A STUDY ON SERUM PROLACTIN IN REDUCED BONE MINERAL DENSITY

Dissertation Submitted for

M.D. BIOCHEMISTRY BRANCH - XIII
DEGREE EXAMINATION

THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600 003.

MARCH 2007

CERTIFICATE

This is to certify that this dissertation in **" A STUDY ON SERUM PROLACTIN IN REDUCED BONE MINERAL DENSITY "** is a work done by **Dr.S.SUMATHY,** under my guidance during the period 2004 - 2007. This has been submitted in partial fulfillment of the award of M.D. Degree in Biochemistry, (Branch - XIII) by the Tamil Nadu Dr.M.G.R. Medical University, Chennai - 600 032.

Prof.Dr.Kalavathy Ponniraivan,B.Sc.,M.D.,
Dean
Madras Medical College,
Govt. General Hospital,
Chennai - 600 003.

Dr.A.Manamalli,M.D.,
Director and Professor
Institute of Biochemistry
Madras Medical College & Hospital
Chennai - 600 003.

Date :

Station :

Date :

Station :

ACKNOWLEDGEMENT

The author wishes to express her profound sense of gratitude to **Dr.A.Manamalli, M.D.,** Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her thoughfull suggestions, constructive criticism and an eye for perfection throughout the study.

The author owes her sincere thanks to **Dr.T.S.Andal, M.D.,DCH.,** former Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for extending her invaluable guidance.

The author owes her sincere thanks to **Dr.Pregna Dohlia. M.D.,** Additional Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her constant guidance and constructive ideas during the study.

The author is highly grateful to **Prof.V.S.Natarajan, M.D., F.R.C.P.,** (Retd) Geriatric Physician, Chennai, and **Dr.Shanthi.G.S.,M.D.,** Assistant Professor of Geriatric Medicine, Government General Hospital, Chennai for their immense help during the study.

The author whole heartedly thanks Additional Professor **Dr.Chandrasekar**, Assistant Professors, **Dr.Shyamraj.M, Dr.Ramadesikan.V.K, Dr.Periandavan. I,** Institute of Biochemistry, Madras Medical College, Chennai, for their constant guidance, encouragement and support in bringing out this study.

The author wishes to thank the technical staff, friends and colleagues of Institute of Biochemistry, Madras Medical College, Chennai for their help.

The author thanks **Mr.Venkatesan** Department of Clinical Epidemiology, Stanley Medical College for the statistical work and evaluation rendered by him.

Sincere thanks are due to the subjects of this study for extending their active support and for co-operating with the requirement of this work.

Last but not the least the author wishes to express her sincere thanks to her husband **Mr.P.Purandhar** and daughter **Miss.P.Harshitha** for extending their co-operation throughout the study and bringing out this booklet.

SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks **Prof. Dr.Kalavathy Ponniraivan,B.Sc.,M.D.,** Dean, Madras Medical College, Government General Hospital, Chennai - 600 003, for granting me permission to utilize the facilities of this institution for the study.

CONTENTS

INTRODUCTION

Reduced bone mineral density is a universal problem of modern society; it may not be the commonest of all diseases, but it is sufficiently common to be important. Osteopenia and osteoporosis are the two different grades of the condition with the density being much more reduced in osteoporosis which is a systemic skeletal disease characterized by low bone mass and micro architectural increase in bone fragility that renders bone more susceptible to fracture.

Osteoporosis is now considered as a major health care problem in India with an estimated 50% of healthy women and 30% of men over 50 years having low bone mass. It is preventable and treatable, but there are no warning signs until fracture occurs. It is a silent risk factor for fracture just as hypertension is for `stroke'.

Hyperprolactinemia is found to be commonly associated with reduced bone mineral density. The osteoporotic effect of excessive prolactin are said to be due to hypoestrogenism, calcium mobilization from bone and direct effect of prolactin on bone.

By the year 2050, osteoporosis is likely to be a major demographic factor due to changes in the life style and the increase in the survival rate of the elderly. The incidence of fracture of the hip is likely to rise across the world to 6.26 millions and 71% of these fractures are likely to occur in the developing world. Therefore the determination of bone mass has to be done constantly. At

present bone mass determination is done only with the help of DEXA Scan. (Dual Energy X-ray Absorptiometry).

A developing nation like India cannot afford to diagnose fracture risk in the general population with the help of DEXA scan due to the high cost. Therefore a test which will be less expensive and reliable should be opted for in our country.

As hyperprolactinemia has been found to be associated with osteoporosis, determination of serum prolactin can be an useful assessment in the determination of reduced bone mineral density. Hence the interest developed to determine this hormone in the elderly who are `high risk' individuals prone to develop osteopenia and osteoporosis and the ensuring complication `fracture'.

The present study is to find out the association of hyperprolactinemia with reduced bone mineral density and plan preventive and treatment protocols to reduce the burden of osteoporosis on society.

REVIEW OF LITERATURE

Bone is a dense, semi-rigid, porous, calcified connective tissue forming the major portion of the skeleton of most vertebrates[1].

The main functions of bone are

1. Mechanical, for locomotion
2. Protective, for organs
3. Metabolic, as a reserve for minerals especially calcium and phosphate[2].
4. Blood cell formation

Bone is composed primarily of an extracellular mineralized matrix with a smaller cellular fraction. Of the total number of 206 bones in adult hood, 60 - 70% dry weight of bone is made up of inorganic salt, 30 - 40% dry weight of bone is made up of organic substance and 10 - 20% of living bone is made up of water[3,18,19]. The organic matrix is primarily type I collagen with lesser amount of non collagenous proteins. The composition of bone is given in Table 1.

