of the calcium binding amino acid carboxy glutamic (which is synthesized by a vitamin- K dependent, post- translational carboxylation of specific glumatic acid residues in a peptide chain. Thus it has been called the vitamin K-dependent protein of bone ***(Johansen et al., 1990).***

It is structurally altered by warfarine administration. This finding had pathophysiological implication for the foetal warfarin embryopathy syndrome, characterized by abnormalities of the growth plate with early closure of the fontanelles and excessive intracranial calcification. This is explained by inhibition of vitamin K- dependent carboxylation by warfarin anticoagulant therapy which was given to the mothers during the first trimester of pregnancy, so the fetuses are born with bone defects. Similar growth plate abnormalities have been reported in rats maintained on warfarin from birth to eight months of age. This was consistent with the concept that the carboxylated form of osteocalcin increases the deposition of hydroxyapetite in the organic matrix of bone ***(Kamihagi et al., 1999).***

The Gla residues enable osteocalcin (OC) to bind strongly to hydroxyapetite, the principal mineral phase of bone. OC is found predominantly in bone matrix and is specially secreted by osteoblasts

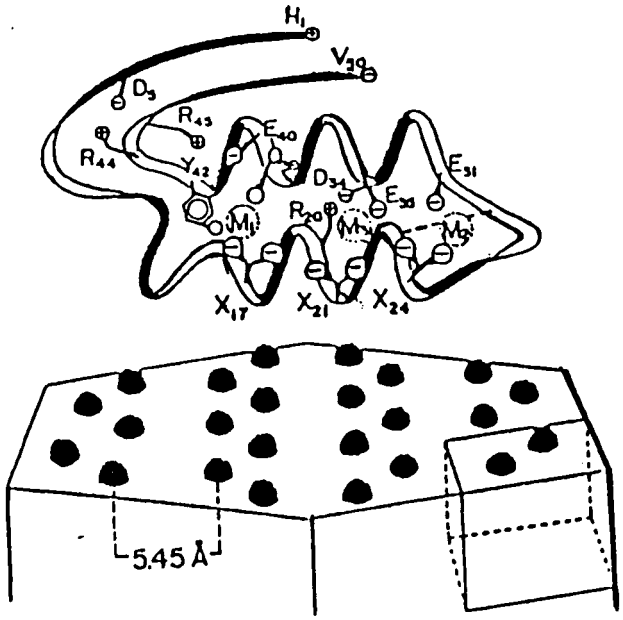
and osteocytes . Most of the circulating OC is produced by new cellular synthesis rather than by bone matrix resorption,so its serum concentration is believed to reflect the rate of bone formation*(Fewtrell et al.,2000).*

Thus the mineralized bone and dentin matrices contain high concentration of osteocalcin, whereas osteoid and predentin do not *(Fewtrell et al., 2000).*

In the presence of Ca++ and the three Gla residues, OC takes the α-helix configuration (38% of carboxylated form take α- helix for 8% in decarboxylated protein) which is in favor to its adsorption to hydroxyapatite crystals and hence in the regulation of mineralizing surface of bone (fig. 2) *(Hauschka et al, 1989).*

The carboxylated (Gla) form is thought to be the active form of the protein, since this is the form that binds strongly to hydroxyapatite. Osteocalain carboxylation in humans is a vitamin K-dependent process *(Jie et al., 1992).*

A proposed model for osteocalcin is shown in fig. (3) to have an unique complementary relationship to hydroxyapatite crystal lattice. The Ca++ promoted adsorption of OC to hydroxyapatite may be understood in term of transition of α-helical structure induced millimolar levels of free Ca++ *(Long et al.,1999).*



Fig(3): A proposed model for osteocalcin

(Long et al.,1999)

C.Synthesis:

Many experimental studies showed that OC is synthesized in bone. ***Lian and Friedman in 1978*** discovered a vitamin K-dependent carboxylation system in the microsomes extracts of chicken bone. The resultant protein had the same molecular weight, the same electrophoretic mobility and a similar isoelectric pH to osteocalcin.

Nishimoto and Price in 1980, proved that the gamma carboxy glutamic acid-containing bone protein (osteocalcin) is synthesiged in calf bone.

Gallagher et al., 1983, demonstrated that the production of osteocalcin by human osteoblast cells. This was evident from the fact that clonal osteosarcoma cell lines which synthesize BGP are also the cell lines with high PTH responsiveness and alkaline phosphatase activity which are the characteristic features of osteoblasts in cell culture.

Most of the circulating osteocalcin is derived from a protein material and is newly synthesized and released from the osteoblastic cell population and not from resorption of existing bone matrix by osteoclasts. Hence serum OC is a specific, sensitive and rapidly responding bone marker of osteoblastic activity ***(Low and law, 1991).***

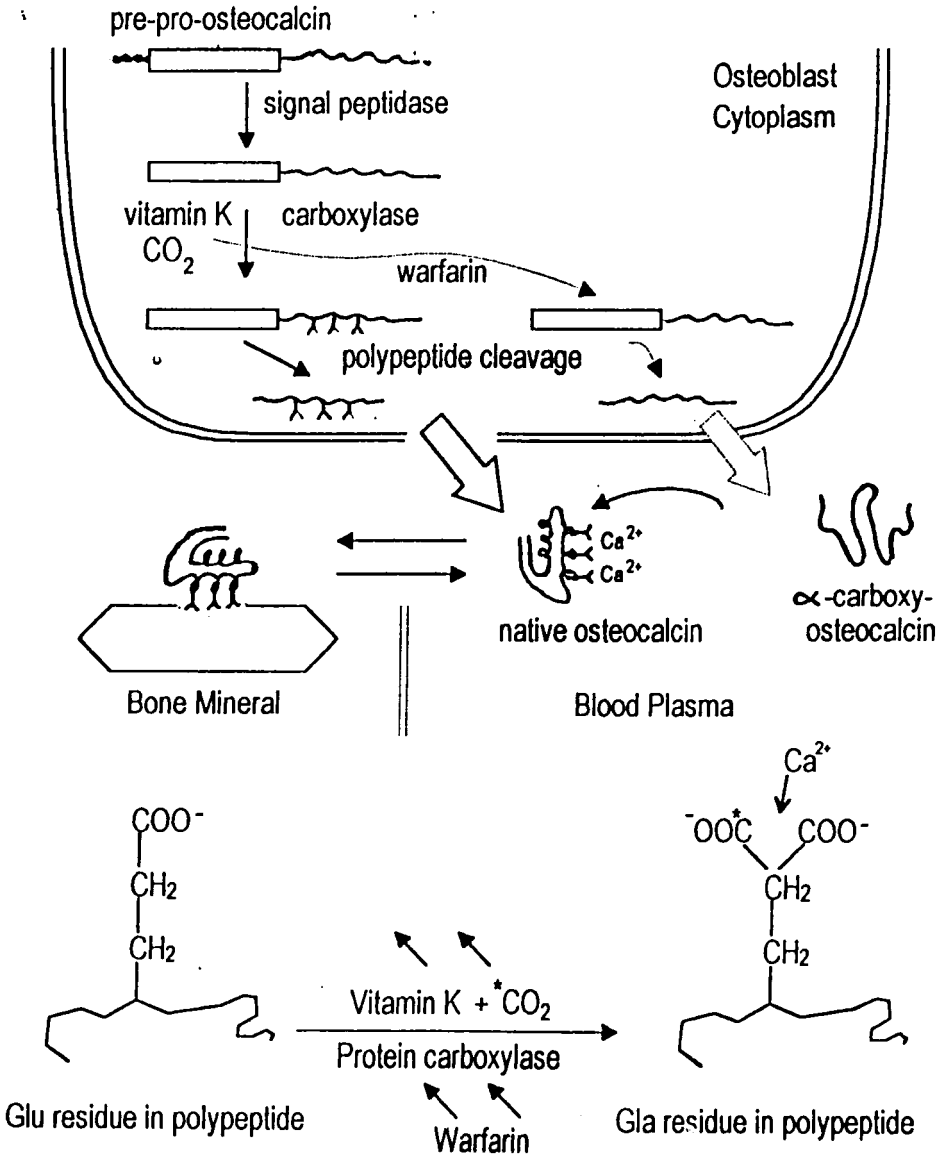
The circulating pool of osteocalcin represents a small fraction of newly synthesized protein which is not absorbed to bone but is released directly into the blood. The synthesis of OC is under the effect of 1, 25 $(OH)_2$ D, active metabolite of vitamin D ***(long et al., 1999).***

Kamihagi et al in 1999, demonstrated that vitamin D injection in rats induced a perceptible elevataion of the osteocalcin plasma level.

Vermeer et al, in 1998, demonstrated that vitamin D deficient chicken have very low serum osteocalcin level. Furthermore ***Long et al., in 1999*** showed that pharmacologic doses of calcifirol (1,25-

dihydroxy vitamin D3) would result in very low birth weight infants (< 1500g).

Osteocalcin is probably synthesized as a higher molecular weight pro-osteocalcin and subsequently proteolytically processed .***Renucci et al.,in 1993*** showed evidence for the existence of an intracellular precursor by detecting 2 immunoreactive components within rat osteosarcoma cells which secrete BGP. One component is identical in molecular weight and electrophoretic mobility with BGP from rat bone, the other component has a higher molecular mass (approximately 9000 Daltons) and about half the electrophoretic mobility of BGP from bone (***Renucci et al.,in 1993).***



Fig(4): Synthesis of vit-k dependent proteins in bone. Vitamin K requirements for synthesis of gla vitamin K-dependent protein carboxyl require vit. K in its reduced form and Co2 for carboxylation of specific gluatmic acid residues

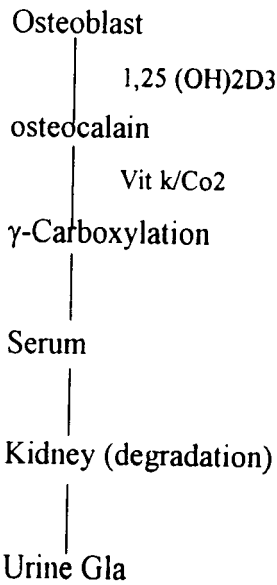
(Hauschka et al., 1989)

D.Metabolism:

During mineralization, osteocalcin is released into the circulation where it can be measured by radioimmunoassay (RIA), a method realised ***by Nishimoto and Price in 1980,*** who utilized the bovine osteocalcin as a tracer, which differs from the human osteocalcin in five amino acids in which there is a cross reactivity.

Later on, a variant assay method was realised ***by Delmas et al.,in 1986*** and many commercial kits for the radioimmunological assay of osteocalcin were thereafter available for laboratory uses.

Osteocalcin is then degraded by the metallo -enzymes present in the hepatic and renal cells(fig. 5) and its half life is very short (about four minutes) osteocalcin is subsequently filtered by the kidney and degraded to its constituent amino acids, hence not present in urine ***(Farrugia and Melick, 1986).***



Fig(5): Metabolism of osteocalcin.

(Farrugia and Melich, 1986)

E. Clearance of osteocalcin:

The clearance of osteocalcin from the blood is very rapid with a half time of a few minutes and most of the OC is cleared by glomerular filtration. It may be catabolized by the liver and /or kidney *(Farrugia and melick, 1986).*

When renal glomerular function is impaired, circulating osteocalcin concentrations are increased.In patients with advanced renal failure, serum osteocalcin levels are invariably elevated. They range from 2 to 200 times higher than normal and are correlated to serum creatinine *(Namgung et al.,1993)*.

Tissues where gamma-carboxy glutamic acid is found:

Apart from plasma coagulation proteins, osteocalcin is found in bone, calcifying cartilage and teeth. Calcified tissues represent a system in which a GLa containing protein might be found, the only calcified tissue that does not have Gla pt. is tooth enamel, which also differs from other calcified tissues in its lack of collagen. Osteocalcin appears in developing bone coincident with the onset of mineralization *(Lindholm, 2002).*

Bone, kidney and liver cells are capable of de novo gamma-Carboxy glutamic acid protein synthesis and in the case of bone and

kidney, gamma carboxy Gla protein appears to continue to remain associated with the tissues, whereas from the liver , the gamma C Glu protein is released to the plasma as a component of the blood clotting system. Intact microsomal carboxylase system has been identified in the placenta and in embryonic bone tissue *(Vermeer et al. , 1998).*

Relation Between Osteocalcin and Blood Coagulation Factors:

Blood coagulation factors were known as the only class of protein to contain Gla, prior discovery of BGP. Osteocalcin is distinctly different from the vitamin K-dependent blood coagulation factors by molecular weight, amino acid composition and sequence *(Kamihago et al.,1999).*

F.Osteocalcin levels in pediatric Age group:

It is found that serum OC concentration in normal children is changing in relation to age and sex from infancy to adolescence, with a pattern resembling the height velocity curves for children. Serum OC concentrations are higher in infants and children than in adults *(Lippincott and Wilkins 2000).*

Osteocalcin is detected in cord blood but levels do not correlate with maternal levels, suggesting that fetal OC metabolism is independently regulated *(Namgung et al ,.1994).*

In neonates, levels rise to 40-60 ng/ml during the first month of life and remain high for the first 6-12 months. Concentrations decline slightly thereafter (range:10-25 ng/ml) and remain relatively constant until the onset of puberty***(Cooper et al., 1997).*** Marked increases in serum OC at puberty show sex related differences that are associated with the adolescent growth spurt; the peak values at the same age as the maximal height velocity. In boys levels decreased to adult levels by 18 years of age and in girls the levels decreased earlier and had a less pronounced maximum. In young adults osteocalcin varies between 2 and 12 ng/ml with a mean of 7.2 ng/ml***(Lippincott and Wilkins 2000).***

Circulating G.H levels also increase during puberty. It is evident that GH stimulates IGF-1 in a variety of tissues, including bone. It is found that IGF-1 stimulates OC synthesis in fetal rat bone culture ***(liliadhall et al., 1994; Trainer, 1994; Johansen et al., 1994).***

Tobume et al., in 1997 reported that recombinant human IGF-1 injection therapy for treating a case of severe idiopathic osteoporosis was associated with an increase in serum OC level by 24%. Thus serum OC measurement has the advantage of reflecting events in the entire skeleton and specially in areas where longitudinal growth takes place.

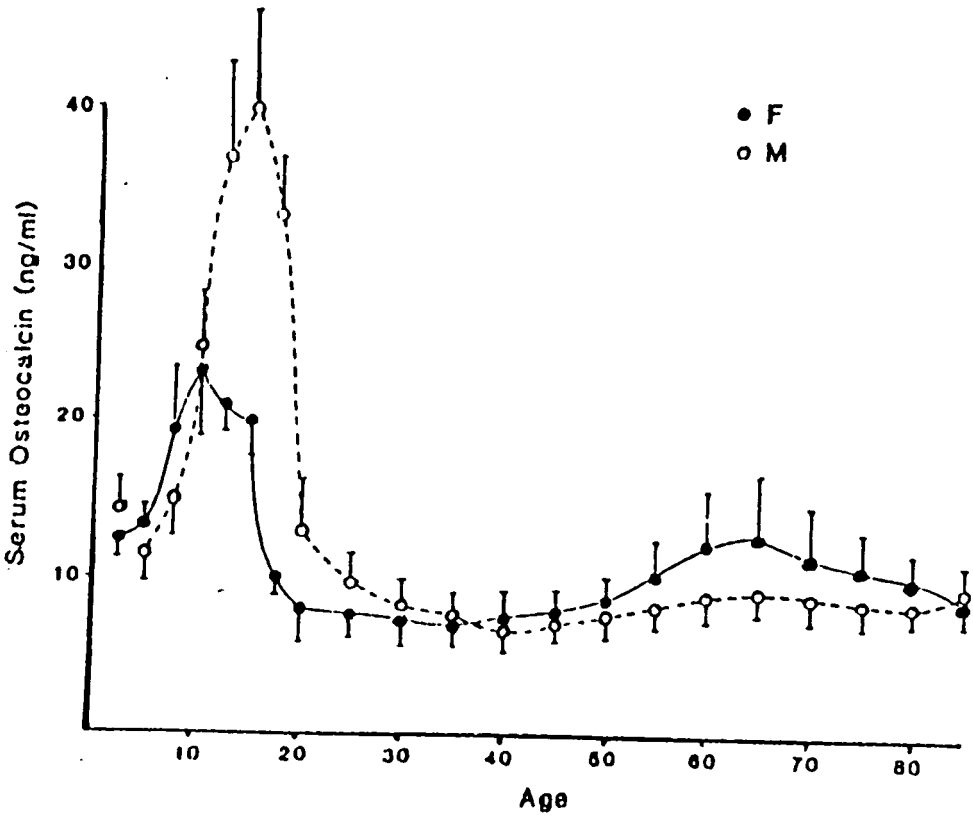


Fig. 6: Human serum osteocalcin concentration of normal subjects. Serum osteocalcin is higher in children than adults. Peak values occur during pubertal growth spurt, which is slightly earlier for girls (F) than for boys (M). Each value represent an(n) at least 10

(Lippincott and Wilkins, 2000).

G. Circadian Rhythm:

Serum OC concentrations show diurnal variations that may be related to circadian rhythm in the rate of bone formation. In young adults on a normal light-dark cycle and meal-schedule, levels of circulating osteocalcin decline during the morning, rise in the

afternoon and early evening and reach a peak nocturnally ***(Haushka et al., 1989).*** This illustrates the importance of regulating the time of blood sampling for osteocalcin determinations in clinical investigations of metabolic bone diseases.

The circadian changes are indicative of existence of biological rhythms in osteoblastic activity rather than changes in metabolic clearance . The physiological aspects on the circadian rhythm are that osteoblastic activity peaks during the early night and decrease in the morning . The circadian osteoblastic activity is relatively stable during seasons and apparently not acutely related to sleep.There are evidence for endogenous cortisol as a regulator of the osteocalcin rhythm especially by mediating the morning decrease in osteocalcin. Osteocalcin synthesis is not completely blocked by glucocorticoids since the effect is blunted by administration of 1,25-(oH)2D3.Although serum osteocalcin may respond to acute doses of several other osteotrophic hormones , it is doubtfull whether endogenous PTH ,1-25(oH)2D3 ,sex hormones or growth hormones platy any role as acute regulators of the circadian rhythm ***(Neilson , 1994)***.