The three major components of bone are osteogenic cells, organic matrix, and minerals. The osteogenic cells include osteoblasts, osteocyts and osteoclasts, while the matrix consists predominantly of collagen and proteoglycans and constitutes approximately one third of the bone mass. The mineral that makes up approximately two thirds of bone is composed of calcium phosphate crystals deposited as hydroxy apatite $[Ca_{10} (Po_4)_6 (OH)_2]$[2]. The other minerals include sodium, magnesium, carbonate, fluoride, Zinc etc.

Approximately 99% of the body's calcium is contained in bone. Hydroxyapatite confers on bone the strength and resilience required by its physiologic roles[32]. Bone contains 85% of phosphates, 55% of magnesium, 50% of sodium and 30% of Zinc along with other trace elements and 20% of water. The concentration of calcium, phosphate and magnesium in plasma are dependent on the net effect of bone mineral deposition and resorption, intestinal absorption and renal excretion. Parathyroid hormone (PTH) and Calcitriol are the principle factors regulating these processes[2].

Structurally two different types of bone matrix are observed. Hard compact cortical bone, found largely in the shafts of long bones that surround the marrow cavities. It is 80 to 90% mineralized by volume and constitutes 80% of the skeleton. It's function is primarily mechanical and protective. Spongy, cancellous or trabecular bone comprises of a network of fine, interlacing partitions. The trabeculae, enclosing cavities contain either hematopoietic or fatty marrow. It is found in vertebrae, flat bones and in ends of the long bones trabecular bone constitutes the remaining 20% of the skeleton. Trabecular bone, which is 15% to 25% mineralized, is more metabolically active.

The organic matter of bone in mainly protein which are enumerated in Table-2. Among the non collagenous protein chondroitin sulfate, proteoglycans III and bone sialoprotein are bone specific.

The major cell types involved in bone resorption and deposition are osteoclasts and osteoblasts. The former are associated with resorption and the latter with deposition of bone. Osteocytes are descended from osteoblasts[4].

Osteoblasts

Osteoblasts are mononucleated cells derived from pluripotent mesenchymal precursors[10,11]. They synthesize most of the proteins found in bone as well as various growth factors and cytokins. They are responsible for the deposition of new bone matrix (Osteoid) and its subsequent mineralization. Osteoblasts control mineralization by regulating the passage of calcium and phosphate ions across their surface membrane. They contain alkaline phosphatase, which is used to generate phosphate ions from organic phosphates[1].

Osteoclasts[12-17]

Osteoclasts are found in sites in which bone is being remodelled. They are large multinucleated cells derived from pluripotent hematopoietic stem cells, typically found on or near bone surfaces within concavities. Acid phosphatase and collagenase are produced by them. They play a key role in bone resorption. Minerals are first removed followed by removal of organic matrix. The degradation products of the matrix enter the cytoplasm of the osteocyte through a process of endocytosis and are then transported across the cell and extruded into the extra cellular space.

Osteocytes

Approximately 10% of the osteoblasts become enclosed in developing matrix and are called osteocytes. The large surface area provided by the osteocytes is responsible for the regulatory mechanism for the exchange of mineral ions between the extra cellular fluid and bone.

Bone is a dynamic structure that undergoes continuing cycles of remodelling, consisting of resorption followed by deposition of new bone tissue. The bone turnover rate is approximately 10% - 30% per year[5].

PATHOPHYSIOLOGY OF OSTEOPOROSIS

BONE REMODELLING[6]

Bone remodelling is a highly integrated process of resorption and successive formation of bone tissue that results in maintenance of skeletal mass with renewal of the mineralised matrix. This is accomplished by focal cell mediated degradation and regeneration of bone tissue with out compromising the overall architecture of the anatomy of bones. In normal remodelling the amount of bone resorbed and laid down is neutral or in positive balance[7].

First precursor cells differentiate into osteoclasts, which erode a cavity on the bone surface. The osteoclasts then disappear and there is a quiescent interval during which the irregular cavity is smoothened off and lined by a layer of cement like substances, which is of similar composition to bone but is mineral rich and collagen poor. A set of osteoblasts is next recruited which refill the excavated cavity with new bone.

Alterations in the dynamics of different parts of the remodelling processes account for the wide variety of age related changes, which occur in skeleton. The remodelling cycle is divided into four discrete phases and is illustrated in Fig. 1.

Quiescence
Activation
Resorption
Reversal and formation

Quiescence

During the quiescent phase, the bone surface is covered by a layer of thin flattened living cells, which arise by terminal transformation of osteoblasts. These cells retain their hormonal receptors, but have lost the ability to synthesize collagen. Between the living cells and the bone is a thin layer of unmineralized connective tissue, which closely resembles osteoid.

Activation

This is the conversion of a small area of bone surface from quiescence to bone resorbing activity.

The precise mechanism by which activation occurs is still unclear. However it is thought that the living cells of a quiescent area are stimulated to digest the unmineralised connective tissue overlying the bone. They then retract to expose the mineralized bone surface, which is chemotactic for osteoclast precursor cells, which undergo fusion into osteoclasts when they reach the surface of the bone.

Resorption

Once assembled on the mineralised bone, the osteoclasts begin to resorb it. The process is by expelling protons across the ruffled border into the resorption area by a proton - trans locating ATP ase. This lowers the local pH

to 4.0 or less, which increases the solubility of hydroxyapatite and allows demineralization to occur. Lysosomal acid proteases are released that digest the now accessible matrix proteins[4]. Resorption is illustrated in Fig.2. This leads to a lacuna in trabecular bone or a cutting cone in cortical bone. When the cavity has reached a depth of between 50μm and 100μm local resorption ceases.

Reversal

This is the period between completion of resorption and the state of formation at a particular location. During this period mononuclear cells and a thin layer of mineral rich cement substance is deposited which smooths the osteoclastic resorption surface.