Histo-physiology of Bone

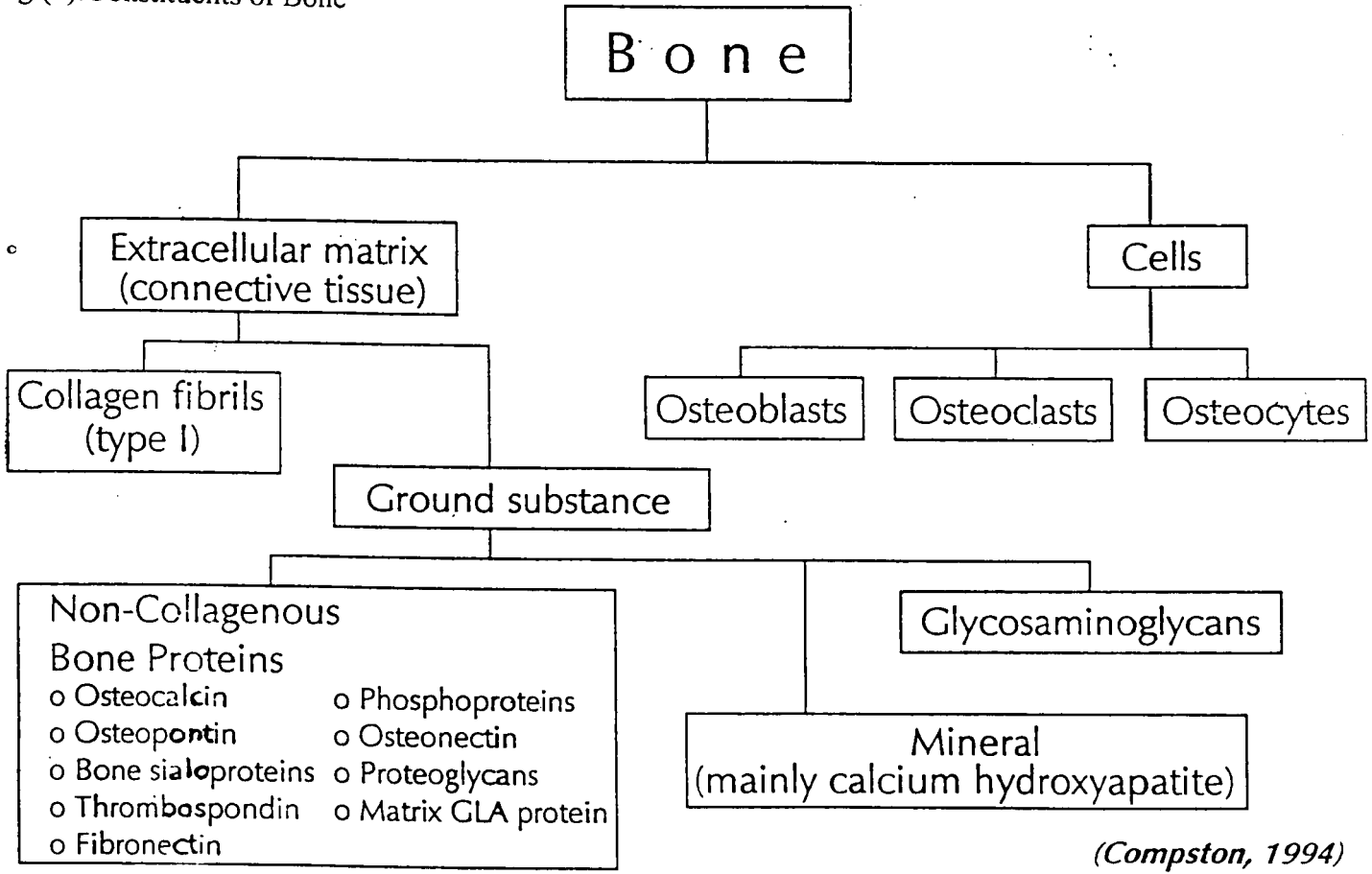
To understand the roles of osteocalcin some physiological points concerning bone must be clarified.

Bone is a complex tissue. Apart from the calcified matrix. It contains a number of cell families of which the bone forming and the bone resorbing cell families are the most important ***(Guyton, 1991).***

The extracellular matrix comprises 35% organic components and 65% inorganic components. The organic components include type I collagen (90%), the gamma carboxyglutamic acid rich protein-osteocalcin-(1-2%), osteonectin, proteoglycans, glycosaminoglycans and lipids. The inorganic component consists mainly of calcium and phosphate in crystals of hydroxyapatite. The skeleton stores more than 99% of the body calcium ***(Peterson et al., 1992).***

Non collagenous proteins comprise 10-15% of total bone protein. Their function is incompletely understood, some are involved in the attachment of osteoclasts to bone matrix, others may play a role in collagen fibrillogenesis, matrix mineralization and regulation bone remodelling ***(Compston, 1994).***

Fig.(7):Constituents of Bone



(Compston, 1994)

A. Bone cells:

i. Osteoprogenitor cells:

Are those cells which give rise to the bone forming cells found in abundance on the endosteal and periosteal surface of bone; these cells resemble fibroblasts in morphology ***(Lindholm, 2002).***

ii. Osteoblasts:

They line the surface of bone and have the capability to produce unmineralized bone matrix which known as osteoid.They also synthesize bone collagen and several of the bone associated non-collagenous proteins and have receptors for parathyroid hormone on their cell surface.The osteoblast family also includes the related osteocytes and bone lining cells. When matrix synthesis is no longer required, osteoblasts lose their synthetic capacity and become lining cells or osteocytes. ***(Hochberg and Karger 2002).***

iii. Bone lining cells:

Are found lining endosteal surfaces and trabeculae. They are flattened against the bone surface and are difficult to distinguish by light microscopy. They have very little cytoplasm or endoplasmic reticulum and possess gap junctions. They have been considered to form a functional membrane separating bone fluid from interstitial fluid ***(Cooper ,. 1997)***.

iv. Osteocytes:

It is the most mature differentiation stage of the osteoblastic line. A certain fraction of the osteocytes incorporate themselves into the newly forming matrix, which is first uncalcified osteoid and at a later stage calcified bone. During this process, the cells become smaller and lose cell organelles. By forming an extensive network of cell processes, the incarcerated osteocytes maintain contact with previously incorporated osteocytes and with the covering layer of osteoblasts and bone lining cells ***(Dawsan et al., 1995)***

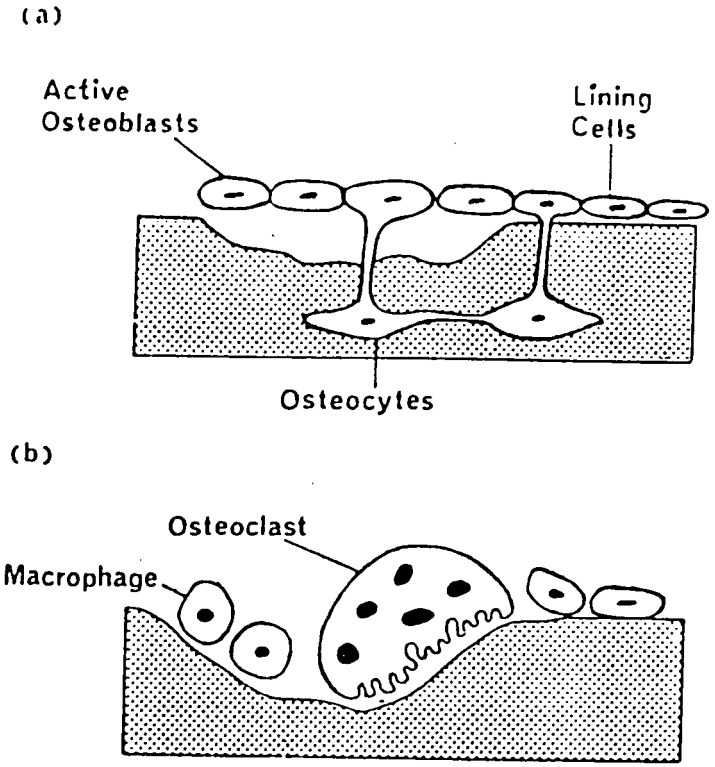
v. Osteoclasts:

They are the most important cells in bone resorption. These multinucleated cells are derived from marrow monocyte stem cells and are believed to form the fusion of mononuclear precursors ***(Burkhardi and Dawson 2001).***

Osteoclasts are located directly on the surface of bone in resorption bays known as "Howship's Lacunae" and have the cellular machinery to release the mineral and degrade the matrix. When in contact with bone surface, their membranes form many processes (ruffled borders) that appear to penetrate the bone surface ***(Dawsan et al., 1995).***

B. Osteogenesis:

The formation of bone cells and bony tissue from more primitive cells is a complex process that always involves cellular differentiation, secretion of calcifiable matrix by differentiated cells, ordered deposition of calcium phosphate mineral upon that matrix and remodelling of that initial mineralized matrix to produce bone *(Cooper et al.,1997).*



Fig(8): Schematic representation of major cells of bone. Relationships of osteoblasts, bone lining cells and osteocytes (a); and osteoclasts and macrophages in a Howship's lacuna (b). *(Dawson, 1995)*

C.Non-collagenous Bone Proteins:

The proteins of bone tissue are many and of diverse characters, as the tissue itself is very heterogenous. They range from collagen found in all connective tissues, to the quantitatively minor but more specific proteins located mainly, if not solely, in bone tissue as result presumebly of synthesis by the osteoblasts. The value of quantitative change in blood contents of some of these bone-related proteins is being assessed as possible indices of bone formations or osteoblastic activity ***(Lindholm, 2002).***

Types of non-collagenous Bone proteins:

i.Bone Sialoprotein (BSP) Osteopontin:

The name "bone sialoprotein" (BSP) was given because of its high sialic acid content. It is a glycoprotein of molecular weight 23000 and comprises 8-12% (W/W) of the total non-collagenous protein of bone. Carbohydrate residues make up about 40% by weight and sialic acid accounts for about half of this value. BSP is not present in any other tissue or organ in vivo, except for its detection at low levels in dentine. Its peculiar presence in bone tissue indicates an unique function in this tissue. It is a matrix protein that enhance cell attachment ***(Fisher et al., 1983 b; Noda et al., 1988).***

ii. Proteoglycans:

Proteoglycans are highly molecular weight polymeric substances with carbohydrate prosthetic groups named glycosaminoglycans (GAGs). ***Engfeldt and Hjerpe in 1976*** showed that the major bone proteoglycans are much smaller in size than the major species present in cartilage.

Fisher et al., (1983a) isolated the proteoglycans of developing fetal bovine bone. It exists as two classes of small proteoglycans. The larger PGI has a protein core of molecular weight 38.000 with 2 attached chondroitin sulphate chains. The smaller PG II has a protein core of similar molecular weight 38.000, but with only one attached chondroitin sulphate.

As in other tissues, proteoglycans may be involved in interactions with collagen fibrils to affect fibrillogenesis. This possible function in bone must precede mineralization, but proteoglycans have also been shown to have inhibitory actions on calcification processes ***(Ibrahim et al., 1993).***

iii. Phosphoproteins:

Proteins containing phosphate groups covalently bound to some of their constituents, serine and threonine residues, were first

described in chicken and bovine bone by ***Spector and Glincher in 1972.*** The strong ionic interactions of phosphate compounds with calcium ions are likely to have some influence on mineralization. It inhibits in vivo hydroxyapatite formation as suggested by ***Menanteau et al. 1982.***

Uchiyama et al., (1986) Succeeded in the isolation of 15 phosphoprtein compounds. The best characterized phosphoprotein extracted from bone is osteonectin.

Osteonectin:

This is a 30000 molecular weight glycoprotein that has affinty for both collagen and hydroxyapatite mineral. It links mineral to collagen fibres. ***Romberg et al., (1986)*** found that it was the most protein inhibitor of hydroxyapatite crystalization, which is likely to be due to linking interaction between appropriately spaced clusters of acidic residues on osteonectin and the hydroxyapatite crystal lattice.

It makes up 2-4% of the total organic matrix and is a major non-collagenous constituent of bovine and porcine bone and dentine. Human bone cells synthesize osteonectin in tissue culture and in vivo, osteonectin is located predominantly in bone and dentine .***(Lindholm 2002).***

iv.Plasma Proteins:

Albumin and alpha2HS glycoprotein were the first serum proteins to be found in abundance associated with bone tissue. Since even dense compact tissue contains variable amounts of blood vessels, it is not surprising to find plasma proteins in extracted solutions. They are also found in extravascular sites of bone both in the tissue fluid and complexed as significant components in the calcified matrix ***(Delmas et al., 1986c).***

a.Albumin:

It makes about 3% of the non-collagenous matrix of bovine bone and probably accumulates by adsorption to the mineral phase during the formation of calcified tissue and remains there until the bone is resorbed ***(Pratico et al., 1997).***

b.Plasma alpha2 hydroxy sulphite glycoprotein:

It has a molecular weight of about 50000 D and is synthesized by the liver and subsequently accumulates in bone and dentine tissues. In bone it is concentrated 30-300 folds relative to albumin, depending on the species. The affinity for bone mineral can explain its concentration from tissue fluids. Evidence that bone may have an influence on plasma levels is given by the fact that calculations show that 40% of the liver production goes to bone and is incorporated in young growing animals. Apart from its possible effect on

mineralization processes, alpha 2 hydroxy sulphite glycoprotein may be implicated in bone resorption ***(Garcia et al., 1997).***

v. γ- Carboxyglutamic Acid containing proteins:

The amino acid, γ- Carboxyglutamic acid, was first found in prothrombin. Particular glutamic acid residues at selected sites on the polypeptide chain are converted to Gla by addition of carboxy group in post-translational events, which have a specific requirement for vitamin K and bicarbonate ion ***(Fewetrell et al.,2000).***

The characteristic interaction of calcium ions with Gla residues, which it was considered, could - by analogy with the blood clotting process -be necessary for mineralization, led to the survey of bone tissues for the presence of this unusual amino acid and independenty two groups isolated low molecular weight constituents containing this amino acid, from different animal species ***(Dawsan et al., 1995).***

The bone constituents is known as bone Gla protein (BGP), or osteocalcin (OC), which will be discussed in details. Another distinct Gla -containing protein named "matrix Gla protein" or "MGP" has been isolated from bovine bone matrix ***(Cioffi et al.,1997).***

Matrix Gla protein (MGP):

It is a low molecular weight (9961) protein with five Gla residues per molecule. This molecule accounts for the majority of the Gla content of newborn rat bone, precedes the accumulation of mineral. Its accumulation appears to be less dependent on Gla residue content than BGP; however, as 27% normal levels MGP occur in warfarin treated animals compared with 1-2% normal levels of BGP (OC) ***(Cioffi et al.,1997).***

The amino acid sequence homology that exists between MGP and OC of various species indicates that these molecules have arisen by gene duplication ***(Nippon et al.,1999).***

Role of Osteocalcin

1- As a marker of bone turnover:

Serum BGP is a sensitive marker of bone formation and it parallels the growth velocity curve during childhood and adolescene ***(Lippincott and Wilkens;2002).***

2- Oseocalcin and bone mineralization:

Bone mineralization occurs in two stages, the first stage is called the primary mineralization, which is characterized by a rapid increase in bone mineral content to reach about 70% of its final density. This occurs as a result of nucleation and crystal multiplication,while the secondstage is called secondary mineralization which is characterized by more gradual completion of mineralization due to crystal growth ***(Capra et al., 1991).***

All three Gla residues lie on the same side of polypeptide chain of BGP and at constant interval. This organization is similar to the spacing of calcium in hydroxyapatite crystals. Accordingly, osteocalcin may bind to the calcium on the hydroxyapatite surface or, alternatively, OC molecule with already bound calcium, may bind to hydroxyapatite, thus, presenting calcium to vacant position in the crystal. Accordingly, OC binds strongly to hydroxyapatite (***Long et al ., 1999***) .

The carboxylated form of osteocalcin (a vitamin K-dependent process) favour the deposition of hydroxyapatite in the organic bone matrix and hence the regulation of bone mineralization (***Fewtrell et al ., 2000***) .

3. Chemotatic action of osteocalcin:

Osteocalcin has a dose dependent chemotatic action on the human monocytes allowing unidirectional migration of activated monocytes ***(Mundy and Poser, 1988).***

OC has also a chemotatic effect on the breast cancer cells and on the sarcomatous cells allowing only their unidirectional migration ***(Kamihagi , 1999).***

4. Stimulation of osteoclasts:

Osteocalcin has an important role in the recruitment and adherence of osteoclast cells. Their stimulation results in a global increase of bone remodelling (***Lian et al., 1987***).

Pathological Variation in Osteocalcin Level

1.Malnutrition (Protein Energy Malnutrition):

Serum OC is believed to be a sensitive marker of bone osteoblastic activity and bone formation. Serum osteocalcin has also been described as a marker of skeletal growth in normal children and in children with growth deficiency ***(Hoschberg and Karger 2002).***

The level of OC has been related to growth velocity and it is reported to be lower with small size children than in controls ***(Namgung et al ., 1993).*** Children with malnutrition are exposed to chronic undernutrition and show stunted growth. This is reflected on serum level of osteocalcin which is found to be low in them ***(Al – Rawhaa et al., 1994).***

In a study was done by ***El Dafrawy et al. in, 1992,*** it was found that serum osteocalcin levels were dramatically decreased in sever protein energy malnutrition and increased four folds during rehabilitation. These change could not be related to 1,25 (OH)2 D3 serum level. The results suggest that serum OC levels are linked to the nutritional status of infants and children .

2- Rickets :

Infants affected with rickets demonstrate a reduction in the blood content of vitamin K -dependent protein, osteocalcin, most

pronounced in diseases of calcipenic and phosphopenic varieties. It has also been established that there is an invasive correlation between OC content and the level of parathyroid hormone. This led ***luk'ianova et al., (1990)*** to suggest that the measurement of blood osteocalcin in children may serve as a diagnostic marker of metabolic disorders and mineralization of osseous tissue.

It is also suggested that serum OC levels may serve as a useful index of active mineralization and provide a clinician with a prompt indication of a successful therapeutic strategy (***El Kholy et al., 1994***).

3- Osteogenesis imperfecta :

A heridetary connective tissue disease characterized by generalized osteoporosis with repeated fractures and bone malformations. There is a significant increase in serum osteocalcin levels in patients with osteogensis imperfecta (***Kamihagi et al., 1999***).

4.Growth Hormone Deficiency (GHD):

Growth hormone (GH) is active in regulating bone mineral deposition and aspects of bone growth and turnover as well as in stimulating longitudinal bone growth. It increases activity of osteoblasts, osteoclasts and osteocytes. Thus GH is needed for proper skeletal maturation (***Ohlsson et al., 1993***).

In children with GHD, osteopenia and bone mass have been reported reflecting the presence of a low bone formation and remodelling activity, in addition to delayed bone maturation and skeletal growth retardation (***Sartorio et al., 1991***).

In basal conditions, serum OC levels were reported to be significantly lower in patients with GHD than in normal children. Before treatment, serum OC concentrations were inversely related to the bone age delay. Basal IGF-1 levels were also reduced. It is suggested that decreased OC levels probably reflect reduced bone turnover and decreased bone formation in patients with GHD. Thus serum OC measurement can be a useful biochemical marker that can be used in conjugation with other biochemical variables to assess the degree which GHD affects bone metabolism (***Johansen et al., 1990***; ***Saggese et al.,1993***).

It is found that the growth rate is significantly increased after institution of rh GH in GH-deficient children. A significant increase in OC level is also found and is coupled with significant increase in serum GF-1 levels. After rh GH therapy a direct correlation between increases in OC concentration and increases in IGF-1 levels is observed. However, the rise in serum OC concentration does not peak except after 3-6 months of rh GH therapy. The delayed peak in serum OC concentration is, therefore, likely to reflect the increased number of functional osteoblasts due

to GF-1 stimulation and could probably also reflect an initiated increase in bone mineral content or density in the GH-deficient children during GH therapy (***Sartorio et al., 1991***; ***Ljunghall et al., 1994***).

It is worth noting that the increment of serum OC concentrations is positively correlated to height velocity increment after 6 months and after 12 months of rhGH therapy. Thus, it is suggested that OC could be particularly useful to monitor treatment. Measurement of changes in serum OC after short term GH administration may help to identify those children who will not responed to therapy (***Tobume et al., 1997***).

5. ***Precocious Puberty :***

The levels of serum OC are elevated in children with central precocious puberty and tends to be progressively normalized after three to nine months treatment by LHRH (Lutenizing hormone releasing hormone) analogue(***Kamihagi et al., 1999***).

6. ***Diseases of bones and joints :***

a) Rheumatoid arthritis:

Falcini et al in 1998 proved that OC levels increase in patients with rheumatoid arthritis as the overall bone turnover is increased in them. Patients with rheumatoid arthritis and treated with low dose glucocorticoids show higher serum level of OC than

those who are treated with gold or non steroidal anti-inflammatory drugs. This is because the low dose of glucocorticoids is accompanied by elevated levels of 1, 25 $(oH)_2D_3$ and PTH (Falcini et al., 1998).

a) Chronic rheumatic diseases:

Reed et al. (1990) proved that OC levels are reduced in patients with chronic rheumatic disease when the diseases are in the active form with high sedimentation rate. These diseases include juvenile arthritis, systemic lupus erythematosis and juvenile dermatomyositis ,on the other hand normal levels were found in the inactive stages of the rheumatic diseases and during remission despite of the use of corticosteroid.The reduced levels of OC were predictive of a reduction in bone mass and osteoblastic activity as well as bone formation in children with rheumatic diseases.

C) Paget's disease of bone:

Deftos et al. (1991) had described the comparison of a new immunoassay that is specific for bone alkaline phosphatase (BAP) to measurement of total alkaline phosphatase (TAP) and BGP (OC) in Paget's disease. In this study, they demonstrated that BAP was increased in serum of patients with Paget's disease. Comparisons with the other measurements revealed that BAP correlated better with TAP than with OC, the lowest correlation occurred between

OC and TAP. These studies indicate that BAP assesses different aspects of bone cell function than OC in Paget's disease and OC measurement is diagnostically less useful than serum alkaline phosphatase estimation in those patients.

7. Liver Diseases :

Fonseca et al. (1987) found that serum osteocalcin levels were significantly decreased in patients with primary biliary cirrhosis when compared to normal control subjects despite of vitamin D replacement.

Also ***Capra et al. (1991)*** suggested that serum osteocalcin levels were significantly decreased in patients with liver cirrhosis in compared to normal subjects.

Serum levels of OC was significantly decreased in patients with acute viral hepatitis when compared to normal control subjects(***Pietschmann et al., 1991***).

Crouzet et al. (1991) suggested significant decrease in serum osteocalcin level in chronic alcoholics when compared to normal control subjects due to direct impact of alcohol on the osteoblasts.

8. Renal diseases :

A small increase in serum osteocalcin was observed in some patients with diminished glomerular filtration rate (GFR) ranging

between 20-30 ml/min/1.73m2. Only those with GFR less than 20 ml/min/1.73m2 who showed a larger increase.In the later group of patients, it could not be demonstrated whether the elevation was entirely due to decreased renal clearance or increased skeletal production as a result of increased bone turnover due to associated secondary hyperparathyroidism ***(Charhon et al., 1986).***

Nishio et al. (1989) found a significant increase in serum osteocalcin level in patients with chronic renal failure under haemodialysis when compared to normal control subjects.

Patients with diabetic nephropathy showed lower OC levels than patients with non diabetic nephropathy ***(Nishio et al., 1989).***

9. Endocrinal diseases:

a) Insulin-dependent Diabetes Melitus (IDDM):

Diaz et al., (1991) suggested that serum osteocalcin level was significantly decreased in comparison with the controls in patients with IDDM without hepatic or kidney disease, or any other pathology with complications in the mineral metabolism. As OC synthesis is directly related to 1, 25 (oH)2D3, it is suggestive to think that in IDDM an abnormal vitamin D metabolism could explain alterations in bone matrix and the diminished bone mineral content observed in these patients.

b)Hypothyroidism:

Leon et al. (1989) studied bone involvement in hypothyroidism. They found that osteocalcin was significantly correlated with T3, TSH and alkaline phosphatase. Serum OC levels were decreased in patients with hypothyroidism and total thyroidectomy. Decreased OC level may reflect a greater pathologic effect on the bone in these patients with a decrease in bone formation.

c)Hyperparathyroidism:

Osteocalcin levels were increased in cases of hyperparathyroidism reflecting increased osteoblastic activity. It was positively correlated to serum alkaline phosphatase being increased in primary and secondary hyperparathyroidism ***(Renucci et al., 1993).***

10. The effect of drugs :

a) Glucocorticoids:

Osteocalcin levels were significantly decreased in pediatric patients who were given high-dose glucocorticoids for a long period. High-dose glucocorticoids decrease the numbers of 1, 25 (OH)2 vitamin D nuclear receptors. This will lead to a direct inhibition of bone formation which is indicated by a marked decrease in serum BGP, while bone resorption remains unchanged.

So, the BGP may be used as a marker for early detection of corticosteroid induced osteoporosis ***(Twasaki, 1991).***

b) Vitamin D3 :

Vitamin D-deficient elderly people with hypocalcemia were injected with vitamin D3. A total dose of 15 mg (600,000 IU) was divided into 3 i.m injections given at one month intervals. The results showed marked increase in serum vitamin D3 level as well as an increase in serum calcium level to the low normal range. Furthermore, a transient significant increase in serum osteocalcin was observed from 10.4 ± 4.1 ng to 14.1±5.9 ng/ml. This demonstrated that the osteocalcin response of osteoblasts to stimulation by vitamin D3 (1,25 (oH) 2 D) is retained in very old people ***(Renucci et al., 1993).***

11. Malignancy :

Osteocalcin could be a valuable bone metastasis marker. ***Onat et al., (1991)*** had measured serum osteocalcin levels by using redioimmunoassay method in 11 healthy subjects in 79 cancer patients. The cancer patients consisted of 36 non-metastatic, 29 with only bone metastasis and 14 with extraoseous metastatic patients and non-metastatic patients in both sexes. There was no significance between non-metastatic patients and patients with other than bone metastasis. This study showed that osteocalcin measurements reflect bone formation rates in bone metastasis and could be used as a bone metastasis marker in suspicious cases.

Genetic effect on osteocalcin :

Osteocalcin Gene :

The human OC gene has been localized to chromosome 1 by analysis of mouse-human somatic cell hybrids. The rat OC gene has been isolated from a rat genomic DNA library. Sequence analysis indicates that m-RNA is represented in a segment of DNA comprised of four exons and three introns. Although the introns in the rat gene are larger, its overall organization is similar to the human gene. Typical sequences associated with most genes transcribed by RNA polymerase II are found in 5-flanking regions of the rat gene . In addition, consensus sequences have been identified for cyclic nucleotide responsive elements and several hormone receptor binding sites (estrogen, thyroid hormone). Also present are AG-rich clusters, the putative vitamin D-responsive elements; within the 1,000 nucleotides immediately upstream from the transcription initiation site are sequences that support 1,25 $(OH)_2$ D -dependent transcription of the rat OC gene ***(Lian et., 1989).***

Twin and family studies suggest serum OC is under strong genetic influence and this is related to the genetic effect on bone mineral density. ***Kelly et al. (1991)*** noted that within dizigotic twin pair, differences in serum OC, presumably reflecting genetic variance, predicted differences in bone mineral density.

Further evidence for genetic effects on bone turnover stems from the observations of ***Tokita et al., (1994)*** which showed the relationship between osteocalcin levels and variation in the vitamin D receptor gene. They have observed common polymorphism in the vitamin D receptor gene in a normal population that predict OC levels. In their study higher OC levels were observed in individuals with a particular gene allele, adding further evidence to the genetic regulation of bone turnover.

Studies on the regulation of OC gene expression provide information on the interaction between autocrine cytokines and the systemic hormone 1,25 (OH)2 D . ***Nanes et al. (1994)*** found that 1,25(OH)2D stimulates OC gene expression by stimulating enhancement of transcription in a dose dependent manner. This occurs through stimulation of steady state OC mRNA. The expression of OC marks the completion of osteoblasts differntiation from their pluripotent progenitors. Therefore, in normal subjects, the evidence to date support a major role for genetic factors in the regulation of bone remodelling***(Kamihagi et al.,1999).***

Vitamin D

Vitamin D (calciferol) is a lipid soluble vitamin. It is a prohormone that exists in two forms: vitamin D3 and vitamin D2. Vitamin D3 is the natural, endogenous compound formed in the skin; the precursor, 7- dehydrocholesterol, is stored throughout the epidermis and is photochemically converted to pro-vitamin D during exposure to ultraviolet radiation. Vitamin D2; used for food supplementation and radiation of ergosterol abtained from yeast or fungi ***(Pitt, 1991).***

I.Physiological Fate of Vitamin D:

Dietary vitamin D is absorbed from the intestine in association with dietary fat and then, in chylomicrons, is rapidly taken up by the liver. In contrast, vitamin D formed in the skin diffuses slowly into the blood so that hepatic uptake and subsequent conversion to 25 (OH) D also occurs more slowly. Therefore, much of vitamin D from the skin remains outside the liver protected from its hepatic inactivation metabolism. This allows continuous prolonged production of 25 (OH) D even when exposure of the skin to sunlight occurs only intermittently ***(Parke, 1994).***

II.Activation of vitamin D:

Vitamin D must undergo two hydroxylation reactions in order to achieve its physiological role. It is hydroxylated in the liver at the 25 position to calcidiol (25-hydroxy cholecalciferol) ***(Gertner, 1990).***

1,25- dihydroxycholecalciferol (1,25 DHCC) is produced in the kidney under the influence of PTH by 1-hydroxylation of 25-hydroxy cholecalciferol (25-HCC). 1-hydroxylase activity has also been demonstrated in placenta and decidua during pregnancy and in bones ***(Ralston, 1993).***

III.Other vitamin D metabolites :

24,25 Dihydroxycholecalciferol:

It is a second activation step in the kidney depending on the physiological state of the subject ***(Deluca and Schnoes, 1983).***

25,26 (OH)2 D and 1,24,25 (OH)3 D :

These are further metabolites that have been detected in normal man. The latter may be produced by 24- hydroxylation of 1,25 (OH)2 D ***(Kains, 1982).***

VI.Sources of vitamin D:

1. Biosynthesis subcutaneously by the action of solar UV radiation of a particular wavelength (290-315 nm) on 7-dehydrocholesterol in the skin. The vitamin is then released into the circulation ***(Pitt, 1991).***

2. Natural diet: egg yolk, fatty fish and to some extent milk ***(Bacon et al., 1990).***

3. Fortified food: like margarine, infant food products and the use of oral supplements ***(Gertner, 1990).***

V.Recommended Daily Dietary Allowances of vit. D.:

The daily requirements of vitamin D is 10 ug or 400 IU ***(Baltrop, 1992).*** Dietary sources of vitamin D are required when clothing, housing, smog and a harsh climate prevent adequate exposure to sunlight.

1, 25 Dihydroxy vitamin D:

1,25 (OH)2 D meets the requirements for classification as a hormone. It is secreted internally by one specific organ, the kidney. Precursors are formed in extrarenal sites. After renal secretion, 1,25 $(OH)_2$ D acts on distant target organs, where it engages in an

intranuclear action, similar to that of other steroid hormones. Its production is controlled by calcium and phosphorus, which are also products of 1, 25 $(OH)_2$ D action ***(Ralston, 1993).***

VI.Action and function of vitamin D:

The major physiological role of 1, 25$(OH)_2$ D is to promote intestinal calcium and phosphate absorption, making these ions available for bone mineralization ***(Gertner, 1990).***

PTH and 1, 25 $(OH)_2$ D form a classical endocrine system under negative feedback control which responds to and acts to prevent changes in the plasma calcium level. The hormones act on three main sites: bone, intestine and kidney. PTH acts on the kidney to increase 1, 25 $(OH)_2$ D synthesis and reduce urinary calcium excretion (by increasing distal renal tubular reabsorption of calcium) PTH and 1, 25 $(OH)_2$ D act in concert to enhance skeletal calcium release by stimulating osteoclastic bone resorption ***(Ralston, 1993).***

In the skeleton 1,25$(OH)_2$ D mobilizes calcium and phosphorus from older bone and promotes the maturation and mineralization of newly formed organic bone matrix. These two skeletal actions, which at first seem contradictory, may be understood by recalling that bone homeostasis involves a continual breakdown of old tissue and a concomitant build up of other areas.

Mineralization of organic bone matrix is accomplished both by the maintenance of normal serum calcium and phosphorus levels and by a direct effect on skeletal tissues ***(Pitt, 1991).***

VII. Physiological significance of 25(OH)D concentration:

Plasma 25 (OH) D concentration is a measure of vitamin D status. It is generally accepted as a useful index, as this metabolite is found in higher concentration in blood than other metabolites, and also has the longest half-life of all vitamin D derivatives. This contrasts with 1, 25 $(OH)_2$ D in blood, whose concentration may be influenced by prevailing concentration of parathyroid hormone ***(Al-Othalmeen, 1993).***

Rickets

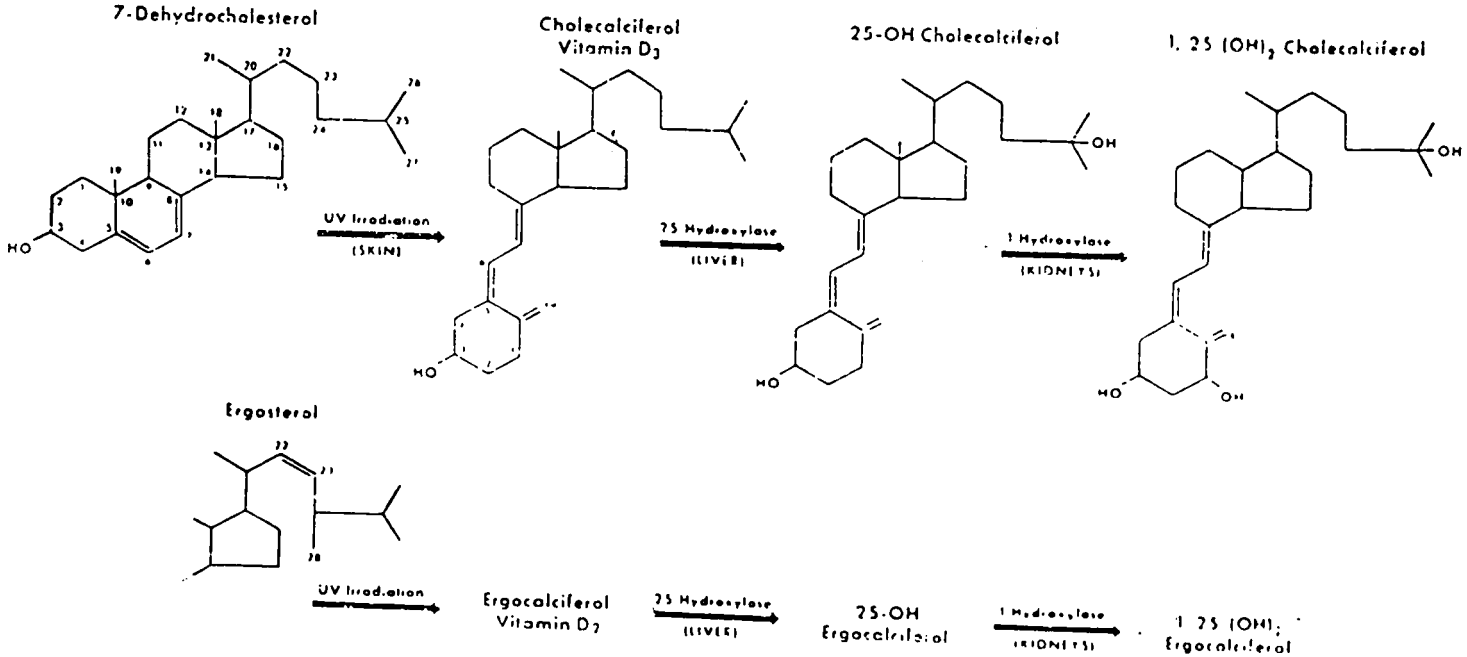
I.Definition of Rickets:

Rickets is a disease of actively growing bone characterized by the presence of non-mineralized osteoid tissue ***(Gertner, 1990).***

Osteomalacia is a consequence of inadequate or delayed mineralization of mature cortical and spongy bone. Children with open physes frequently exhibit the features of both rickets and osteomalacia ***(Rosenberg, 1991).***

The term rickets and osteomalacia describe gross, histologic and radiologic abnormalities common to a number of different diseases. In fact more than 50 diseases of varied etiology and with different clinical presentations have rickets, osteomalacia or both as a common pathologic denominator ***(Pitt, 1991).***

Fig(9): Metabolism of vitamin D2 and D3 ***(Bacon et al., 1990)***



II. Classification of rickets:

Baltrop (1992), classified rickets into 4 main groups:

1 Nutritional rickets (vitamin D dificiency).

2 Non-nutritional rickets:

a. Malabsorptive states

b. Familial hypophosphatemic rickets (vitamin D resistant rickets).

C. Hereditary hypophosphatemic rickets with hypercalciuria.

d. vitamin D dependent rickets (type I and type II).

e. Fanconi syndrome.

3. Concomitant disease:

a. Long term anticonvulsant therapy.

b. Chronic renal failure.

C. Chronic liver disease.

4. Substrate deficiency:

a.Neonatal.

b.Post neonatal.

A.Nutritional rickets (Vitamin D Deficiency) :

Nutritional rickets is a systemic disorder characterized by growth failure, bony deformity, hypotonia, listhessness and delayed motor development. Nutritional rickets is primarily attributed to inadequate supplies or availability of vitamin D. The skeletal manifistations of

rickets are due to inadequate mineralization of osteoid, which gives rise to bones that are less rigid and liable to be deformed by the normal physical forces acting on them ***(Teotia, 1997).***

Pathogenesis

Rickets develops when inadequate amounts of calcium and/or phosphorus in the extracellular fluid upset the critical ratio of calcium to phosphorus necessary for normal mineralization.The bone matrix or osteoid continues to be produced at its usual rate and its accumulation becomes disproportionate to the amount of calcification ***(Stamp, 1994).***

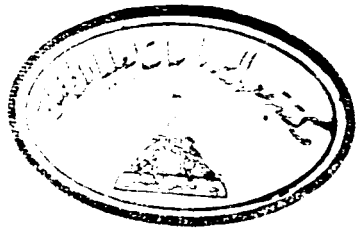
Structure of Normal Growth Plate:

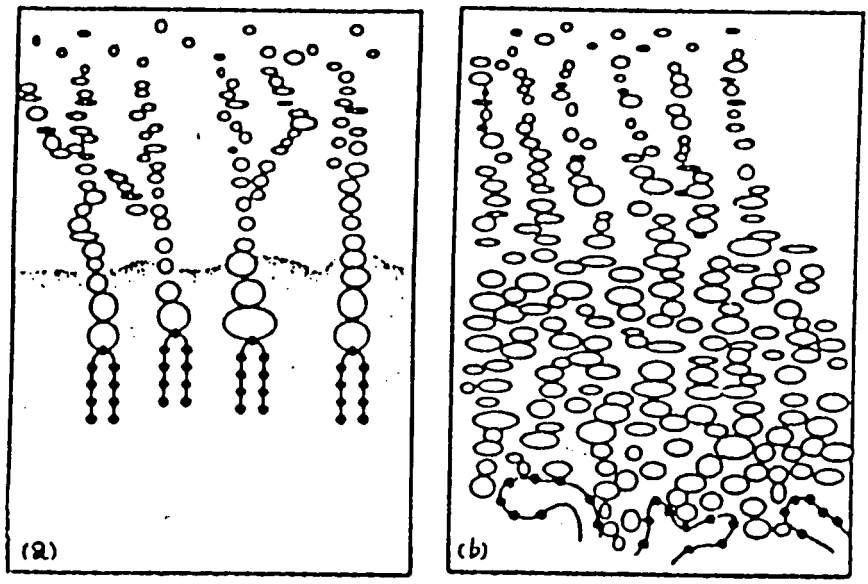
The general structure and organization of the normal growth plate must be understood before the changes of rickets may be appreciated. The growth plate is located at the ends of long bones and situated between the epiphysis and metaphysis. Although the growth plate is adjacent to the epiphysis, functionally it is part of the shaft. Therefore, the commonly used term epiphysis plate is inaccurate. The usual growth plate is not flat, but shows various combinations of concavities and convexities ***(Lannotti, 1990).***

The growth plate is divided into the regions known as the reserve or resting zone, proliferative zone, hypertrophy zone and zone of calcification, which is the base of growth plate. In actuality, these zones merge into one another. Portions of the calcified zone are resorbed by osteoclasts leaving vertical struts or bars of calcified cartilage ***(Cooper et al.,1997)*** . These act as templates for bone to be deposited on their surfaces. The structure composed of a central core of calcified cartilage covered by bone is known as the primary spongiosa. Eventually, the cartilage core is removed and the trabeculae become known as secondary spongiosa (ancellous bone is formed through this sequence).***(Fewtrell ,2000).***

Pathologic Changes of The Rackitic Growth Plate:

There is elongation and distortion of the normal columnar arrangement of chondrocytes in the zone of hypertrophy. In the underlying zone of maturation, provisional calcification is delayed or absent and vascularization via defective or obliterated channels is impaired and irregular. The primary spongiosa is also abnormal as a result of excessive parathyroid hormone activity ***(Compston, 1994).***





Fig(10): Diagram of longitudinal section of the cartilaginous growth plate in health and in rickets . ***(Stamp, 1994)***

Clinical picture:

Nutritional rickets may be manifested between the ages of 6 and 24 months and somewhat earlier in premature infants. Deficiencies in bone mineralization are particularly evident in regions of rapid bone growth. In the first year of life they are the cranium, wrists and ribs ***(Aurbach et al., 1992).***

At birth, the skull is growing most rapidly and neonatal rickets may therefore present as craniotabes. In which the cranial vault has the consistency of a ping-pong ball. In the first year of life, rickets produces swollen epiphysis at the wrist and swelling, beadling of the costochondral junctions, the so called "rickety rosary". The inward pull of the diaphragm produces a groove in the rib cage, called Harrison's sulcus ***(Stamp, 1994).***

Other manifistations include delayed eruption of teeth and enamel defects, pigeon chest, scolliosis, kyphosis, dwarfism, painful bones, fractures, anorexia and weakness ***(Teotia and Teotia , 1997).***

Generally, bow legs (genu varum) develop in rackitic children in the toddler years, knock-knees (genu valgum) are seen in affected preteen agers and adolescents ***(Vermeer, 1998).***

Roentographic Changes:

The major specific roentographic manifistations of rickets are seen at the growth plate. Areas of most active growth show the most dramatic changes. Disordered mineralization and maturation at this site lead to lengthening and widening of the plate. The zone of provisional calcification is diminished in density and irregular. Enlargement of the growth plate results in flaring, cupping and fraying of the metaphysis ***(Vermeer et al., 1998).***

Stages of vitamin D deficiency:

The plasma biochemical changes of rickets depend in part on the stage of the disorder classified rickets into 3 stages ***(Bacon et al, 1990).***

1- Stage I:

In the early stages, the serum calcium is low but serum phosphate and PTH are normal and amino aciduria is absent.