The osteoblast precursors are also assembled prior to bone formation. The mechanism of coupling bone formation to resorption is unclear, however the phase of bone formation is an inevitable consequence of the preceding bone resorption.

Formation

Bone formation occurs in two stages. Soon after the cement substance has been deposited the newly formed osteoblasts begin to deposit a layer of unmineralised bone matrix. After deposition, the collagen fibrils making up the matrix aggregate and cross - link before they become mineralised. The new matrix begins to mineralise after approximately one week.

Regulation of Bone Metabolism

Many factors are involved in the regulation of bone metabolism which are enumerated in Table - 3.

Two products of osteoblasts namely receptor activator of nuclear factor (RANK) and osteoprotegerin (OPG) have been identified that appear to be the final common pathway in coordinating osteoblasts and osteoclast activity[3]. RANK - ĸB ligand binds to a receptor on osteoclast progenitor cells and increase osteoclast differentiation and activity. OPG serves as a decoy receptor for RANK ligand. When OPG binds to RANK ligand, the osteoclast stimulation activity is prevented. The relative ratios of these two molecules determine bone turnover[2]. Biochemical markers of bone turnover are ennumerated in Table-4[2,20-2,110,121].

BONE MINERAL DENSITY

Bone Mineral Density (BMD) is the amount of mineralised bone tissue in a given area, usually calculated as grams per square centimeter. It is a measure of bone density which is the amount of bone tissue in a certain volume of bone and it reflects the amount of calcium in bones[64].

Peak bone mass in males and females occur by the third decade of life and plateaus for about 10 yrs, during which time, turn over of bone is constant with bone formation equaling bone resorption. This is followed by net bone loss of about 0.3% to 0.5% per year[27]. Decreased bone mass and increased fragility can occur because of

1. Failure to achieve optimal peak bone mass.
2. Bone loss caused by increased bone resorption
3. Inadequate replacement of lost bone as a result of decreased bone formation[23].

Factors which play a role in determining peak bone mass, remodelling and fracture risk are given in Table-5.[24-26].

Bone mass is increased by physical exercise, intake of good calcium during childhood and puberty and also in obesity.

Bone mass is reduced[27], by late menarche, early menopause, nulliparity caffeine ingestion, cigarette smoking, alcohol use, immobilisation and insufficient dietary intake of calcium, phosphate and Vitamin D.

Bone mineral density is calculated from the bone mineral content and the area of the bone scanned and expressed in units/gm/cm^2, which can be plotted in relation to age with respect to reference population. Bone mineral density decrease with age when the fracture risk rises rapidly[150-154]. There is a decrease in mineral to matrix ratio (degree of mineralization in reduced) in osteoporotic tissue[155-162]. When BMD is reduced, depending upon the degree of reduction it is either known as osteopenia or osteoporosis.

Given the increasing aging of population, osteoporosis and fracture are expected to increase. Large bone sizes may be associated with lower risk of fracture[28]. Every standard deviation of decrease in bone mineral density increase fracture risk two or three folds. Beginning with menopause, women have increased bone loss of 3 - 5% per year for about 5-7 years[27]. Over a 10 year period 18% of 65 year old women with osteoporosis will experience a fracture, increasing to 33% among those with bone mineral density more than four standard deviations below the peak mean. The life time hip fracture risk for a 50 year old women is nearly 15%[29].

Reduction in BMD can be measured based on the following criterias[30,31].

1. Normal range plot : Here the subjects BMD and age are plotted with respect to reference population

2. **T**-scores : It measures, the deviation of the subjects BMD value from the mean BMD for young adult population, in units of the population standard deviation (SD).

$$\textbf{T score} = \frac{\text{Measured BMD - Young adult BMD}}{\text{Young adult SD}}$$

3. **Z** - scores : It is the mean BMD and standard deviation for a healthy age matched population which is used as a reference value instead of the mean BMD and SD for a young normal group.

$$\textbf{Z score} = \frac{\text{Measured BMD - Age matched mean BMD}}{\text{Population SD}}$$

Categorisation[32] of the normal and reduced mineral density groups are shown in Table - 6 and also illustrated in Fig.3[33].

OSTEOPOROSIS[36,37]

There are many definitions for osteoporosis and most of them attempt to describe bone mass as it relates to fracture risk.

Definition

In April 1993 the Consensus Development Conference defined Osteoporosis as a "Systemic Skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture". The World Health Organisation defines Osteopenia as a bone density which is less than normal but not 2.5 SD below normal bone density and osteoporosis as a `bone mineral density less than 2.5 SD below mean peak value in young adults'. While this definition is less descriptive it is the one most often used by radiologists when they measure bone density and it gives the physician an idea of fracture risk

Bone Remodelling and Osteoporosis[36-43]

The hall mark of osteoporosis is reduction in skeletal mass caused by an imbalance between bone resorption and bone formation. Loss of gonadal function and aging are the two most important factors that contribute to the development of osteoporosis. Starting around the 5^{th} or 6^{th} decade of life, loss is at the rate of 0.3 - 0.5% / yr. In women after menopause and in men after castration the rate of bone loss increases by 10 fold. Thus there is increased rate of bone resorption compared to bone formation.

The bone loss that accompanies aging is its association with a progressive decline in the supply of osteoblasts in proportion to the demand for them. While age related bone loss affects cortical bone, in postmenopausal women in whom there is excess osteoclast activity it is the trabecular bone that is lost.

Causes of Osteoporosis[44,46]

Several hypothesis regarding the pathogenesis of osteoporosis are available but the basic multicellular unit theory put forward by Frost et al., (1962 - 63) has been gaining wide acceptance. As per their hypothesis osteoporosis can be due to any one of the following six mechanisms.

1. Resorption and formation remain constant (but unequal with aging).
2. Resorption increases but formation remains constant.
3. Resorption remains constant but formation decreases.
4. Resorption and formation both decrease but do so unequally.
5. Resorption and formation both increase but do so unequally.
6. Resorption increases but formation decreases.

PATHOGENETIC FACTORS

Systemic Factors

Two features of osteoporosis suggest a role for local factors in pathogenesis.

1. Systemic hormones that influence the skeleton, including estrogen and parathyroid hormone alter the production of local factors e.g. cytokines, prostaglandins and growth factors.

Gonadal hormone deficiency is the cause for highest incidence of osteoporosis in post menopausal women, young women with low estrogen level and in hypogonadal men.[47,48,97,105,107,112] Low estrogen levels are associated with vertebral fractures in men[49]. Similarly androgen deficiency may be important in women as well as in men[50,51]. Androgens are the source of estrogen, both in the gonads and in the periphery, and may have a direct effect on bone. Rarely osteoporosis can also occur in the absence of any evidence of gonadal hormone deficiency. Osteoporosis is associated with high levels of sex hormone binding globulin (SHBG)[95]. This association of sex hormone with SHBG may reflect decreased availability of sex hormones to the tissues when SHBG levels are high[52].

Increase in PTH levels which increases with age, is found to accelerate bone loss[109,118,124]. The increase of the hormone is probably due to decreased dietary intake of and impaired intestinal absorption of calcium, often associated with vitamin D deficiency[149]. Estrogen deficiency may also play a role in the increase of PTH[53].

Differential bone loss occurs in different parts of the skeleton. Production of IL-1, TNF - α and IL-6 may be increased in estrogen deficient and osteoporotic patients[54-57]. However, there have been negative results in studies of the role of cytokines in osteoporosis in humans.

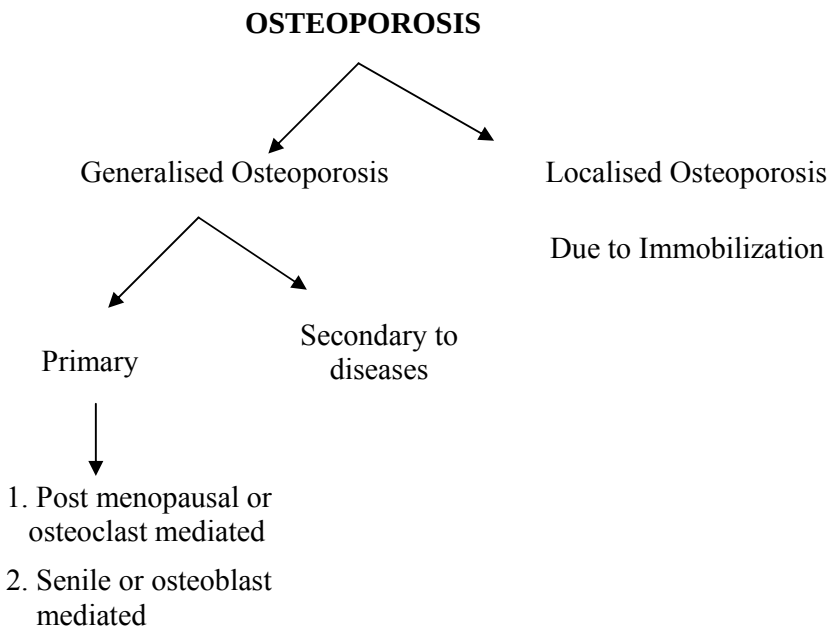
Calcitonin deficiency does not appear to play a role in osteoporosis[58] although pharmacologic doses of calcitonin can prevent bone loss or even increase bone mass in patients with high bone turn over.

Glucocorticoid excess can produce secondary osteoporosis but does not appear to play a major role in primary osteoporosis[113-115].

Growth hormone secretion and circulating IGF-1 decreases with age and their levels are low in osteoporotic patients, particularly males[61]. Thyroid hormone excess has been found to exacerbate osteoporosis[59,60]. Risk factors for osteoporosis is illustrated in figure - 7 and that for osteoporotic fractures are enumerated in Table 7.[62,77-86,119,122].

CLASSIFICATION OF OSTEOPOROSIS[44]

On the basis of the modified classification by Nordin (1964), which takes account of findings by the World Health Organisation (WHO) on causes of osteoporosis, it is classified as



Generalised Osteoporosis

1. Primary

On the basis of the pattern of bone loss and fractures, Riggs and Melton (1988) identified two types of osteoporosis.

Type 1 postmenopausal or osteoclast mediated

Type 2 senile or osteoblast mediated.

Type 1 Primary Osteoporosis is characterised by a rapid bone loss seen in recent postmenopausal women. The turnover of trabecular bone is accelerated. Therefore distal radial and vertebral fractures are common.

Type 2 Primary osteoporosis is characterised by age related bone loss, calcium deficiency and or hyperparathyroidism. Fractures of the proximal femur, especially fracture of the neck of femur and inter trochanteric fracture are more common in this type of osteoporosis. Osteoporosis secondary to diseases are enumerated in Table -8[63,88-93].

Diagnosis[65,66]

As indicated by WHO, osteoporosis can be diagnosed before fracture occurs by measuring bone density. The frequency of diagnosis, therefore, depends on the frequency, site, and timing of bone density measurements.

The history should indicate detailed analysis of calcium intake and nutrition, change in height or weight, physical activity and life style, smoking history, mensural and reproductive history, personal or family history of fragility fracture, or other metabolic or endocrine disorders that may affect the skeleton.