2- Stage II:

later, the plasma calcium level may lie in the normal range, but the plasma inorganic phosphorus level is reduced and the alkaline phosphatase level is increased.

3- Stage III:

Finally, in florid rickets, the fall in level of plasma phosphorus and rise in level of alkaline phosphatase persists and the plasma calcium level is decreased, making tetany more likely. Such vitamin D deficiency is accompanied by increasing reversible generalized amino acidosis and increased levels of circulating parathyroid hormone.

Chemical Pathology:

In healthy infants the inorganic phosphorus concentration is 4.5-6.5 mg/dl, whereas 1.5-3.5 mg/dl. The alkaline phosphatase of

serum, which in normal children is less than 200 Iu/dl, is elevated in mild rickets to more than 500 Iu/dl. The serum calcium level is usually normal, but under certain conditions it is reduced and tetany may develop. Normally, the total serum calcium ranges between 8.8 and 10 mg/dl with an average of 10 mg/dl ***(Teotia and Teotia , 1997).***

Complications:

Respiratory infections such as bronchitis and bronchopneumonia are common in rackitics associated with severe deformities of the chest. Tetany also occurs when serum calcium falls below 7-7.5 mg/dl ***(Stamp et al., 1994).***

Prevention:

Ten ug of cholecalciferol or 400 Iu/24h are appropriate for children up to 18 years of age. In an adolescent as is pregnancy or lactation an additional 200 IU (5 ug) is recommended ***(Rosemberg, 1991).***

Therapy:

The usual dose of vitamin D recommended in the therapy of rickets is 2000 to 5000 units daily ***(Teotia andTeotia , 1997).***

B. Non nutritional Rickets:

Several disorders may result in clinical rickets inspite of normal dietary intake and normal endogenous production of vitamin D.

i. Malabsorption states:

Gastrointestinal malabsorption by inhibiting the absorption of the fat soluble vitamin D, may give rise to rickets even in apparently adequately nourished children. Childhood vitamin D deficiency is seen particularly in steatorrhea due to biliary obstruction (usually biliary atresia),less commonly in celiac disease and least frequently in the pancreatic insufficiency of cystic fibrosis *(Gertner, 1990).*

ii. Familial Hypophosphatemic Rickets(vitamin D.resistant rickets)

X-linked familial hypophosphatemic rickets (XLH) is the most common form of heridetary hypophosphatemic rackitic disease. The abnormality in vitamin D metabolism is limited to the proximal convoluted tubule and is probably a secondary phenomenon and not a primary genetic derangement. Affected patients variably present with lower extremity deformity, short stature, bone pain, dental abscesses, rickets and/or osteomalacia *(Eccons 1992).*

The classical syndrome is genetically transmitted as an X-linked dominant trait. The syndrome is characterized by life long hypophosphatemia that is secondary to renal tubular phosphate loss. Inspite of the resultant hypophosphatemia, a known stimulus of calcitirol production, affected patients maintain only normal or marginally decreased serum 1, 25- dihydroxy D levels ***(lobaugh and Drezner, 1983).***

The defective regulation of vitamin D metabolism is due to decreased 25-hydroxyvitamin D-1α-hydroxylase activity in the renal proximal tubule. Serum calcium levels are in the normal range and rickets generally appears between 12 and 18 months of age. Remission usually follows growth closure, but recurrence of symptoms is common later in life. Combined oral phosphate and low dose 1α (OH) D3 therapy, coupled with stringent biochemical monitoring has proved to be an effective approach which will allow correction of the osseous abnormalities and enhance growth rates ***(Balson and Tieder, 1990).***

iii. Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH):

Hereditary hypophosphatemic rickets with hypercalciuria occurs most probably as an autosomal recessive trait, it has been described in closely related members of a Bedouin tribe ***(Trieder et al., 1987).***

The disorder comprises of rickets (or osteomalacia), hypophosphatemia due to decreased tubular reabsorption of phosphate and elevated serum concentrations of 1,25-dihydroxyvitamin D, which leads to increased intestinal absorption and hypercalciuria ***(Baltrop , 1992).***

The elevation in circulating 1,25-dihydroxyvitamin D concentrations in HHRH patients is in marked contrast to the decreased concentrations of 1,25 dihydroxyvitamin D observed in X-linked hypophosphatemic rickets. Thus in HHRH patients, the renal 1α hydroxylase enzyme appears to be responding normally to the hypophosphatemic stimulus, which is the result of a renal tubular phosphate reabsorption defect ***(Garcia , 1997).***

The clinical, biochemical and radiological abnormalities are reported to respond to treatment with oral phosphate preparations alone, although the underlying renal tubular defect remains unchanged ***(Garcia , 1997).***

iv. Vitamin D -Dependent Rickets:

Two types are recognized:

Type I:

It results from a deficiency (or response) of the enzyme 1α hydroxylase, which converts 25 (OH)D to 1,25 (OH)2 D. serum levels of vitamin D and 25 (OH) D are normal, but serum levels of

1,25 (OH)2 D are low. It is inherited as an autosomal recessive trait. This responds well to physiological doses of 1,25 (OH)2 D or 1α HCC ***(Ralston, 1993).***

Type II:

It is due to abnormalities of the calcitriol receptors, which either fails to bind to the hormone, or having bound to calcitriol, fails to interact normally with chromosomal DNA ***(Gartner, 1990).***

Abnormalities of the hair in type II may be a useful clinical sign. Generalized alopecia of early onset is present in approximately one half of the reported kindreds. Other patients have spare hair, while approximately one third have normal hair growth ***(Hughes et al., 1988).*** In contrast to type I, serum 1, 25 $(OH)_2$ D level is elevated in type II disorder ***(Pitt, 1991).***

Symptoms may be present as early as 3 months of age (in contrast to the later onset of nutritional rickets), with most patients being symptomatic by 1 year of age. The primary rackitogenic factor is hypocalcemia, which results from a decrease in intestinal calcium absorption, often apparent soon after birth, secondary hyperparathyroidism follows rackitic bone changes may be severe, rapidly progressive and accompanied by pathologic fractures ***(Gartner, 1990).***

v. Fanconi Syndrome:

The term "Fanconi syndrome" is applied to a group of conditions in which varying combinations of proximal renal tubular defects occur in association with rickets or osteomalacia. The proximal tubular defects usually result in generalized aminoaciduria, hyperphosphaturia and hypophosphatemia, and a systemic acidosis due to an excessive urinary bicarbonate loss. In addition, these proximal tubular defects may also occasionally cause renal glycosuria, kaliuresis and hypokalemia, polyuria and hypouricemia ***(Morris and Sebastian, 1983).***

The etiology of fanconi syndrome is complex. The syndrome may arise in the absence of any generalized metabolic disease and may occur sporadically or be inherited ***(Thakher and O'Riordan, 1988).***

The syndrome may also occur in association with certain inherited metabolic diseases, for example cystinosis, wilson's disease and lowe's syndrome; or it may occur in association with chronic hypocalcemia and secondary hyperparathyroidism, as in rickets of vitamin D deficiency and of 1 α-hydroxylase deficiency. Fanconi syndrome may also be acquired, for example, in association with heavy lead poisoning multiple myeloma and degraded tetracycline administration ***(Teotia and Teotia, 1997).***

C. Concomitant Diseases:

There is a number of well recognized associations between rickets and other disorders or treatments.

i.Long term Anticonvulsant Therapy:

It causes vitamin D deficiency and rickets probably due to increased hepatic elimination of vitamin D and its metabolites after enzyme induction by phenobarbital and diphenylhydantoin ***(Gartner ,1990).***

ii.Chronic Liver Disease :

Both hepatocellular and biliary diseases can be associated with defective bone mineralization and rickets. In hepatocellular disease, defective hydroxylation of vitamin D leads to decreased production of the metabolite, 25 (OH)D. In extrahepatic biliary atresia, 25 (OH) D has been found to prevent rickets inspite of severe malabsorption of vitamin D itself ***(Heuli et al, 1990).***

iii.*Chronic Renal Failure:*

Renal osteodystrophy is the term used to designate the spectrum of skeletal changes associated with renal disease. Its manifestations are influenced by the cause of the renal disease, severity of the renal insufficiency, degree of compensatory hyperparathyroidism,

deficiency of vitamin D metabolism, mode and length of dialysis, corticosteroid therapy *(MC Carthy and Kumar 1990).*

The secondary hyperparathyroidism, which occurs early but intermittently in the course of untreated renal failure is provoked by phosphate retention, and leads to depression of serum calcium, which in turn stimulates the release of PTH . Depressed levels of 1,25 $(OH)_2$ D are a result of impaired conversion of 25 (OH) D to 1, 25 $(OH)_2$ D, resulting from the loss of renal mass and 1 α- hydroxylase activity *(MC Carthy and Kumar, 1990).*

In children, the major findings are usually those of rickets. Resorptive changes of secondary hyperparathyroidism can also usually be identified. Skeletal maturation and growth may be delayed or diminished. These children are also prone to develop slipped epiphysis. This complication is most common in- but not restricted to- the proximal femoral epiphysis *(MC Carthy and Kumar, 1990).*

D. Substrate Deficiency:

The term "substrate deficiency" has been used to denote disorders in which the availability of the bone minerals, calcium and phosphorus, is insufficient to sustain normal mineralization in the presence of adequate amounts of vitamin D and its metabolites *(Baltrop, 1992).*

i.Neonatal:

The occurrance of clinical features of classical vitamin D deficiency rickets in young infants and particularly in preterm infants (neonatal osteopenia, neonatal rickets) has been increasingly recognized. Congenital rickets occurs in infants of mothers with nutritional disorders ***(Baltrop, 1992).***

Infants fed with a "preterm formula", as opposed to human milk, showed low plasma phosphours values. In very low birth weight infants (< 1500 gm), the development of rickets has been attributed to prenatal phosphate deficiency secondary to placental insufficiency, which could be prevented by means of postnatal phosphate supplementation ***(Holland et al., 1990).***

ii.Post Neonatal:

Substrate deficiency in the post neonatal age group has been described in relation to calcium deficiency; which occurred in infants receiving a prolonged special diet as a therapy of chronic diarrhea. Severe rickets is also reported in children who were fed a soy-based formula with a very low calcium content. A combined vitamin D and calcium deficiency was reported ***(Davidovits et al., 1993).***

Rickets And Osteocalcin

Modulation of osteocalcin synthesis by vitamin D:

OC synthesis directly involves three vitamins: vitamin K for Gla formation, vitamin C for hydroxylation and vitamin D for stimulation of osteocalcin production ***(Jie et al., 1992).***

Vitamin D has well known involvement in bone metabolism and Ca^{++} homeostasis. It is thus of great interest to find a connection between osteocalcin and vitamin D. The first suggestion that vitamin D modulated osteocalcin synthesis is derived from the observation of decreased bone and serum osteocalcin concentrations in vitamin D-deficient chicks and rats ***(Price, 1993).***

Silve et al. (1986) found out by study of bone derived cell cultures from children that the unstimulated cultures produced only low levels of BGP.However, after preincubation with 1, 25 $(oH)_2$ D_3, all cell cultures released easily detectable amounts of BGP.

OC gene transcription was studied by ***Demany et al (1990),*** who found that osteocalcin gene transcription was directly regulated by 1, 25 $(OH)_2$ D_3.

Mechanism of Action of Vitamin D on Osteocalcin :

The osteocalcin gene is the only gene for which a defenitive direct interaction between the vitamin D receptor and target gene promotor occurs. The vitamin D receptor is the most potent known regulator of the osteocalcin gene ***(Morrison et al., 1992).***

Uses of osteocalcin Measurement in cases of Rickets:

1. Diagnostic:

Infants with rickets demonstrate a reduction in the blood content of vitamin K dependent protein, osteocalcin, most pronounced in disease of calcipenic and phosphopenic varieties. It has also been established that there is an inverse correlation between OC content and the level of PTH.This led ***luk'Lanova et al., (1990)*** to suggest that the measurement of blood OC in children may serve a diagnostic marker of metabolic disorders and mineralization of osseous tissues.

2.Index of response to therapy:

Unchanged or even elevated levels of alkaline phosphatase are frequently observed during the early healing phase of effective antirackitic therapy.Biochemical improvement in nutritional rickets may become evident after a few days, but radiological healing may not be recognized for 2-3 weeks ***(El Kholy, 1994).***

So serum osteocalcin levels may serve as a useful index of active mineralization and provide the children with a prompt indication of a successful therapeutic strategy.

SUBJECTS & METHODS

Subjects , Patients and Methods

Subjects :

The present study was conducted on 73 infants whose ages ranged from 6 to 24 months.All these infants were collected from the pediatric clinics El-Khalifa hospital.They were 51 males (69.9 %) and 22 females (30.1 %) with a male to female ratio 5.1:2.2.All infants have faint coloured skin.

Fifty one infants were chosen randomly among those who were complaining of minor illness and did not receive vitamin D and calcium supplement.With exclusion of those who were suffering from chronic disease that may affect bone growth or serum osteocalcin level and those who were having symptoms or signs of metabolic or hormonal diseases or malnutrition disorders.

Based on the season during which the samples collected, they were classified into two groups :

Group I :

Infants were examined in autumn and winter (from september to february): It includes 21 infans whose ages ranged from 6 to 24 months with mean of 16.857± 5.179 months and male to female ratio 5:2.

Group II :

Infants were examined in spring and summer(from Mars to August) : It includes 30 subjects whose ages ranges from 6 to 24 months (mean ± SD = 17.3 ±5.414). They were 17 males and 13 females .

We also examined 22 infants who were suffering from active rickets. They were classified into other 2 groups .

Group III :

Rackitic patients in autumn and winter : It includes 13 patients. They were 10 males and 3 females whose ages ranged from 6 to 19 months (mean ± SD = 10.153 ±3.531).

Group IV :

Rackitic patients in spring and summer : It includes 9 patients, all were males whose ages ranged from 10 to 19 months with mean ± SD = 13.0 ± 3.6 .

Methods :

The infants were subjected to the following:

1. Full history: With special emphasis on dietary history, supplementation of the diet with vitamin D, exposure to sun and a full history of motor milestones of development.

2. Thorough clinical examination : for signs of rickets, presence of any bone deformities and chest complications.
3. Anthropometric examination: measurement of height, weight and head circumference and referring to percentile according to their ages and sex.
4. Plain X-ray of the wrist.
5. Venous blood samples were collected at the same time (12 p.m), serum was separated and investigated for serum calcium, inorganic phosphorus,alkaline phosphatase and serum osteocalcin by ELISA.

6.Statistical analysis: All data were statistically analysed using student t –test, chi square test and correlation matrix test. All statistics were done using Standard Computer Programme.

A. Estimation of Serum Calcium :

By (Calcium colorimrtric test) ELI Tech.

Principle :

Colorimetric measurement with orthocresolphtalein complexon. The 8-hydroxyquinoline prevents Mg^{2+} from interference up to 4 mmol/L (100 mg/L).

Reagents :

Reagant 1:	Diethylamine	360	mmol/L
Reagant 2:	O- Cresolphtalein complexon	0.15	mmol/L
	8- Hydroxyguinoline	17.2	mmol/L
Standerd :	Calcuim	10	mg/d

Procedure :

Wave length: 270 nm

Temperature: 37 ° C

Cuvette : 1 cm light path

Read against reagent blank

	Blank	Standerd	Sample
Reagent	1 ml	1 ml	1 ml
Distilled Water	10 uL	---	---
Standard	---	10 uL	
Sample	---	---	10 ul

Mix and the optical density (OD) was read after 5 minutes incubation. The final colour is stable for at least 1 hour.

Calculation:

$$\frac{\text{OD sample}}{\text{OD standard}} \quad X\ n$$

n = Standard concentration

n = 10 mg/dl

Reference Values:

Serum, plasma : 8.8-10.2 mg/dL

ELT Tech Diagnostic zone. Industrielle 61500 Sees. France.

B. Estimation of Serum Inorganic Phosphorus:

By (Fiske-Subbarow,Direct method).

Principle :

Phosphates ion reacts with molybdate to produce phosomolybdate , which is finally reduced to a molybdenum blue, which is photometrically measured.

Reagent :

A. Fiski-Subarow reagent 3 x 100 ml

B. Standard 1 x 10 ml

Reagent composition is as follow :

Sulfuric acid 1.7 N.

Ammonium molybdate 7 mM

Iron sulphate 8 mM

Procedure :

	Blank	Standard	Sample
Sample	-	-	٠,١ ml
Standard	-	0.1	- ml
Reagent	3.0	3.0	3.0 ml

Mix well and let stand for 10 min. at room temperature (20-25 ° C).

Wave length : 650 nm

Colour stability : a minimum of 2 hours.

Calculations :

$$\frac{SA \quad O.D}{ST \quad O.D} \times 4 = \text{mg of phosphorus / dl}$$

Normal Values :

3.5 – 6.5 mg/dl

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C. Estimation of Serum Alkaline Phosphatase :

By (direct colorimetric method) Bio-Analysis

Principle :

The present Alkaline Phosphatase (ALP) procedure is based on hydrolysis of thymolphthalein monophosphate by ALP theraby liberating thymolphthalein.The addition of alkali terminates the enzymatic activity and converts thymolphthalein, an alkaline indicator, to a blue-green chromophore, the absorbance of which measured photometrically at 590 nm.

Reagents :

1. Substrate reagent :

   Sodium thymolphthalein monophosphates 10 mmol.

2. Buffer reagent :

   2-amino-2 methyl-1 propanol, magnesium chloride and surfactant.

3. Color Developer :

An aqueous solution of sodium hydroxide and sodium carbonate.

4. Buffered substrate :

The solution is prepared by mixing 1 volume of substrate with 1 volume of buffer.

5. Reference standard :

Solution of thymolphthalein.

Procedure :

	Blank	Standard	Test
Buffered substrate	0.5	0.5	0.5 ml
Standard	-	0.05	- ml
Serum	-	-	0.05 ml
D.W	0.05	-	- ml
Colour developer	2.5	2.5	2.5 ml

Read the absorbance of each test and standard tube against reagent blank at W.L : 540 nm.