1. Physical examination : should include careful height measurement, assessment of the spine, evaluation for thyroid and adrenal disease.

2. Radiographic examination : Plain radiographs are not sensitive enough to diagnose osteoporosis until total bone density has reduced by 50% but bone densitometry is useful. Single and dual photon densitometry has been used. They are less accurate analysis and radiation exposure is more than x-ray absorptiometry.

3. Assessment of BMD : The technique used to assess BMD are :

1. Conventional skeletal radiography
2. Radiographic photodensitometry
3. Radiogrammetry
4. Single energy absorptiometry
5. Dual energy x-ray absorptiometry
6. Quantitative computed tomography
7. Photon scattering methods
8. Neutron activation analysis
9. Ultrasound evaluation of bone

Most widely used techniques of assessing BMD are dual - energy x-ray absorptiometry - DEXA and quantiative computerised tomography - QCT. Dexa is more precise and the diagnostic measure of choice. The measuring device by Dexa has already been ennumerated.

4. Ultrasound Evaluation of Bone[44]

Several methods have been developed that variously examine the velocity attenuation or reflection of ultrasound in bone.

Ultrasound attenuation

A short burst of ultrasound is passed through the heel (os calsis), the frequency varying from 200 to 1000 KHz. The amplitude spectrum is compared with that from water alone to give a plot of attenuation in the os calcis against frequency, and the slope of the linear portion of this graph is taken to characterise the bone.

Attenuation is related both to the amount of bone in the path of the ultrasound and to the trabecular structure. The technique discriminates patients with or without osteoporosis as well as measurements of BMD.

This is advantagenous when compared to QCT and DEXA examinations which require more space and investment.

The calcaneum has many advantages for the assessment of osteoporosis. First of all it is very easy to access. It is a weight bearing bone like the neck of the femur and the vertebral bodies. It is approximately 90% trabecular bone. The posterior half of the bone has parallel surfaces on the medial and lateral aspects and hence the passage of ultrasound waves is most unhindered and best suitable for BMD measurements as illustrated in Figure 3(a).

Additional biochemical markers in the blood are given in Table -9.[34,35,45,95].

Management of Osteoporosis is given in Table 10[67].

Recommendation for Prevention[71,123]

The National Osteoporosis Foundation has made the following recommendations to physicians.

1. Counsel all women on the risk factors for osteoporosis
2. Perform an evaluation for osteoporosis on all postmenopausal women who present with fracture, using BMD
3. Recommend BMD testing to all women younger than 65 years who have one or more risk factors for osteoporosis in addition to menopause.
4. Recommend BMD testing to all women 65 years and older regardless of additional risk factors.
5. Advice all patients to obtain an adequate dietary intake of calcium (atleast 1200 mg/day)
6. Recommend regular weight bearing exercise and muscle - strengthening exercise to reduce the risk of fall and fracture.
7. Advice patients to avoid smoking and reduce alcohol intake.
8. Consider all postmenopausal women who present with vertebral or hip fracture to be candidates for osteoporosis treatment.

9. Indicate therapy to reduce fracture risk in women with T score below 2 in the absence of risk factors and in women with Tscore below 1.5 if other risk factors are present.

10. Pharmacological options for osteoporosis prevention and treatment are :

    Hormone replacement therapy

    Alendronate

    Raloxifenes

    Calcitonin

GOALS FOR THERAPY[67]

1. Prevention of fracture

2. Optimization of skeletal development and maximization of peak bone mass at skeletal maturity.

3. Prevention of age related and secondary causes of bone loss.

4. Preservation of the structural integrity of the skeleton

5. Improvement in the quality of life

6. Decrease in morbidity and mortality

Osteoporosis therapy can reduce the risk of fracture by as much as 50%[108].

HYPERPROLACTINEMIA AND IT'S ASSOCIATION WITH BONE MINERAL DENSITY

In the last 20 years several biochemical works on bone mineral density have suggested that hyperprolactinemia has been associated with reduced bone mineral density[72-75,97-102,111,116,117].

The osteoporotic effect of excessive prolactin may be due to hypoestrogenism, calcium mobilization from the bone, prolactin receptors in the bone and prolactin dependent increase in parathyroid hormone related peptide level[76].

The anterior pituitary hormones are classified into 3 categories.

1. The Growth hormone, prolactin, chorionic somatomammotropin group.
2. The glycoprotein hormone group
3. The pro-opiomelanocortin peptide family.

Growth hormone, prolactin and chorionic somatomammotropin are a family of protein hormones having considerable sequence homology[4].

Prolactin[125]

Prolactin is a protein hormone of the anterior pituitary gland that was originally named for its ability to promote lactation in response to the suckling stimulus of hungry young mammals. It appears in a multiplicity of post translational forms ranging from size variants to chemical modifications such

as phosphorylation or glycosylation. It is not only synthesized in the pituitary gland but also within in the central nervous system, the immune system, the uterus, and its associated tissues of conception and the mammary gland It's biological role is not only in reproduction but it also controls a variety of behaviors and even plays a role in homeostasis. Prolactin - releasing stimuli are nursing stimulus, light, audition, olfaction and stress. Dopamine of hypothalamic origin provides inhibitory control over the secretion of prolactin.

Prolactin Chemistry and Molecular Biology

Prolactin Gene

Based on its genetic, structural, binding and functional properties prolactin belongs to the prolactin / growth hormone / placental lactogen family. (Group I of the helix bundle protein hormone).

Genes encoding the 3 hormones evolved from a common ancestral gene by gene duplication. In the human genome, a single gene, found on chromosome 6 encodes prolactin. The prolactin gene is 10 Kb in size and is composed of 5 exons and 4 introns. The mature human prolactin is composed of 199 amino acids as illustrated Figure -4.

Structure

The prolactin molecule is arranged in a single chain of amino acids with 3 intramolecular disulfide bonds between 6 cysteine residues in humans.

Cys^{4} - Cys^{11}

Cys^{58} - Cys^{174}

Cys^{191} - Cys^{199}

Secondary and Tertiary Structure of Prolactin

50% of amino acid chain is arranged in α - helices while the rest of it forms loops. The 3 dimensional model of prolactin contains 4 long α - helices arranged in anti parallel fashion. This is illustrated in figure -5.

Prolactin Variants

The major form of prolactin found in the pituitary gland is 23kDa. Variants of prolactin have been found. These are a result of alternative splicing of the primary transcript, proteolytic cleavage and other post translational modification of the amino acid chain.

Monomeric prolactin	-	Little prolactin 23Kd
Dimeric prolactin	-	Big Prolactin 48 to 56 Kd
Polymeric prolactin	-	Big - Big Prolactin > 100 Kd

The monomeric form is the most bioactive prolatin. A glycosylated form of prolactin has also been identified and it is less biologically active than little prolactin.

Site of synthesis and secretion of prolactin

The cells of the anterior pituitary gland which synthesize and secrete prolactin are called lactotrophs or mammotrophs. They constitute 20 - 50% of the anterior pituitary cells depending on the sex and the physiological status of the animal. Most of the prolactin expressing cells appears to arise from growth hormone producing cells. Two cell forms expressing the prolactin gene are large polyhedral cells found through out the gland and smaller angulated or elongated cells clustered mainly in the lateral wing and median wedge. Prolactin and growth hormone can also be secreted from the intermediate cell population called mammosomatotrophs of the pituitary.

Prolactin immunoreactivity was found in telencephalon in the cerebral cortex, hippocampus, amygdala, septum, caudate putamen, brain stem, cerebellum, spinal cord and choroid plexi. Placenta, amnion, decidua, uterus, mammary gland, immune system, cells of thymus, spleen and peripheral lymphocytes contain prolactin.

Prolactin Receptors[96]

The prolactin receptor is a single membrane - bound protein that belongs to class I of the cytokine receptor superfamily. The gene encoding the human prolactin receptor is located on chromosome 5 and contains at least 10 exons. The distribution of prolactin receptors are given in Table 11.

Activation of prolactin - receptor is by means of extracellular domain and Intracellular domain. The intracellular domain, is the key player in the initiation of the signal transduction mechanism associated with the prolactin receptor. Although the intracellar domain of the prolactin receptor is devoid of any intrinsic enzymatic activity, ligand - mediated activation of prolactin

receptor result in tyrosine phosphorylation of cellular protein including the receptor itself. The membrane proximal region of the intracellular domain is constitutively associated with a tyrosine kinase termed Janus kinase 2 (JAK 2). Phosphorylation of JAK 2 occurs within 1 minute after prolactin binding, suggesting a major role for JAK 2.

Biological Action of Prolactin

Reproduction

The best known effect of prolactin is on the mammary gland. It is also important for the maintenance and secretary activity of the corpus luteum. It also affects other actions related to reproduction such as mating and maternal behaviours.

Lactation

The effect of prolactin on the mammary gland (mammogenesis), synthesis of milk (lactogenesis) and maintenance of milk secretion (Galactopoiesis).

In the process of lactogenesis, prolactin stimulates uptake of amino acids, synthesis of milk proteins casein and α - lactalbumin, uptake of glucose and synthesis of milk sugar lactose and milk fats.

Active lactation is due to a reduction in estrogen and progesterone and in prolactin levels after delivery. Suckling increases milk production after parturition and is essential for continued lactation. In the absence of suckling, prolactin concentrations which rise through out gestation return to normal by the 7th postpartum day. Suckling increases prolactin levels by 8.5 fold in

nursing mothers . Lactational amenorrhea is a form of contraception that depends on the frequency and duration of breast feeding.

Homeostasis

Prolactin plays a role in maintaining the internal environment by regulating the immune system, osmotic balance and angiogenesis.

Immune Response

Prolactin is a common mediator of the immunoneuroendocrine network, where nervous, endocrine and immune systems communicate with each other. Prolactin plays a role in humoral and cellular immune response. Effect of prolactin on lymphocytes may involve interleukin - 2.

Osmoregulation

Prolactin regulates solute and water transport across cell membranes. It decreases the transport of sodium and increases the transport of potassium across mammary epithelial cells. It is responsible for fluid, sodium, chloride and calcium transport across intestinal epithelial membranes. Prolactin acts on the proximal convoluted tubules to promote sodium, potassium and water retention.

Angiogenesis

Angiogenesis, the development of blood vessels is inhibited by prolactin.

Prolactin Secretion

The calculated production rate of prolactin ranges from 200 to 536 µg / day/ m^2. Reference values are given in Table -12[143].

Factors Influencing Secretion

Circadian Rhythm[125-127]

Prolactin is detectable in plasma at all times during the day but is secreted in discrete pulses superimposed on basal secretion and exhibits a diurnal rhythm with peak values in the early morning hours. There is a true circadian rhythm in humans because it is maintained in a constant environment independent of the sleep rhythm. Lowest concentration is found at mid day.

External Stimuli[128]

The suckling stimuli is the most important physiologic regulator of prolactin secretion. Within 1 - 3 minutes of nipple stimulation, prolactin levels rise and remain elevated for 10 - 20 minutes. This reflex is distinct from the milk let - down, which involves oxytocin release from the neurohypophysis.

Stress affects prolactin secretion though the significance of it is uncertain. It may be related to the action of prolactin on cells of the immune system or some other aspects of homeostasis.