Calculation :

$$\frac{\text{Abs of Ts}}{\text{Abs of Std}} \times \text{conc. Of Std (IU/L)} = \text{ALP in test (IU/L)}$$

Abs. of Std : Absorbance reading of standard.

Abs. of Ts : Absorbance reading of specimen.

Expected Values :

< 200 IU/L

D. Estimation of Serum Osteocalcin :

By Enzym-Linked Immunosorbant Assay (ELISA), Active Human Osteocalcin DSL-10-7600 :

Priciple :

The DSL (Diagnostic Systems Laboratories) Osteocalcin Assay is an enzymatically amplified "One-step" Sandwich-type immunoassay. In the assay, standards and unknown diluted serum samples are incubated with anti-osteocalcin polyclonal detection antibody Labelled with the enzym Horseradish Peroxidase in microfilteration wells coated with an affinity purified anti-osteocalcin mouse monoclonal antibody. After incubation and washing, the wells are incubated with the substrate tetramethyl Benzidine(TMB).An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm.

The absorbance measured is directly proportional to the serum concentration of osteocalcin. A set of osteocalcin standards is used to plot a standard curve of absorbance versus osteocalcin concentration . The unknown samples can be calculated from the curve.

Reagents :

a. Anti-osteocalcin Coated Microtitration Strips : One strip holder containing microfiltration wells coated with anti-osteocalcin mouse monoclonal antibody.

b. Osteocalcin standard A/sample diluent: One bottle 55 ml, containg 0 ng/ml osteocalcin in a protien-based buffer with a non-mercury preservative.

c. Osteocalcin standards (Lyophilized) : Five vials, labelled B-F, containing approximate concentrations of 4,20,60,120,240 ng/ml osteocalcin in a protien-based buffer with a non mercury preservative. Reconstitute each vial with 1.0 ml of deionized water.

d. Osteocalcin controls : Two vials, levels I and II containing low and high concentrations of osteocalcin in a protien based buffer.

e. Osteocalcin Assay Buffer : A protien based buffer.

f. Osteocalcin Antibody-Enzyme Conjugate Concentrate: Purified anti-osteocalcin poly clonal antibody conjugate to the enzyme horseradish peroxidase in a protein buffer.

g. TMB Chromogen Solution : Solution of tetramethylbenzidin (TMB) in citrate buffer with hydrogen peroxidase.

h. Wash Concentrate : Buffered saline with a nonionic detergent

.

i. Stopping Solution : 0.2 M. sulfuric acid.

Procedure :

1. Mark the microfilteration strips to be used according to the protocol.
2. Pipet 100 uL of each standard, control and diluted unknown to the appropriate well.
3. Prepare the Antibody-Enzyme Conjugate Solution by diluting the conjugate concentrate with the assay buffer.
4. Add 100 uL of Antibody-Enzyme Conjugate Solution to each well using a semi automatic dispenser .
5. Incubate the wells, shaking at a fast speed (500-600 rpm)on an orbital microplate shaker for 2 hours at 22-28°C.
6. Aspirate and wash each well five times the wash solution.
7. Add 100 uL of the TMB chromogen solution to each well using a semi automatic dispenser .
8. Incubate the wells, shaking at a fast speed (500-600 rpm) on an orbital microplate shaker for 10 min. at 22-28°C.
9. Add 100 uL of the 0.2 M sulfuric acid (stopping solution) to each well using a semi automatic dispenser .
10. Read the absorbance of the solution in the wells withen 30 min., using a microplate reader set to 450 nm.

Calculation :

a. Calculate the mean absorbance for each standard, control or unknown.

b. Using a linear- or semi-log graph paper, plot the mean absorbance reading for each of the standard along the y-axis versus the osteocalcin concentrations in ng/ml along the x-axis.

c. Draw the best fitting curve through the mean of the duplicate points

d. Determine the osteocalcin concentrations of the controls and unknown from the standard curve by making their mean absorbance reading with the corresponding osteocalcin.

Normal Values in children : 10-25ng /ml

Diagnostic System Laboratories, Inc. Corporate Headquarters
445 Medical Center BLVD,webster , Texas. USA

RESULTS

Results

The present study comprised 73 infants whose ages ranged from 6 to 24 months .

Fifty one apparently healthy infants were classified according to season of examination into 2 groups :

group I : includes 21 infants who were examined in winter and autumn.

group II: includes 30 infants who were examined in summer and spring.

Twenty two rackitic infants were classified into another 2 groups:

Group III : includes 13 rackitic infants who were examined in winter and autumn.

group IV : which includes 9 rackitic infants who were examined in summer and spring.

The clinical data of the four studied groups are illustrated in table (1) which shows that group I comprised 21 healthy infants

examined in winter and autumn whose ages ranged from 6 to 24 months with mean of 16.85±5.17 months, 71.4% of them were males and 28.6% were females.

Table (1) : clinical data of the studied groups

Group	No.	Sex				Age in months	
		M.	%	F.	%	Range	Mean
Group I (healthy infants examined in winter & autumn)	21	15	71.4%	6	28.6%	6-24	16.857 ±5.179
Group II (healthy infants examined in summer and spring)	30	17	56.7%	13	43.3%	6-24	17.3 ±5.414
GroupIII(rackitic infants examined in winter and autumn)	13	10	76.9%	3	23.1%	6-19	10.153 ±3.531
Group IV (rackitic infants examined in summer and spring)	9	9	100%	0	0%	10-19	13 ±3.6

Group II includes 30 healthy infants examined in summer and spring whose ages ranged from 6 to 24 months with mean of

17.3±5.41 months, 56.7% of them were males and 43.3% were females.

As regards group III, it includes 13 rackitic infants examined in winter and autumn, their ages ranged from 6 to 19 months with mean of 10.15 ± 3.53 months 76.9% were males and 23.1%were female.

Group IV comprised 9 rackitic infants examined in summer and spring, their ages ranged from 10 to 19 months with mean of 13± 3.6months , all of them were males.

Table (2)
The anthropometric data of the studied groups

Group	No.	Height percentile for Age & sex			Weight percentile for Age & sex			Head circumference percentile for age & sex		
		<5th	5th –10th	≥25th	<5th	5th-10th	≥25th	<5th	5th-10th	≥25th
Group I (healthy infants examined in winter and autumn	21	4.7%	9.5%	85.7%	9.5%	47.7%	42.8%	4.8%	14.2%	80.9%
Group II (healthy infants examined in summer and spring)	30	53.3%	13.3%	33.3%	16.7%	40.0%	43.3%	16.7%	6.7%	76.7%
Group III (rackitic infants examined in winter and autumn	13	23.1%	30.8%	46.2%	7.7%	46.2%	46.2%	0%	0%	100%
Group IV (rackitic infants examined in summer and spring)	9	44.4%	11.2%	44.4%	44.4%	11.1%	44.4%	0%	0%	100%

The anthropometric data of the studied groups are illustrated in ***table (2)***which shows that 4.7% of group I (healthy infants examined in winter and autumn) had height percentile less than 5^{th} percentile while 53.3% of group II (healthy infants examined in summer and spring) had height percentiles less than the 5^{th} percentile for their age and sex. 23.1% of group III (rackitic infants examined in winter and autumn) had height percentile less than the 5^{th} . While 44.4% of group IV (rackitic infants examined in summer and spring) had height percentiles less than the 5^{th} percentiles for their age and sex.

As regards weight, 9.5% of group I had weight percentiles less than 5^{th} for age and sex. 16.7% of group II had weight percentiles less than the 5^{th} percentile for age and sex.While group IIIhad 7.7 % less than the 5^{th} percentile for age and sex, and 44.4 % of group IV had weight percentiles less than the 5^{th} for age and sex.

Table (3)

Statistical comparison between height Percentiles in group I&II

Group I		Group II		t-test	P
Range	Mean	Range	Mean		
$<5^{th}-90^{th}$	50.714 ±5.45	$<5^{th}-90^{th}$	21.166 ±3.5	14.524	0.001

P <0.01= high significant

When height percentiles of group I were statistically compared to group II as shown in ***table (3)*** it was found that height percentiles of healthy infants examined in winter and autumn (group I) were significantly higher than height percentiles of healthy infants examined in summer and spring (group II).

Table(4)
Statistical comparison between weight percentiles in groupI and group II

Group I		Group II		t-test	P value
Range	Mean	Range	Mean		
$<5^{th}$-90^{th}	24.76± 10.54	$<5^{th}$-90^{th}	20.5± 12.04	-1.982	0.53

P> 0.05 = non significant.

Table (5)
Statistical comparison between head circumference in group I and II

GroupI		GroupII		t-test	P-value
Range	Mean	Range	Mean		
$<5^{th}$-90^{th}	42.619 ±13.02	$<5^{th}$-90^{th}	47.5± 8.99	-1.769	0.324

P> 0.05 = non significant

While there was no statistically significant difference between the two groups as regards weight or head circumference percentiles ***(tables 4&5)***

Table (6)
Laboratory findings of group I
(Infants examined in winter and autumn)

Parameter	Minimum	Maximum	Mean	S.D
Serum calcium (mg/dl)	8.00	11.0	9.3714	±0.935
Serum inorganic phosphorus (mg/dl)	4.1	6.4	4.686	±0.604
Serum alkaline phosphatase (U/L)	28.5	260	70.261	±51.915
Serum osteocalcin(ng/ml)	9.00	59.0	25.523	±13.302

The laboratory findings of group I are illustrated in ***table (6)*** which shows that serum total calcium level of this group ranged from 8 to 11 mg/dL with mean of 9.371 ±0.935 (the normal total serum calcium is 8.8-10.7mg/dL). Serum inorganic phosphorus ranged from 4.1 to 6.4 mg/dL with mean of 4.686±0.604 mg/dL (the normal serum inorganic phosphorus is 4.5 to 6.5mg/dL).

Serum alkaline phosphatase ranged from 28.5 to 260 U/L with mean of 70.26±51.92 U/L (normal serum alkaline phosphatase is <200 U/L). Serum osteocalcin level ranged from 9 to 59 ng/ml with mean of 25.52±13.302 ng/ml (normal serum osteocalcin is 40-60ng/ml in neonates with gradual decline to reach 10-25ng/ml in children)

Table (7)
Laboratory findings of group II
(Healthy infants examined in summer and spring)

Parameter	Minimum	Maximum	Mean	±S.D
Serum calcium (mg/dl)	8.0	11.0	9.763	±0.96
Serum inorganic phosphorus (mg/dl)	4.2	5.5	5.526	±0.361
Serum alkaline phosphatase (U/L)	50	267	92.633	±38.227
Serum osteocalcin(ng/ml)	0.0	52.0	15.860	±10.938

Table (8)
Statistical comparison between the laboratory findings
In group I and II

Parameter	Group I				Group II			
	Range	Mean	SD	Range	Mean	±SD	t	P
S.calcium (mg/dl)	8-11	9.371	0.935	8-11	9.763	±0.960	-1.45	0.153
S.inorganic phosphorus (mg/dl)	4.1-6.4	4.686	0.604	4.2-5.5	5.526	±0.361	1.6	0.001
S.alkaline phosphatase(U/L)	28.5-260	70.262	51.915	50-267	92.633	±38.277	-1.84	0.072
S.osteocalcin(ng/ml)	9-59	25.524	13.302	0-52	15.86	±10.938	2.721	0.009

P> 0.05 = non significant.

P< 0.01 = high significant

Table (7) presens the laboratory findings of healthy infants examined in spring and summer (group II). Serum calcium level ranged from 8 to 11mg/dl with mean of 9.763 ±0.960 mg/dl. There

was insignificant difference of serum calcium levels between group II and group I as shown in ***table (8).***

Serum inorganic phosphorus of group II ranged from 4.2 to 5.5mg /dl with mean of 5.526+0.326.It is significantly higher in group II(healthy infants in spring and summer) than group I(healthy infants in autumn and winter ,as shown in ***table (8).***

As regards serum alkaline phasphatase, its levels ranged from 50 to 267 U/L with mean value of 92.633 ±38.227 U/L. There was insignificant difference between serum alkaline phosphatase level of group II and group I as shown in ***table (8).***

Serum osteocalcin of group II ranged from 0 to 52 ng/ml with mean value of 15.86±10.938 ng/ml.Serum osteocalcin levels of group II shows high significant decrease when compared to group I as shown in ***table (8)*** .

Table (9)
Laboratory findings of group III
(rackitic infants examined in winter and autumn)

Parameter	Minimum	Maximum	Mean	±S. D
Serum Calcium(mg/dl)	7.6	9.9	8.780	±0.669
Serum inorganic phosphorus (mg /dl)	2.7	5.2	4.084	±0.838
Serum alkaline phosphatase (U/L)	75	381	166.692	±96.813
Serum osteocalcin (ng/ml)	21	68	35.369	±15.226

Table (9) illustrates the laboratory investigations of rackitic infants who were examined in winter and autumn (group III) . It shows that serum calcium ranged between 7.6 and 9.9 mg/dL with mean value of 8.780 ± 0.669 mg/dl .Serum inorganic phosphorus of this group ranged between 2.7 and 5.2mg /dL with mean value of 4.084 ±0.838. While serum alkaline phosphatase ranged from 75 to 381U/L with mean of 166.692±96.813U/L .Serum osteocalcin ranged from 21 to 68 ng/ml with mean value of 35.369±15.226.

Table (10)
Statistical comparison between laboratory Findings of group I and group III

Parameter	Group I			Group III				
	Range	Mean	SD	Range	Mean	±SD	t	P
S.calcium (mg/dl)	8-11	9.371	0.935	7.6-9.9	8.780	±0.669	-2.137	0.009
S.inorganic phosphorus (mg/dl)	4.1-6.4	4.686	0.604	2.7-5.2	4.084	±0.838	1.805	0.080
S.alkaline phosphatase (U/L)	28.5-260	70.262	51.915	75-381	166.692	±96.813	4.385	0.001
s.osteacalin (ng/ml)	9-59	25.524	13.302	21-68	35.369	±15.226	5.029	0.001

P > 0.05 = non significant
P < 0.01 = high significant

When means of serum calcium, serum phosphorus, serum alkaline phosphatase and serum osteocalcin of group III are compared to group I as shown in ***Table (10)***, it is found that there is high significant decrease in serum calcium level in group III as compared to group I .But there is insignificant difference between serum inorganic phosphorus of the two groups. While serum alkaline phosphatase is highly significant increased in group III as compared to group I. As regards serum osteocalcin of group III, it shows high significant increase when compared to group I.

Table (11)
Laboratory findings of group IV
(rackitic infants examined in summer and spring)

Parameter	Minimum	Maximum	Mean	±S. D
Serum Calcium (mg/dl)	7.7	8.9	8.266	±0.421
Serum inorganic phosphorus (mg IdL)	2.9	5.7	4.344	±0.881
Serum alkaline phosphatase (U/L)	59	321	186.111	±112.246
Serum osteocalcin (ng/ml)	4.5	45	18.6 66	±12.906

Table (11) illustrates the laboratory investigations of group IV (rackitic infants who were examined in summer and spring). It shows that serum total calcium level of this group ranged between 7.7 and 8.9 mg/dl with mean value of 8.266 ±0.421. Serum inorganic phosphorus of this group ranged from 2.9 to 5.7 mg/dl with mean value of 4.344 ± 0.881mg/dl .Serum alkaline phasphatase of group IV ranged between 59 and 321 with mean value of 186.111 ±112.246. While serum osteocalcin level ranged between 4.5 and 45 ng/ml with mean value 18.666 ±12.906.

Table (12)
Statistical comparison between laboratory findings of group III and group IV

Parameter	Group III			Group IV				
	Range	X`	SD	Range	Mean	±SD	t	P
S.calcium (mg/dl)	7.6-9.9	8.780	0.669	7.7-8.9	8.266	±0.421	0.232	0.070
S.inorganic phosphorus (mg/dl)	2.7-5.2	4.084	0.838	2.9-5.7	4.344	±0.881	-0.700	0.492
S.alkaline phosphatase (U/L)	75-381	166.692	96.813	59-321	186.111	±112.246	-0.434	0.669
S.osteocalcin (ng/ml)	21-68	35.369	15.226	4.5-45	18.666	±12.906	2.685	0.014

P > 0.05 = non significant.
P< 0.05 = significant

Statistical comparison between the laboratory finding of group III (rackitic infants examined in winter and autumn) and those of group IV (rackitic infants examined in summer and spring) is shown in ***Table (12).*** There was insignificant difference in serum total calcium, serum inorganic phosphorus or alkaline phosphatase of group IV as compared to group III. But serum osteocalcin was significantly decreased in group IV than group III.

Table (13)
Statistical comparison between laboratory findings of group II (healthy in summer and spring) and group IV (rackitic in summer and spring)

Parameter	Group II			Group IV				
	Range	X`	S.D	Range	X`	±S.D	t	p
S.calcium (mg/dl)	8-11	9.763	0.96	7.7-8.9	8.266	±0.421	-3.104	0.004
S.inorganic phosphorus (mg/dl)	4.2-5.5	5.526	0.361	2.9-5.7	4.344	±0.881	3.689	0.001
S. alkaline phosphatase (U/L)	50-267	92.633	38.227	59-321	186.111	±112.246	7.003	0.001
S.osteocalcin (ng/ml)	0-52	15.860	10.938	4.5-45	18.666	±12.906	2.167	0.037

$P < 0.05$ = significant.
$P < 0.01$ = high significant.

Table (13) shows statistical comparison between laboratory findings of group IV (rackitic infants examined in summer and spring) and group II (healthy infants examined in summer and spring).

There was high significant decrease of total serum calcium level in group IV as compared to group II.Serum inorganic phosphorus was high significant lower in group IV than group II . There was high significant higher level of alkaline phosphatase in group IV as compared II .Serum osteocalcin was significantly increased in group IV as compared to group II .

Table(14)
Statistical correlation between serum osteocalcin and serum inorganic phosphorus in group I & II.

S.Osteocalcin X`± S.D	S.inorg.phos. Mean± S.D	r value	P value	significance
20.090±12.779	4.83±0.481	-0.322	< 0.05	S

S = significant

Statistical correlation was done between serum osteocalcin and other studied parameters in healthy groups(I and II) as illustrated in ***tables (14,15,16)*** which show significant negative correlation between serum osteocalcin level and serum inorganic phosphorus level as r=-0.332 with P value less than 0.01 (table 14).Also there was negative correlation between serum osteocalcin and serum total calcium level as r =-0.305 with P value less than 0.o5 ***table (15).***

Table(15)
Statistical correlation between serum osteocalcin and serum Calcium in group I & II.

S.Osteocalcin X`± S.D	S.Calcium Mean± S.D	r value	P value	Significance
20.090±12.779	9.9±0.96	-0.305	< 0.05	S

S = significant

While there was no correlation between serum osteocalcin and serum alkaline phosphatase as r = 0.20 and P value more than 0.05 ***table (16).***

Table(16)
Statistical correlation between serum osteocalcin and serum Alkaline Phosphatase in group I & II.

S.Osteocalcin **X`± S.D**	S.Alk. Phos. **X`± S.D**	r value	P value	Significance
20.090±12.779	83.890±45.603	0.220	> 0.05	N.S

N.S = non significant

Table (17) shows correlation matrix between serum osteocalcin total serum calcium , serum inorganic phosohorus and serum alkaline phosphatase.

Table (17)
correlation matrix between serum osteocalcin total serum calcium , serum inorganic phosohorus and serum alkaline phosphatase in group I & II using r value.

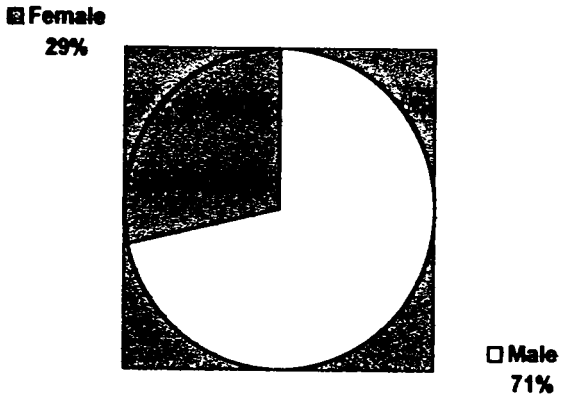
	S.Osteocalcin	S.Inorg.Ph.	S.Calcium	S.Alk.Phosph.
S.Osteocalcin		- 0.332 S	- 0.305 S	0.220 N.S
S.Inorg.Ph.			0.457 H.S	0.035 N.S
S.Calcium				0.350 S

S = Significant. N.S = Non significant. H.S = High Significant.