Regulation of prolactin Secretion

Prolactin secretion is regulated by physiological states like mensural cycle, pregnancy, lactation and stimuli like light and stress.

Intra Pituitary Regulation

The secretion of prolactin is regulated by autocrine - paracrine factors within the anterior lobe. Local regulators of prolactin are given in Table - 13[129-133.]

Neural Control

It is well known that prolactin secretion, unlike the secretion of other pituitary hormones, is primarily under tonic inhibitory control by the hypothalamus. Dopamine is the principle, physiological prolactin inhibiting factor[134] (PIF) released from the hypothalamus. Dopamine inhibits prolactin secretion from lactotrophs both in vivo and vitro[135]. Secretary bursts of prolactin are caused by the acute withdrawal of dopamine inhibition or stimulation by prolactin releasing factors or combination of both events.

Neuro Endocrine Regulation[125,134,136]

Secretion of prolactin, like that of other anterior pituitary hormones, is regulated by hormonal feed back and neural influences from the hypothalamus as illustrated in figure 6 and Table - 14. In the elderly it is found that there is a rise in serum prolactin levels at the rate of 5.3% per year due to the loss of hypothalamic pituitary regulatory function due to aging.

Feed Back Control[137]

Feed back is exerted by prolactin itself at the level of the hypothalamus. Negative feed back control of prolactin secretion is mediated by a unique short loop mechanism within the hypothalamus. This is by increasing the concentration of tyrosine hydroxylase activity in the neurons that produce dopamine. Short feed back-loop inhibition of Gonadotropin releasing hormone

secretion by prolactin has been suggested as the reason for the inhibition of gonadotropin secretion that occurs in women who are nursing and in patients with prolactin secreting adenomas of the pituitary gland[2].

Prolactin Clearance

[138]Prolactin clearance rate ranges from 40 - 71 ml/min/m^2 prolactin is cleared rapidly with a half-life of 26 to 47 minutes. Prolactin secretion is episodic in 4 to 14 secretary pulses during the day each lasting 67 to 76 minutes over 24 hrs[139-141]. Prolactin reaches its highest level during sleep and lowest is at mid noon[142].

Prolactin stimulation

Other than the local regulators given in Table 13 for prolactin secretion there are other stimulators and inhibitors, which are given in Table 15.

HYPERPROLACTINEMIA

In the absence of a prolactinoma, hyperprolactinemia may be caused by other pituitary or sellar tumours that inhibit dopamine because of pressure on the pituitary stalk or interruption of the vascular connection between the pituitary and hypothalamus.

Idiopathic hyperprolactinemia[144,145]

An elevated circulating prolactin level in patients in whom no cause is identified is considered idiopathic and they are resistant to dopamine.

Macroprolactinemia[146]

Large circulating prolactin molecule with markedly reduced bioactivity.

Clinical Significance[147]

Hyperprolactinemia is the most common hypothalamic pituitary disorder encountered in clinical endocrinology. The signs and symptoms of hyperprolactinemia are given in Table -16.

The etiology of hyperprolactinemia is given in Table -17.

Mechanism of reproductive dysfunction due to hyperprolactinemia[106]

Inhibition of pulsatile Gonadotrophin Releasing Hormone (GnRH) secretion, interference with gonadotropin action in ovary, interference with estrogen positive feed back, inhibition of Follicle Stimulating Hormone (FSH) directed ovarian aromatase, inhibiton of progesterone synthesis by granulosa cells, and inhibition of 5 - a reductase enzyme in men thereby reducing conversion of testosterone to dihydro testosterone.

Hypogonadism is a well established cause for secondary osteoporosis. Hypogonadism results in premature ovarian failure or acquired GnRH deficiency and results in reduction in bone density with preferential trabecular bone lose. Clinically it leads to amenorrhoea and a state of functional menopause.

Association of hyperprolactinemia with reduced bone mineral density[148]

The precise means by which estrogen deficiency causes increase bone turnover is not known, possible mechanism includes a direct effect on osteoblasts via estrogen receptors. Trabecular bone is found to be more affected than cortical bone. There is a 15-30% reduction in trabecular bone density and 17% reduction in cortical bone density. Estrogen reduces calcium and phosphorus excretion. Estrogen deficiency accelerates, the bone turnover. Deficiency during puberty can impair the attainment of normal peak bone density there by making women vulnerable to skeletal fracture.

Androgens play an important role in the maintenance of normal bone mass in women. It's effect is similar to that of estrogen, its deficiency slower in producing calcium loss. Cortical osteopenia was significantly related to the duration of hyperprolactinemia but not to the absolute level of prolactin or androgen.

Patients of both sexes with hyperprolactinemia have reduced bone density and treatment not only stops bone loss but also reverses it. Hypogonadism should be treated not only to restore fertility but also to prevent osteoporosis[107,117]

Bone mineral density was studied in hyperprolactinemic women and compared with normal subjects. The mean bone mineral density in gm/cm^2 in hyperprolactinemic women was 9% less than is normal subjects. Negative correlation was found between bone mineral density and the duration of hyperprolactinemia. The analysis of bone metabolic parameters, bone turnover, bone formation and bone absorption showed an increase in hyperprolactinemic states.

AIM OF THE STUDY

On having reviewed about reduced bone mineral density especially osteoporosis and its association with hyperprolactinemia, the work on prolactin in reduced bone mineral density has been taken up with the view of establishing the following .

1. Reference range for serum prolactin for the study.

2. To analyse the level of serum prolactin and other biochemical parameters namely s.calcium, s.phosphorus, s.alkaline phosphatase, in subjects with reduced Bone Mineral Density i.e. osteopenic subjects and osteoporotic subjects and to assess whether their level differed from their reference range statistically.