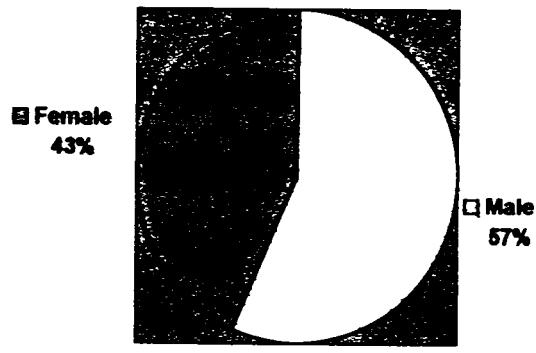
Total serum calcium was highly significant positively correlated to serum inorganic phosphorus as r =0.457 and P value is less than 0.01 ***(table 17)***.

While there was no correlation between serum inorganic phosphorus and serum alkaline phosphatase in group I and II ***(table 17)***.

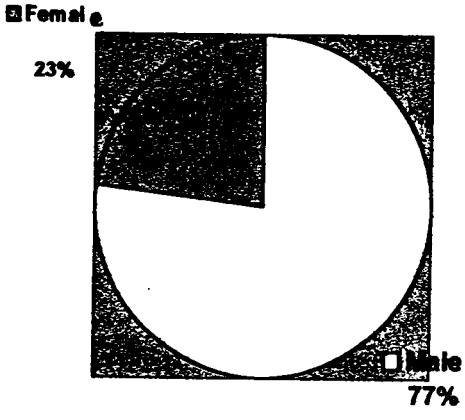
Group I (healthy in winter & autumn)



Group II (Healthy in summer & spring)



Group III (Rackitics in winter & autumn)



Group IV (rackitics in summer & spring)

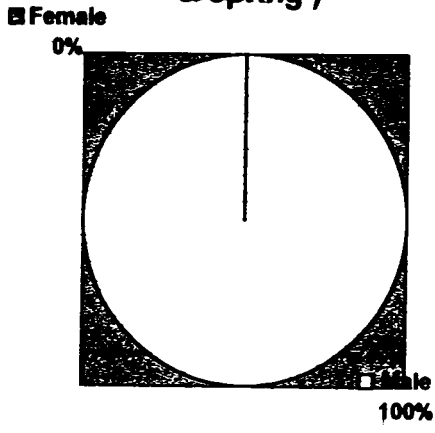


Fig. (12) Anthropometric data of the studied groups.

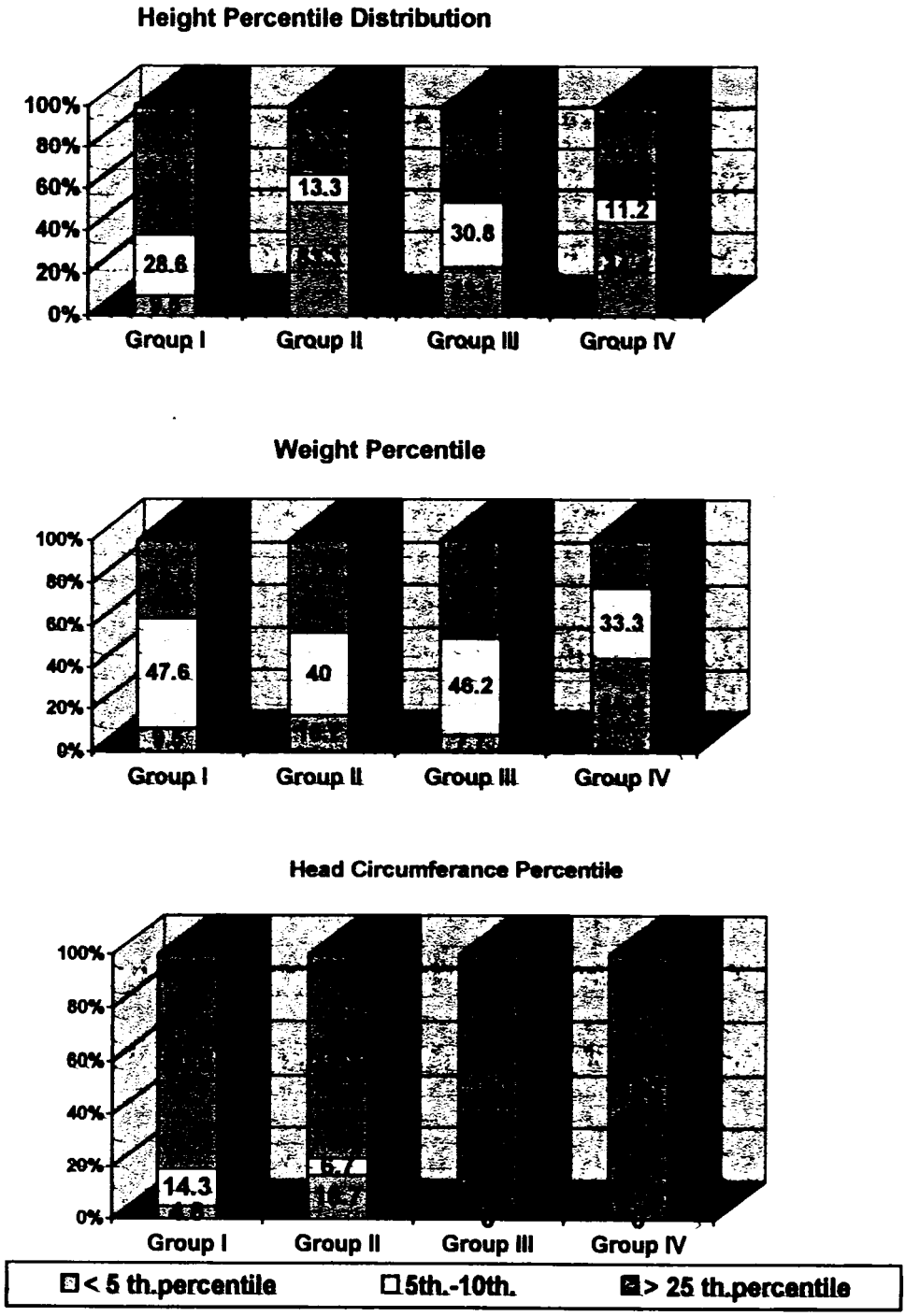
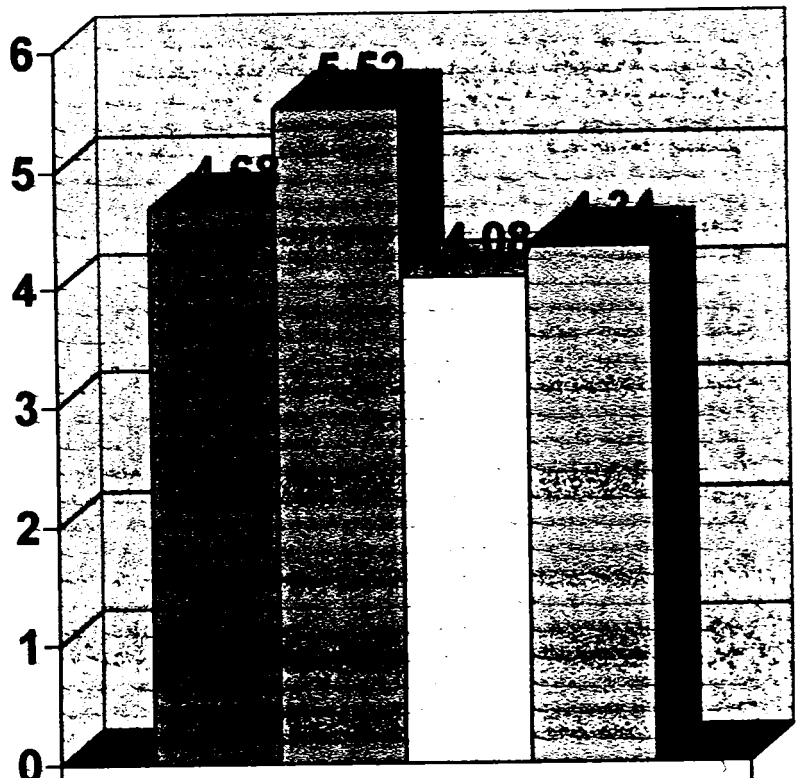
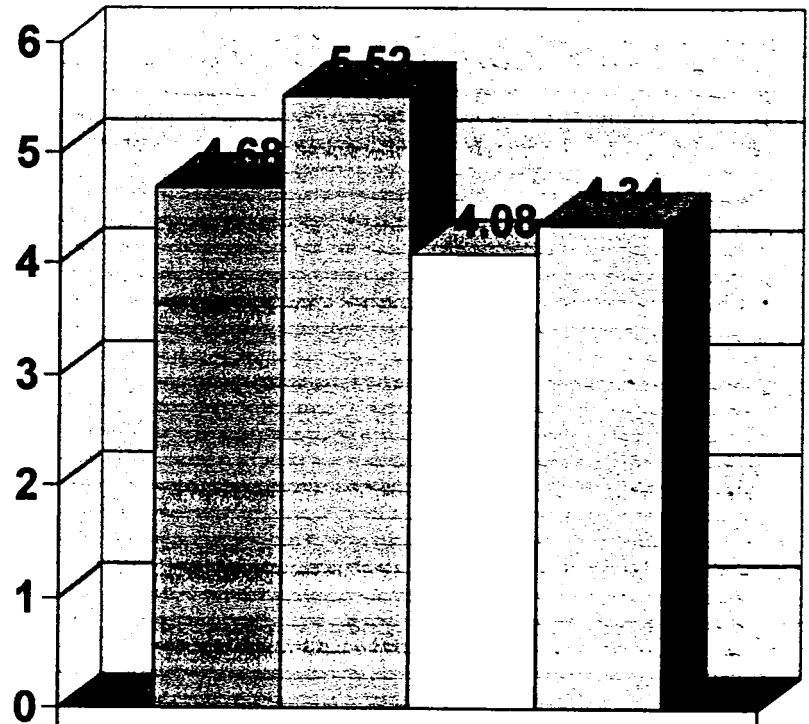


Fig. (13) Comparative study between all studied groups regarding mean serum calcium.



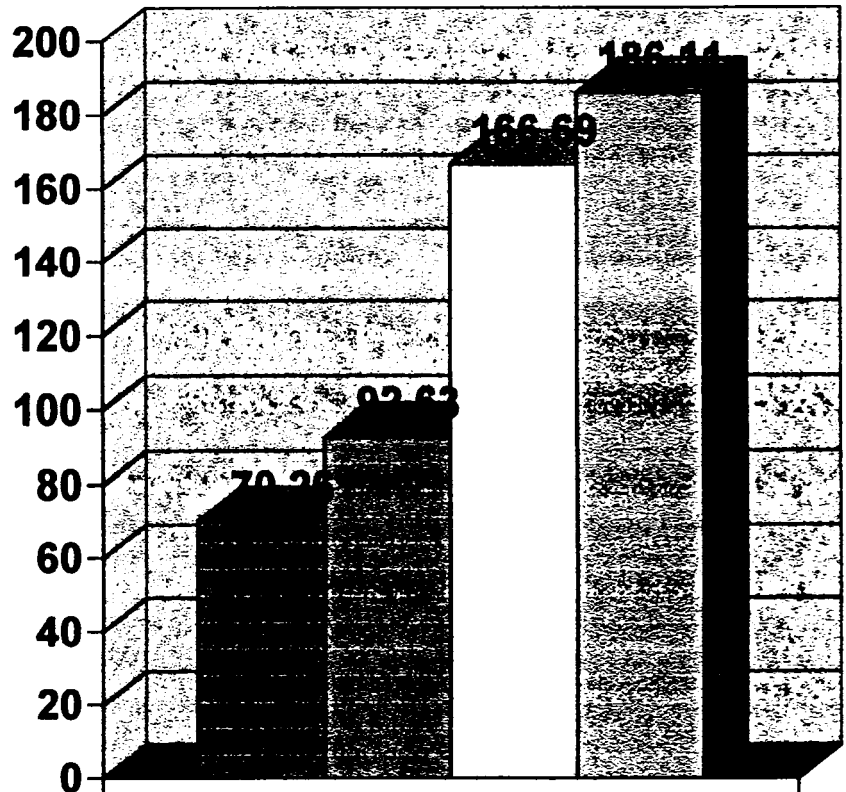
	Mean S.Inorg.Phosphorus
Group I	4.68
Group II	5.52
Group III	4.08
Group IV	4.34

Fig. (14) Comparative study between all studied groups regarding mean serum inorganic phosphorus.



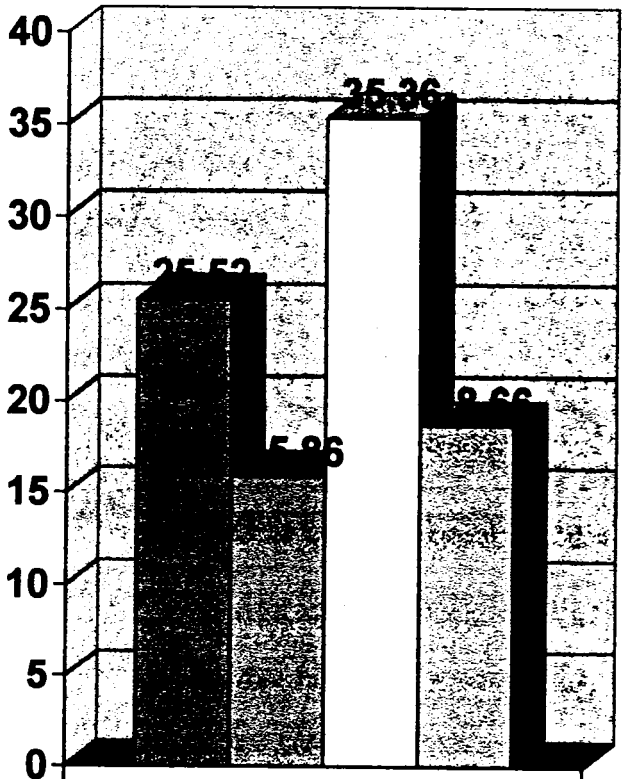
	Mean S.Inorg.Phosphorus
Group I	4.68
Group II	5.52
Group III	4.08
Group IV	4.34

Fig. (15) Comparative studies between all studied groups regarding mean serum alkaline phosphatase.



	Mean S. Alkaline Phosphatase
Group I	70.26
Group II	92.63
Group III	166.69
Group IV	186.11

Fig.(16) Comparative study between all studied groups regarding mean serum osteocalcin .



	Mean S. Osteocalcin
Group I	25.52
Group II	15.86
Group III	35.36
Group IV	18.66

DISCUSSION

Discussion

Before the use of biochemical markers of bone formation , it was hard to know the actual mechanism of bone growth , mineralization and bone metabolism ***(Renucci et al. ,1993)***.

Osteocalcin (bone gamma carboxyglutamic acid containing protein) is a protein synthesized by osteoblasts and incorporated in the bone matrix. It is a sensitive and specific marker of bone formation reflecting osteoblastic activity , since a portion of newly synthesized osteocalcin is released into circulation from bone ***(Fewtrell et al., 2000)***.

Osteocalcin accounts for approximately 25% of the non - collagenous protein found in adult bone and is therefore the most abundant protein in the human skeleton (about 15 g/kg body weight of man). Mineralized bone and dentin matrices contain high concentration of osteocalcin , whereas osteoid and predentin do not ***(Hochberg and karger 2002)***.

The synthesis of osteocalcin is under the effect of 1,25(OH)2 D3 , the active metabolism of vitamin D ***(long et al. , 1999)***.

The carboxylated form of osteocalcin (a vitamin K dependent process) favour the deposition of hydroxy apatite in the organic

bone matrix and hence the regulation of bone mineralization ***(fewtrell et al., 2000).***

Infants with rickets demonstrate a reduction in the blood content of osteocalcin. It has also been established that there is an inverse correlation between osteocalcin content and the level of PTH. This led ***luk'lanona et al., (1990)*** to suggest that the measurement of blood osteocalcin in children may serve as a diagnostic marker of metabolic disorders and mineralization of osseous tissues.

The aim of this study was to investigate possible effect of seasons on the level of serum osteocalcin in infants to determine their effect on bone growth. .

The present study was conducted on 73 infants whose ages ranged between 6 and 24 months . They were divided into four groups ,group I : apparently healthy infants who were examined in winter and autumn , group II :apparently healthy infants who were examined in summer and spring , group III : rackitic infants who were examined in winter and autumn and group IV : rackitic infants who were in summer and spring .

As regards anthropometric data , our study showed that height percentiles of infants who were examined in summer and spring

were significantly lower than those examined in winter and autumn . In summer and spring 53.3% of infants had height percentiles less than 5th while in winter and autumn 4.7% only had height percentiles less than 5th . It was found that infants in winter or autumn have significantly higher height percentiles than infants in summer or spring. This can be explained by bone remodeling which increase in winter and autumn so bone formation is increased and this is reflected by increase height growth velocity (Iong et al., 1999). This fact is one of the causes of seasonal variation of osteocalcin which showed highly significant decrease in summer and spring than in winter and autumn as we will discuss later . While the study showed no significant difference in weight between infants examined in summer or spring and those in winter or autumn as in winter and autumn 9.5%of infants had weight percentiles less than 5 th and 16.7% was the ratio in summer and spring .In agreement with our study , ***Specker et al. in 1994*** stated that there was no significant seasonal effect on newborn weight. Also ***Bhandari and others in 1999*** reported that there were no significant difference in weight of low birth weight infants born in summer or spring than born in winter or autumn . Even ***Cooper et al. in 1997*** who stated that there were statistically significant association between weight at one year and bone mineral content, they found infant weight was not significantly associated with either biochemical markers of bone turnover.

Also there was no effect of seasons on head circumference ,as this study showed no significant difference in head circumference between infants who were examined in winter or autumn and infants and those examined in summer or spring. In agreement with our result ***,Sierra et al.in 1993*** stated that head circumference of SGA and AGA infants was not affected by seasons.

This study showed that levels of serum inorganic phosphorus in apparently healthy infants who were examined in summer or spring were highly significant increased than those of apparently healthy infants who were examined in winter or autumn .This can be explained by the lower levels of serum osteocalcin in summer and spring so bone mineralization will decrease and inorganic phosphorus level will increase in serum. In contrast to our result ***Specker et al. , in 1993*** stated that there were no differences in serum phosphorus of newborn infants born in summer and those born in winter and they did not explain their results.

The present study there was no significant difference in total serum calcium of apparently healthy infants who were examined in summer or spring and apparently healthy infants who were examined in winter or autumn. In agreement with our results ***Seirra et al., in 1993*** found that there was no significant difference in serum calcium of newborn infants by change of season of birth.

Also this study shows no significant difference between apparently healthy infant who were examined in summer or spring and those who were examined in winter or autumn as regards serum alkaline phosphatase level. Our results were in agreement with ***specker et al., (1994)*** who stated that there were no difference in serum alkaline phosphatase of newborn infants by season of birth. Serum alkaline phosphatase is derived from several sources other than bone so it doesn't reflect appositional bone growth and doesn't change with seasons. However in adult scale ***Douglas et al., 1996*** reported that alkaline phosphatase activity in serum were higher in autumn than in spring in postmenopausal women.This can be explained by bone resorption in postmenopausal women which increase in autumn and winter.

The present study demonstrated high significant decrease in serum osteocalcin level in apparently healthy infants who were examined in summer or spring than in apparently healthy infants who were examined in winter or autumn. In contrast to our study ***specker et al., 1994*** studied seasonal difference in bone mineral content in relation to osteocalcin and $1,25(OH)_2$ D and they found significant lower bone mineral content and higher serum osteocalcin levels in summer born versus winter born newborn infants. As regards bone mineral content data of small for gestational age infants, ***Namgung and other in 1993*** found significant lower bone mineral content and higher serum

osteocalcin level in summer born than in winter born newborn SGA infants.In this study when we refer to the birth date of studied infants, it is found that almost all of winter and autumn examined children (the group of higher serum osteocalcin levels) were born in summer.

Reduced bone mineral content and high serum osteocalcin in summer born newborns is related to reduced maternal vitamin D status in the preceding winter, that is mean sunshine deprivation in winter alters vitamin D metabolism in the mother and fetus during an important early fetal skeletal development resulting in low bone mineral content and high osteocalcin level at birth (6 months later),so it is an early fetal effect(Specker et al.,1994). In studies of normal preterm and term infants, mean cord serum osteocalcin concentrations were higher than ,and not correlated with maternal concentration, supporting fetal production of osteocalcin. So high serum osteocalcin concentrations in summer-born infants may be an adaptive response to the lower BMC of these infants, secondary to elevated plasma 1,25(OH)2D ***(Namgung et al.,1993).***

By reviewing the literature till now,we can not find similar study on the same age,however on adults level ***Berstralh et al., in 1999*** have shown significantly higher bone mineral content and lower serum osteocalcin concentrations in adults in summer than in

winter. This seasonal variation of osteocalcin is the same variation which was found in this study on infants.

Bhandari etal.in 1999 demonstrated that in vivo and vitro study, 1,25 $(OH)_2$ D stimulates basal levels of osteocalcin. The low bone mineral content in winter has been thought to be related to seasonal variation in sunshine exposure and hence vitamin D metabolism, consequently 1,25 $(OH)_2$ D was elevated in winter as an adaptive mechanism for low vitamin D status.

Fewtrell et al., (2000) supported the fact that bone remodeling was the cause of seasonal variation. As bone remodeling increased in winter and autumn together with decreased bone mineral density. So seasonal differences in serum osteocalcin and 1,25 (oH)2 D may represent compensatory responses to BMC changes.

Seasonal variation of serum osteocalcin could be due to change in calcitropic hormones. These hormones act directly influencing the production rate or metabolic clearance rate. Human growth hormone, cortisol and thyroxine exhibit significant seasonal variations through their seasonal changes in bone remodeling ***(Kamihagi et al., 1999).***

Osteocalcin levels were significantly decreased and height growth was markedly suppressed in pediatric patients who were given prednisolone for a long period. So cortisol affect bone formation which is reflected by the serum level of osteocalcin ***(long et al., 1999).***

Osteocalcin serum levels in children with total growth hormone deficiency are significantly lower than control subjects. The treatment of these children by growth hormone accelerates growth velocity and increase the levels of plasma osteocalcin ***(Cooper et al., 1997).***

So, growth hormone increase bone remodeling activity, while cortisol might lower serum osteocalcin level due to decrease production rate ***(Fewetrell et al., 2000)*** .

In a study was done on post-menopausal women, ***Douglas et al., in 1996*** reported that serum osteocalcin concentration was higher in spring than in winter. This was explained by increase bone resorption in winter .

As regards rackitic infants in our study,there was significant decrease in total serum calcium in rackitic infants than healthy infants who were examined in the same season. These results were in agreement with ***El-kholy et al.,(1994)***who explained

hypocalcemia to be due to vitamin D deficiency whose major action is to enhance calcium absorption.

Serum inorganic phosphorus of rackitic infants in winter and autumn wasn't significantly changed than healthy infants who were examined in the same season .while rackitic infants who were examined in summer or autumn had high significant lower level of serum inorganic phosphorus than healthy infants who were examined in the same season .This is in consistent with previous studies by ***El kholy et al., (1994)*** who explained hypophosphatemia to occur due to the development of hyperparathyroidism leading to hyperphosphaturia and consequently hypophosphatemia.

As regards serum alkaline phosphatase in rackitic infants , it was highly significant increased in them than in apparently healthy infants who were examined in the same season .These results are in accordance with previous studies by ***Sabry (1990) and El Kholy (1994).*** A well established fact is a progressive increase in the plasma alkaline phosphatase value, reflecting increasing but ineffective activity of osteoblastic cells ***(Baltrop et al., 1992)***.

Serum osteocalcin levels in rackitic infants were significantly higher than in apparently healthy infants who were examined in the same season .These results are in contrast with ***Wergedal et***

al.,(1992)and El Kholy et al., (1994). One of the best known effects of vitamin D (1,25(OH) 2 D3) on bone cells is to stimulate osteocalcin production ***(park et al., 1998).*** So in rickets ,low serum 1,25 (oH)2 D3 concentration leads to low serum osteocalcin . But in our study rackitic infants started treatment with vitamin D supplementation so osteocalcin level were high as a response to stimulation of osteoblastic activity .Other biochemical markers of bone mineralization didn't show any signs of recovery , also radiological investigation of all rackitic infants didn't show any manifestations of healing process. These results are in agreement with ***Baltrop in 1992*** who stated that unchanged or even elevated levels of alkaline phosphatase are frequently observed during the early healing phase of effective anti-rackitic therapy. While serum osteocalcin levels may increased after a few days of treatment but radiological healing may not be recognized for 2-3 weeks.

The effect of seasons on rackitic infants was obvious in our study. Serum osteocalcin was significantly lower in summer or spring than in winter or autumn.

While there was no effect of seasons on serum calcium,serum inorganic phosphorus and serum alkaline phosphatase levels as there were no significant difference between rackitic infants who were

examined in summer or spring and those who were examined in winter or autumn.

Our finding of a significant negative correlation between osteocalcin and serum inorganic phosphorus in healthy ggroups (I and II) is in accordance with previous studies by ***Gunberg et al.,in 1999,*** who found that hypophosphatemia may be a general regulator of osteocalcin synthesis.

The significant negative correlation between serum osteocalcin and total serum calcium in group I and II is in agreement with ***Gunberg et al.(1999)*** who demonstrated an inverse relationship between serum calcium concentration and serum osteocalcin levels, as calcium induces alpha –helical conformation of osteocalcin and promote adsorption of osteocalcin on hydroxyapatite.

Absence of correlation between serum osteocalcin and serum alkaline phosphatase is in accordance with previous report by ***Duda et al. ,(1998)*** who recorded dissociation between serum levels of osteocalcin and alkaline phosphatase. ***Epstien in 1998*** suggested that although alkaline phosphatase and osteocalcin are both indices of osteoblastic activity ,yet these two markers sometimes correlate poorly.

SUMMARY & CONCLUSION

Summary & Conclusion

The conventional clinical indices of bone metabolism involve the measurement of serum alkaline phosphatase and urinary hy - droxyproline excretion. However, serum alkaline phosphatase is derived from several sources other than bone and urinary hydroxyproline reflects the turnover of extra-skeletal as well as skeletal proteins. Furthermore, alkaline phosphatase is mainly related to bone mineralization and isn't a sensitive index of bone growth.

Osteocalcin (or bone gamma carboxy glutamic acid containing protein) is a protein synthesized by osteoblasts and incorporated in the bone matrix. During mineralization, osteocalcin is released into the circulation where it can be measured .

Serum osteocalcin is a sensitive marker of bone formation and it parallels the growth velocity curve during childhood and adolescence.

The carboxylation form of osteocalcin is thought to be the active form. Osteocalcin carboxylation in human is a vitamin K dependent process, while the synthesis of the protein is under the effect of 1,25 $(OH)_2$ D_3.

This study was carried out on 73 infants. Fifty one healthy infants grouped into two groups the first group was examined in spring and summer while the second group was examined in autumn and winter. Twenty two rackitic infants were grouped into two groups, one group was examined in summer and spring, and the other group was examined in autumn and winter.

All infants were subjected to anthropometric messuring and evaluation of total serum calcium, inorganic phosphorus, alkaline phosphatase and serum osteocalcin levels.

Our results showed no effect of seasons on total serum calcium and serum alkaline phosphatase of healthy infants as there was no significant difference in their levels between infants who were examined in summer or spring and infants who were examined in winter or autumn. While there were obvious effects of seasons on serum inorganic phosphorus and serum osteocalcin levels as there was high significant increase in serum inorganic phosphorus in spring and summer than in healthy infants examined in autumn or winter. And there was high significant decrease in serum osteocalcin level in healthy infants examined in spring and summer than in healthy infants examined in autumn and winter. This seasonal variation was explained by many factors . The first is sunshine exposure which decrease in winter so 1,25 $(OH)_2D_3$ elevated as an adaptive mechanism for low vitamin D status. 1,25

$(OH)_2 D_3$ stimulates basal levels of osteocalcin. The second factor is bone remodeling which increase in autumn and winter together with decrease bone mineral density and increase osteocalcin level. The third factor could be the change in calcitropic hormones which act directly influencing the production rate or metabolic clearance rate of osteocalcin.

As regards anthropometric data this study showed high significant decrease in height percentile of healthy infants who were examined in spring and summer than those who were examined in autumn or winter. But there was no significant difference in weight or head circumference percentiles between infants examined in different seasons. Seasonal effect on height percentile is due to seasonal variation of bone remodeling which affects longitudinal bone growth and is reflected by serum level of osteocalcin.

The effect of season on rackitic infants was obvious on serum osteocalcin level which was significantly lower in summer or spring than in winter or autumn .While there was no seasonal effect on serum calcium, inorganic phosphorus or alkaline phosphatase.

In conclusion there is no effect of seasons on serum total calcium and serum alkaline phosphatase in healthy children while serum inorganic phosphorus decreases in winter and autumn .

Serum osteocalcin increases in winter and autumn reflecting appositional bone growth which increases in winter and autumn. So we must focus on summer or spring born infants (will be at age of six months in the next winter or autumn) as regards vitamin D Supplementation to allow sufficient and healthy longitudinal bone growth.

RECOMMENDATIONS

Recommendations

1. Monitoring of serum osteocalcin level must be used to estimate bone growth in children ;specially those who are suffering from any disease that may affect bone growth ; as it is a sensitive and specific marker of bone formation .

2. In winter there is more need of vitamin D and calcium supplementation for bone mineralization, as it is known that serum osteocalcin level is increased as an adaptive mechanism for low vitamin D status due to seasonal variation of sunshine exposure.

3. Spreading common medical knowledge about the importance of vitamin D supplementation, the ideal regime for nutrition of infants and the importance of sunshine exposure to warn people from rickets and its complication. These knowledge must be spread through different mass media especially the television.

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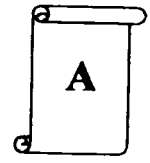

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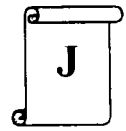

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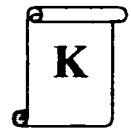

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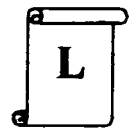

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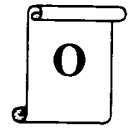

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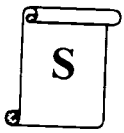

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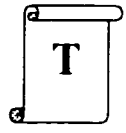

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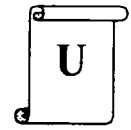

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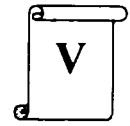

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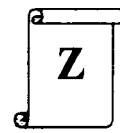

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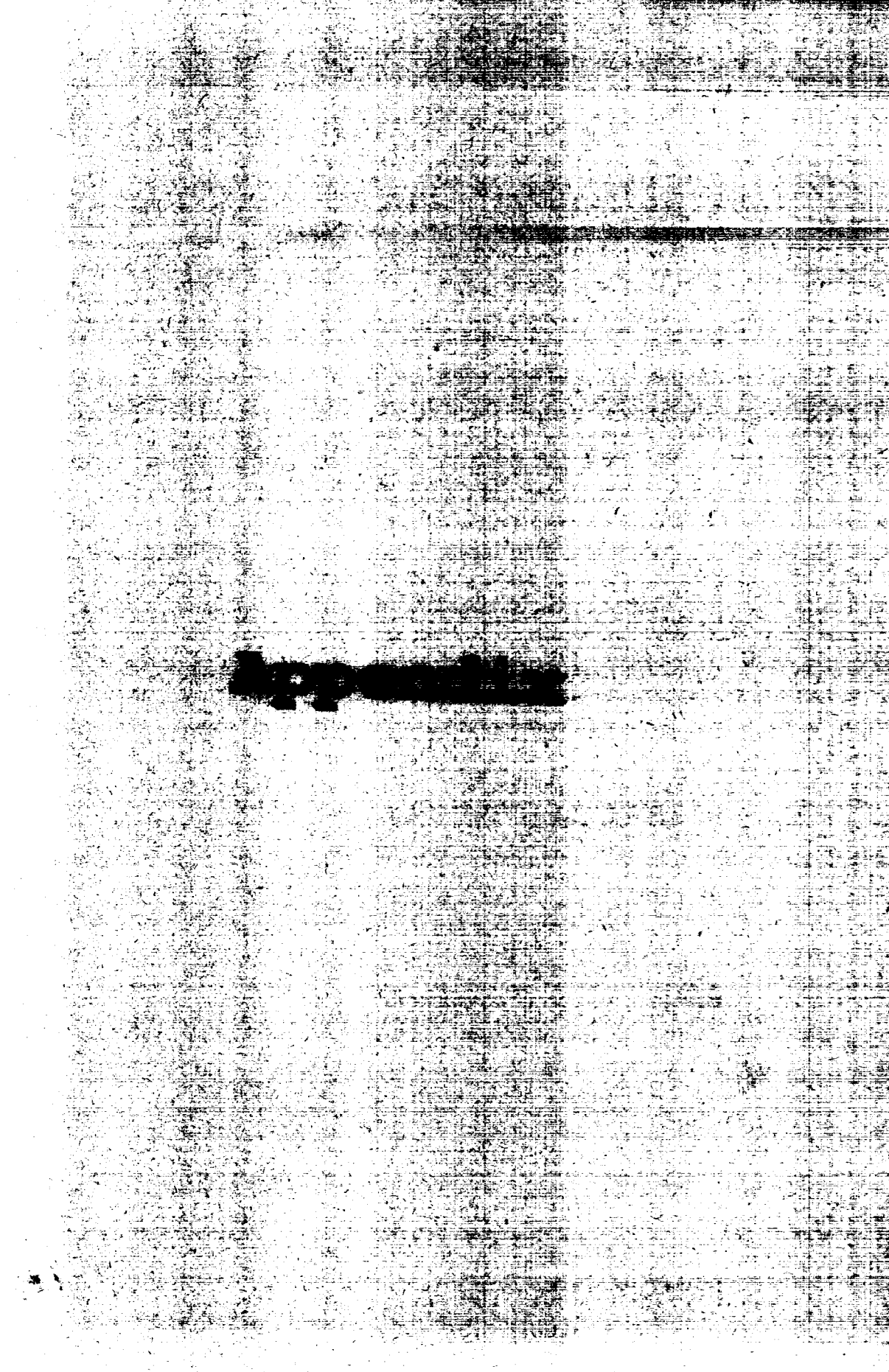


Results of group I (healthy infants in winter and autumn) :

Patient No.	Sex	Age in months	Weight Percentile	Height Percentile	Head circum .Percentile	T. Serum Calcium (mg/dl)	S. Inorg. Phosphorus (mg/dl)	S. Alkaline Phosphatase (U/L)	Serum Osteocalcin(ng/ml)
1	Male	14	10 th	95 th	75 th	8.6	4.4	61	10.5
2	Male	18	10 th	75 th	5 th	8.4	4.4	54	25
3	Male	18	10 th	90th	75 th	8.9	4.6	62	24
4	Female	19	5 th	< 5 th	< 5 th	8.8	4.8	28.5	9
5	Female	18	5o th	25 th	5 th	8.5	5.8	50	10.5
6	Male	18	25 th	50 th	75 th	8.6	5.8	32	40
7	Male	23	25 th	5 0th	50 th	10.6	4.3	50	59
8	Female	19	25 th	10 th	5 th	10.5	4.5	75	21
9	Male	24	< 5th	25 th	25 th	11	4.5	60	33
10	Male	10	10 th	90th	75 th	8.8	5.1	114	21
11	Male	18	< 5th	25 th	50 th	10	4.4	62	23
12	Male	23	25 th	75 th	50 th	10.5	4.1	46	42
13	Female	24	50 th	50 th	50 th	9	4.2	35	16
14	Male	18	75 th	75 th	75 th	10.6	4.5	37	40
15	Male	6	10 th	50 th	25 th	10	4.5	60	13
16	Male	10	5 th	25 th	25 th	10.5	4.3	65	11
17	Female	18	75 th	75 th	75 th	8	4.1	39	21
18	Female	12	10 th	10 th	25 th	8.4	4.5	46	18
19	Male	10	5 th	25 th	25 th	8.8	4.5	150	36
20	Male	12	10 th	25 th	25 th	9	4.7	89	22
21	Male	22	50 th	90 th	75 th	9.3	6.4	260	41
Mean		16.4	24.76	50.71	42.619	9.37±0.93	4.68± 0.60	70.26±51.91	25.52± 13.30

Results of Group II (healthy infants in Summer and Spring)

Patient No.	Sex	Age in months	Weight Percentile	Height Percentile	Head circum .Percentile	T. Serum Calcium (mg/dl)	S. Inorg. Phosphorus (mg/dl)	S. Alkaline Phosphatase (U/L)	Serum Osteocalcin (ng/ml)
1	Female	16	50 th	25 th	< 5 th	8	4.9	71	8
2	Male	14	25 th	50 th	90 th	10	5.3	87	10.5
3	Male	24	< 5th	<5 th	50 th	8.7	5.5	125	5
4	Female	18	25 th	10 th	5 th	11	5.2	98	38
5	Female	24	5 th	< 5 th	25 th	10.8	4.9	85	9
6	Female	18	< 5th	50 th	<5 th	10	4.8	66	13
7	Male	20	5th	<5 th	25 th	11	5.3	71	18
8	Female	15	25 th	50 th	50 th	11	5.2	83	23
9	Male	12	10 th	50 th	>95 th	10	5.3	85	12
10	Male	21	5 th	<5 th	<5 th	10.7	5.5	68	7
11	Female	19	5 th	25 th	< 5 th	10.2	4.9	65	12
12	Female	22	5 th	<5 th	25 th	10.9	5.4	97	52
13	Male	21	5 th	10 th	90th	8.6	4.80	120	27
14	Female	18	25 th	<5 th	50 th	8.7	5.5	64	8.5
15	Female	17	25 th	75 th	25 th	10.5	5	78	25
16	Male	24	5 th	< 5th	50 th	9	4.7	85	11
17	Male	18	25 th	5 th	75 th	8.6	5.5	76	22
18	Female	11	10 th	90 th	50 th	10.7	4.5	50	15
19	Male	15	75 th	<5 th	75 th	9	5	89	6
20	Male	23	10 th	<5 th	95 th	8.8	5.5	71	23
21	Female	22	10 th	10 th	75 th	9.5	5.1	80	30
22	Male	15	95 th	<5 th	25 th	8.8	5.5	125	7
23	Male	22	<5 th	<5 th	50 th	8.9	5.5	100	16
24	Male	7	25 th	<5 th	50 th	8.8	5.4	98	0

Results of Group II (healthy infants in Summer and Spring)(cont.):

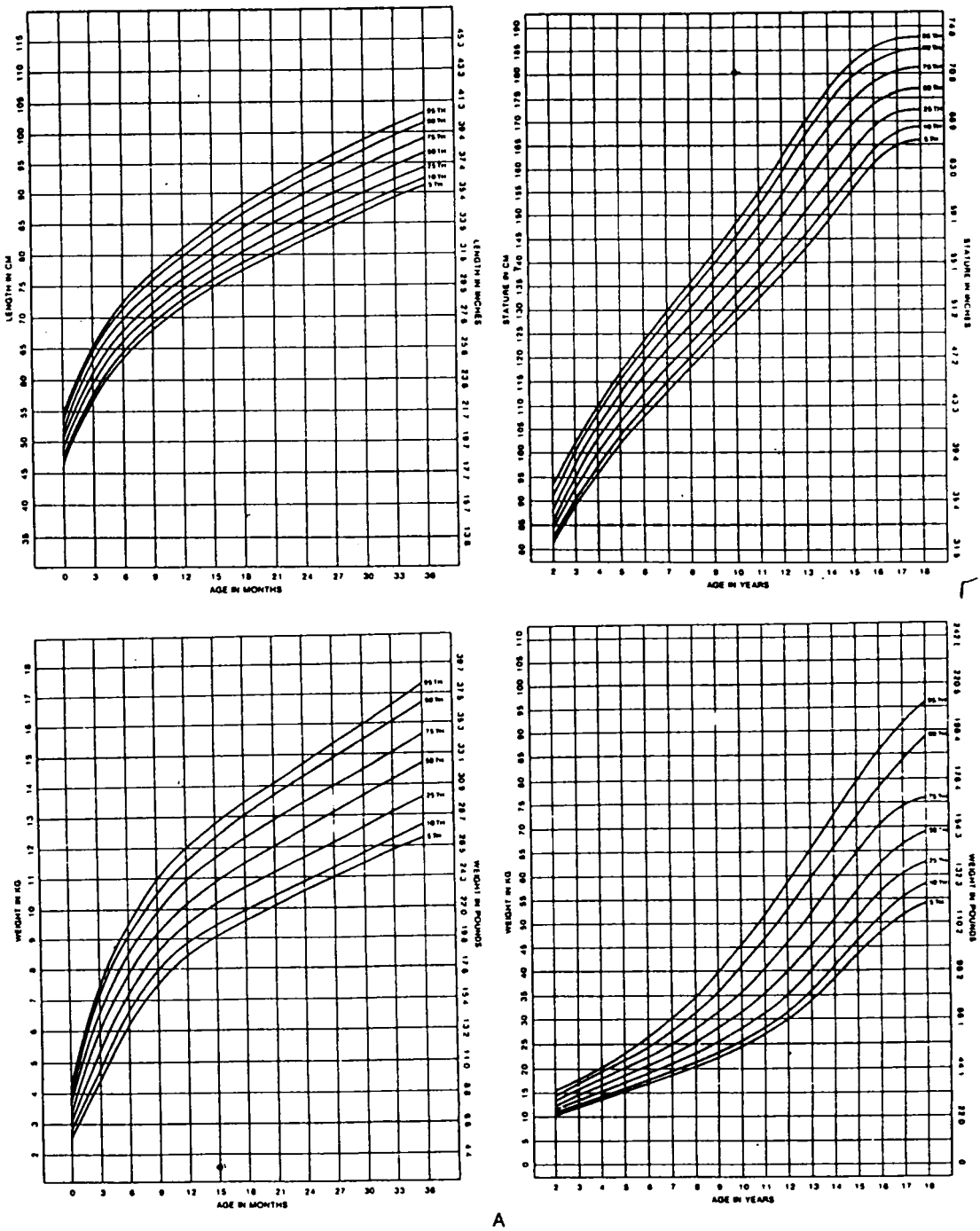
Patient No.	Six	Age in months	Weight Percentile	Height Percentile	Head circum .Percentile	T. Serum Calcium (mg/dl)	S. Inorg. Phosphorus (mg/dl)	S. Alkaline Phosphatase (U/L)	Serum Osteocalcin (ng/ml)
25	Male	23	<5 th	<5 th	75 th	9.4	4.8	267	13
26	Male	7	10 th	<5 th	75 th	10.9	5.3	97	7
27	Male	8	25 th	>95 th	75 th	8.9	5.5	100	20
28	Male	22	<5th	<5th	5^{th}	11	5.5	120	21
29	Female	17	25 th	<5th	<5th	10	5.2	98	9.5
30	Female	6	50 th	75 th	>95th	10.5	5.4	60	7.3
Mean		17.3	20.5	21.16	47.5	9.76±0.96	5.52±0.36	92.63±38.27	15.86±10.93

Results of Group III (Rachitic infants in Winter and Autumn)

Patient No.	Sex	Age in months	Weight Percentile	Height Percentile	Head circum Percentile	T. Serum Calcium (mg/dl)	S. Inorg. Phosphorus (mg/dl)	S. Alkaline Phosphatase (U/L)	Serum Osteocalcin(ng/ml)
1	Male	15	95 th	75 th	>95 th	8.7	3.6	75	22
2	Male	8	25 th	25 th	>95th	9.0	4.5	98	21
3	Male	9	10 th	5 th	>95 th	9.5	5.0	100	26
4	Female	10	10 th	<5 th	50 th	8.4	5.0	125	59
5	Female	9	25 th	<5 th	75 th	8.5	5.2	128	68
6	Male	10	5 th	50 th	> 95 th	8.0	3.5	220	27
7	Male	11	5 th	50 th	>95 th	9.0	4.1	190	38
8	Male	10	25th	10th	9o th	7.6	3.8	250	23
9	Male	19	5th	75th	25th	9.0	4.0	381	53
10	Male	8	<5th	<5th	50th	9.0	2.7	105	29.5
11	Female	6	5th	10 th	90 th	9.4	2.7	98	25
12	Male	6	75 th	25th	>95th	9.9	5.0	87	34
13	Male	11	50 th	10 th	95 th	8.1	4.0	310	21
Mean		10.15	26.15	26.92	80.38	8.78±0.669	4.08+-0.83	166.69±96.81	35.36±15.22

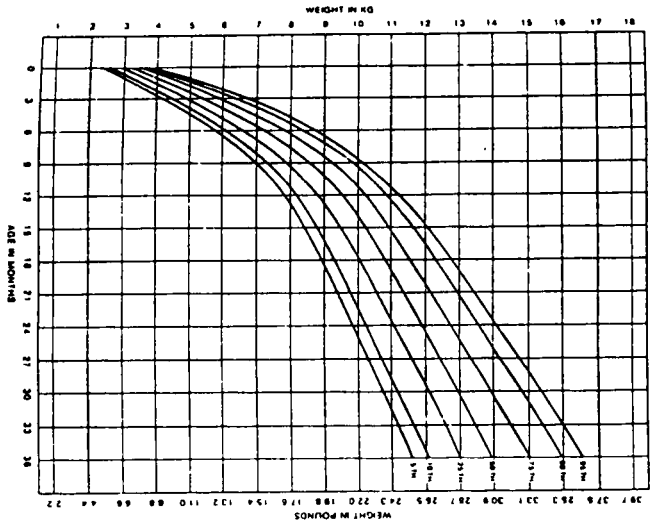
Results of Group IV (Rachitic infants in Summer and Spring)

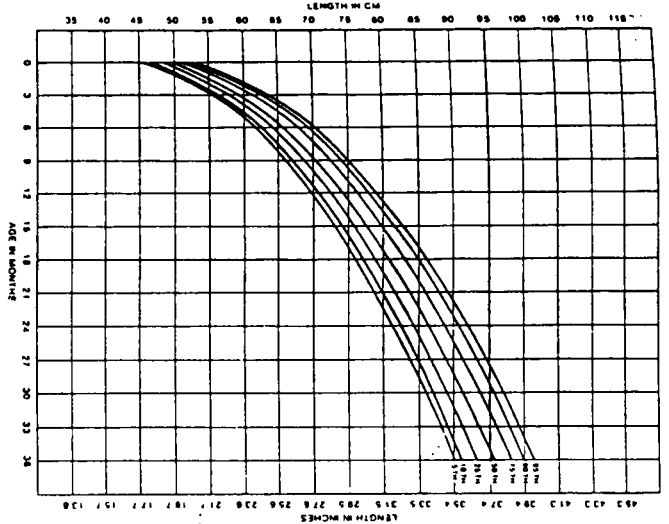
Patient No.	Sex	Age in months	Weight Percentile	Height Percentile	Head circum .Percentile	T. Serum Calcium (mg/dl)	S. Inorg. Phosphorus (mg/dl)	S. Alkaline Phosphatase (U/L)	Serum Osteocalcin(ng/ml)
1	Male	17	<5 th	5th	>95 th	8.9	3.8	71	8.5
2	Male	11	<5th	<5th	75th	8.5	4.5	80	45
3	Male	10	<5th	<5th	>95th	8.7	4.8	82	8.6
4	Male	11	5th	<5th	95 th	7.7	3.9	253	26
5	Male	11	<5th	75th	75th	7.8	5.5	321	25
6	Male	10	10th	<5th	>95th	8.1	5.7	315	26.5
7	Male	17	50th	90th	>95 th	8.1	4	283	5.4
8	Male	16	50th	75th	>95 th	8.6	4	211	11
9	Male	19	10th	25th	95th	8	2.9	59	12
Mean		13.55	16.11	32.2	90.55	8.26±0.42	4.34±0.88	186.11±112.24	18.66±12.90

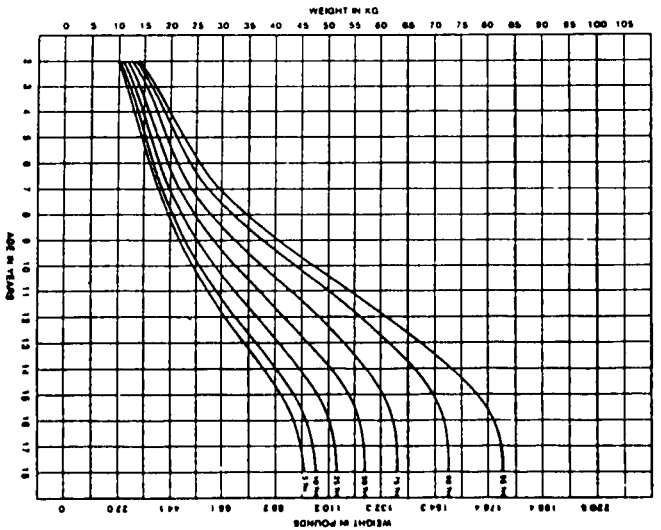


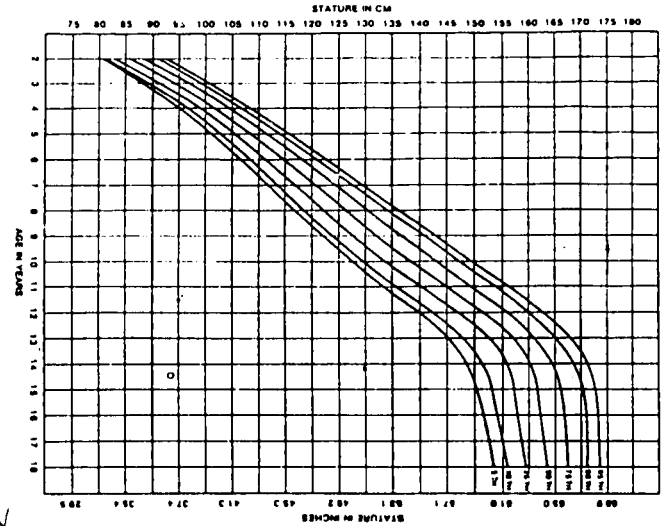
A

A (*above*) and *B* (*opposite*). Charts for BOYS (*A*) and GIRLS (*B*) of length (or stature) by age (*upper curves*) and weight by age (*lower curves*), each curve corresponding to the indicated percentile level. These charts are based on the data in Tables 3–3 and 3–4. (*A* and *B*, From Hamill PVV, Drizd TA, Johnson CL, et al: Physical growth: National Center for Health Statistics percentiles. Am J Clin Nutr 32:609–610, 1979.)


WEIGHT IN KG
AGE IN MONTHS
WEIGHT IN POUNDS


LENGTH IN CM
AGE IN MONTHS
LENGTH IN INCHES


WEIGHT IN KG
AGE IN YEARS
WEIGHT IN POUNDS


STATURE IN CM
AGE IN YEARS
STATURE IN INCHES

ARABIC SUMMARY

الملخص العربى

إن الدلالات الاكلينيكية التقليدية لبناء العظام تشـــمل قيـاس مسـتوى الفوسفات القلوي بالدم وقياس الهيدروكسي بروليـن في البول. لكن الفوســفات القلوي في الدم له مصادر عديدة غير العظام. وكذلك الهيدروكسي بروليـن فـي البول يعكس تجديد البروتينات العظمية وأيضا البروتينات في أنســجة أخــرى غير عظمية.

الاستيوكالسين هو بروتين يصنع بواسطة الخلايا العظمية ويدمج في العظـــام وجزء منه يخرج الى الدم حيث يمكن قياسه.

ان الاستيوكالسين في الدم دالة حساسة لبناء العظام ومستواها في الدم يتـوازى مع سرعة النمو خلال مرحلتي الطفولة والمراهقة.

تهدف هذه الدراسة الى توضيح تأثير التغيرات الموسمية على نمـــو العظـــام ومستوى الاستيوكالسين في الاطفال الاصحاء وكذلك الذين يعانون من مرضى لين العظام.

وأجريت هذه الدراسة على واحد وخمسين طفلا من الاصحاء تم تقسيمهم الـــى مجموعتين:

الاولى تم فحصها في فصلي الشتاء والخريف والثانية تم فحصها في الربيـــع والصيف وكذلك اثنين وعشرون طفلا يعانون من لين العظام تم تقسيمهم أيضا الى مجموعتين الاولى تم فحصها في الخريف والشتاء والثانيـــة فـــي الربيـــع والصيف. وجميع الاطفال خضعوا لقياسات النمو وتحديد مســتوى الكالســيوم والفسفور والفوسفات القلوي والاستيوكالسين بالدم.

وقد أوضحت النتائج ان تغيير الفصول لم يؤثــر علـــى مســتوى الكالســيوم والفوسفات القلوي في الدم على الاطفال الاصحاء بينما لوحظ أن هناك تـــأثيرا

واضحاً لتغيير الفصول على مستوى الفسفور والاستيوكالسين في الدم. حيث اتضح إزدياد مستوى الفسفور في الدم في فصلي الربيع والصيف ازدياداً واضحاً بالمقارنه بمستواه في الخريف والشتاء. وهناك إنخفاض في مستوى الاستيوكالسين في الدم للاطفال الاصحاء في فصلي الربيع والصيف انخفاضاً واضحاً بالمقارنة بمستواه في الخريف والشتاء.

هذه التغيرات الموسمية ترجع لعدة عوامل :-

العامل الاول: هو التعرض لاشعة الشمس الذي يقل في الشتاء وبالتالي ارتفاع مستوى ٢٥،١ هيدروكسي فيتامين د(كوسيلة تعويضية عن انخفاض مستوى فيتامين"د") مما يؤدي الى زيادة افراز الاستيوكالسين.

العامل الثاني: فهو تجديد العظام الذي يزداد في الخريف والشتاء بينما تقل كثافة المعادن في العظام فيزداد افراز الاستيوكالسين.

العامل الثالث: يمكن أن يكون تأثير الهرمونات التي تتحكم في سرعة انتاج او سرعة تكسير الاستيوكاسين.

وبالنسبة لقياسات النمو فان هذه الدراسة اوضحت انخفاضاً ملحوظاً في طول الاطفال الاصحاء في فصلي الربيع والصيف مقارنة بفصلي الخريف والشتاء، بينما لم يؤثر تغيير الفصول على وزن هؤلاء الاطفال. وهذا التأثير الموسمي على الطول يرجع لتأثر عملية تجديد العظام بتغيير الفصول.

أما الاطفال المصابين بلين العظام فقد لاحظنا التأثير الموسمي على مستوى الكالسيوم والاستيوكالسين في الدم فقد انخفض انخفاضاً واضحاً في الصيف والربيع مقارنةً بالشتاء والخريف.

وقد أوصت هذه الدراسة باستخدام قياس مستوى الاستيوكالسين في الدم كمؤشر لنمو العظام في الاطفال حيث انه دالة حساسة ومؤشر خاص يدل على بناء العظام.

مستخلص

فى هذه الدراسة تمت مقارنة مستوى (الكالسيوم – الفوسفور – الفوسفات القلوي – الأستيوكالسين) فى الدم وكذالك قياسات النمو في الأطفال في الفصول المختلفة .

أوضحت النتائج أن تغير الفصول لم يؤثر علي مستوي الكالسيوم والفوسفات القلوي بينما إنخفض مستوى الفسفور في الدم في فصلي الخريف والشتاء عنه في الصيف والربيع وإرتفع مستوى الاستيوكالسين في الدم في فصلي الشتاء والخريف وكذلك إزداد نمو العظام الطولي في هذين الفصلين مقارنة بفصلي الصيف والربيع .

وقد أوضحت هذه الدراسة بإستخدام قياس مستوى الاستيوكالسين كمؤشر لنمو العظام في الأطفال و أوصحت كذلك بالتركز علي إعطاء الأطفال مواليد الصيف والربيع فيتامين (د) كي تنمو عظامهم النمو السليم .

الكلمات الكاشفة

- التغيرات الموسمية
- أستيوكالسين
- نمو العظام

جامعة عين شمس
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

شـــكـــــر

أشكر السادة الأساتذة الذين قاموا بالإشراف
وهم :

١ - أ.د / منى حسين السماحي
٢ - الوظيفة : أستاذ طب الأطفال – جامعة عين شمس

١ - أ.د / فريدة الباز محمد
٢ - الوظيفة : أستاذ طب الأطفال – جامعة عين شمس

١ - أ.د / نادية عبد الستار
٢ - الوظيفة : أستاذ التحاليل الطبية – جلمعة عين شمس

١ - أ.د / إيهاب محمد عيد
٢ - الوظيفة : أستاذ.م الأطفال - معهد الدراسات العليا للطفولة

جامعة عين شمس
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

رسالة دكتوراه

أســم الطـــالـــب : منال فهمي جوهر

عنوان الـرســالة : **دراسة تأثير التغيرات الموسمية علي نمو العظام أو مستوى الأستيوكالسين في الأطفال المصريين**

أسم الدرجة : دكتوراه

لجنة الأشراف

١ - أ.د / منى حسين السماحي	٢ - الوظيفة : أستاذ طب الأطفال - جامعة عين شمس
١ - أ.د / فريدة الباز محمد	٢ - الوظيفة : أستاذ طب الأطفال - جامعة عين شمس
١ - أ.د / نادية عبد الستار	٢ - الوظيفة : أستاذ التحاليل الطبية - جلمعة عين شمس
١ - أ.د / إيهاب محمد عيد	٢ - الوظيفة : أستاذ.م الأطفال - معهد الدراسات العليا للطفولة

تاريخ البحث : ١١ / ٢ /

الدراسات العليا

ختم الإجازة

أجيزت الرسالة بتاريخ ٧ / ٦ /٢٠٠٣

موافقة مجلس الجامعة

/ / ٢٠٠

موافقة مجلس الكلية

٢٠٠٢/٩٨٢

جامعة عين شمس
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

صفحة العنوان

أســـم الطـالب : منال فهمي جوهر
الدرجة العلميـة : دكتوراه
القسـم التابع له : قسم الدراسات الطبية
أســـم المعهـد : معهد الدراسات العليا للطفولة
الجـامعـــــة : عين شمس
سنـــة التخـرج : ٢٠٠٣/م
سنـــه المـنـح : ٢٠٠٣/م

دراسة تأثير التغيرات الموسمية على نمو العظام و مستوى الأستيوكالسين في الأطفال المصريين

توطئة

للحصول على درجة الدكتوراه

في دراسات الطفولة-القسم الطبي

مقدمة من

الطبيبة / منـــــــــــال فهمي جوهر

تحت اشراف

الأستاذة الدكتورة / منى حسين الســـــماحى

أستاذ الأطفال - جامعة عين شمس

الأستاذة الدكتورة / فريـــــدة الباز محمـــد

أستاذ الأطفال - جامعة عين شمس

الأستاذة الدكتورة / ناديـــة عبـد الســـــتار

أستاذ التحاليل الطبية - جامعة عين شمس

الأستاذ الدكتور / ايهـــاب محمـــد عيـــد

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