3. To correlate the level of serum prolactin with s.calcium, s.phosphorus, s.alkaline phosphatase, and Bone Mineral Density.

4. To determine whether it is possible to established a cut off level for serum prolactin to differentiate subjects of reduced bone mineral density from normal subjects.

MATERIALS AND METHODS

A total of 124 subjects who attended an `Osteoporosis Camp', for the purpose of screening for osteoporosis, were included in the present cross sectional study of hyperprolactinemia and its association with reduced BMD.

The subjects were grouped as follows :

Group I	:	1.	Apparently normal elderly men
		2.	Apparently normal elderly women
Group II	:	1.	Elderly men with osteopenia
		2.	Elderly women with osteopenia
Group III	:	1.	Elderly men with osteoporosis
		2.	Elderly women with osteoporosis

BMD was estimated using ultrasound of the os calcis and depending on the speed of sound the subjects were diagnosed as having either osteopenia or osteoporosis. Bone densitometry reports provide a T Score - the number of standard deviations above or below the mean bone mineral density matched to young controls.

Normal	:	A value for BMD ± 1 SD of the young adult reference mean
Osteopenia	:	A value for BMD > 1SD & -2.5 SD lower than the young adult mean

Osteoporosis : A value for BMD > 2.5 SD lower than the young adult mean

Severe Osteoporosis : A value for BMD > 2.5 SD lower than the young adult mean in the presence of one or more fragility fractures

The following inclusion and exclusion criteria were applied while selecting the subjects.

Inclusion Criteria

1. Apparently healthy elderly subjects
2. Both male and female subjects
3. Subjects between 55 - 80 years of age

Exclusion Criteria

1. Diabetes Mellitus
2. Chronic Renal failure
3. Acute and chronic liver disease
4. Alcoholism
5. Drugs - anticonvulsants

SPECIMEN COLLECTION AND STORAGE

After obtaining informed oral consent of the subjects five ml of peripheral venous blood samples was collected from each of the 124 subjects under strict aseptic precaution into clean dry test tubes with out adding any anticoagulant and the samples were allowed to stand for half an hour. The serum was separated by centrifugation at 2500 rpm for 10 min. Samples were labeled and allotted identification number. 1 ml of the serum from each sample was transferred with the help of a micro pipette into clean dry tubes and preserved at - 20°C for analysis at a later date. Samples were analyzed in two batches within 30 days of collection. Serum prolactin levels were estimated in two batches by ELISA. The remaining specimens were analyzed for s.calcium and s.phosphorus and s.alkaline phosphatase.

ESTIMATION OF SERUM PROLACTIN

Serum prolactin was estimated by ELISA method using pathozyme prolactin of OD 427 of Omega Diagnostics.

This test has been calibrated against in house standards and against the World Health Organisation 1st International Reference Preparation (WHO 1st 1RP 75 / 504).

PRINCIPLE OF THE TEST

Pathozyme prolactin assay is a solid phase sandwich enzyme immuno assay in which specific anti prolactin antibodies coated on to micro filtration wells form an antigen antibody complex with prolactin present in the specimen. On addition of the conjugate ie monoclonal anti prolactin labelled Horse radish peroxidase enzyme, the prolactin is sand witched between the solid phase anti prolactin antibodies and antiprolactin labeled enzyme conjugate forming a Antibody - Antigen - Antibody complex. After incubation and washing off of the unbound antibody the substrate (Tetra Methyl Benzidine) is added and incubated. After stopping the reaction by the addition of stop solution (1ml HCl) the colour developed is measured colorimetrically at the wavelength of 450 nm. The concentration of prolactin in the sample is directly proportional to the colour intensity of the test sample.

MICROTITRE PLATE

Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.

REAGENTS AND CONSUMABLES

Calibrators	Concentration	Contents	Quantity
A	0 ηg/ml	Reference standard Human serum free of prolactin lyophilised	1 ml
B	5 ηg/ml	Reference standard prolactin diluted in human serum lyophilised	1 ml
C	15 ηg/ml	Reference standard prolactin diluted in human serum lyophilised	1 ml
D	50 ηg/ml	Reference standard prolactin diluted in human serum lyophilised	1 ml
E	100 ηg/ml	Reference standard prolactin diluted in human serum lyophilised	1 ml
F	200 ηg/ml	Reference standard prolactin diluted in human serum lyophilised	1 ml

Conjugate		Anti-prolactin HRP conjugate Anti-prolactin conjugated to HRP Ready to use Pink	11 ml
Substrate - TMB		Substrate solution 3, 3', 5, 5' tetra methyl benzidine in a citrate buffer ready to use colourless	11 ml
Stop solution		Hcl 1 ml stop solution : hydrochloric acid (diluted in purified water) Ready to use colourless	11 ml

REAGENT PREPARATION

All reagents were brought to room temperature (20°C to 25°C) and mixed gently prior to use with out inducing foaming.

1 ml of distilled water is added to each standard vial in order to reconstitute the lyophilised standards. Allow to stand for at least 20 minutes and mix gently store at - 20°C when not in use. Rehydrated standards can be stored for 30 days at 2°C to 8°C. For long term storage aliquot and freeze at - 20°C. Freeze thaw only once. Thawed standards must be mixed prior to testing.

ASSAY PROCEDURE

1. Bring all the kit components and the samples to room temperature (20°C to 25°C) prior to the start of the assay.

2. One set of multi level calibrator / standards should be run with each batch of sample. Secure the desired number of coated wells in the holder. Record the position of the standard and the test serum on the EIA Data Recording sheet provided.

3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip - lock before being replaced at 2°C to 8°C.

4. Dispense 50 µl of standards and test serum into the assigned wells.

5. Dispense 100 µl of Anti - prolactin conjugate into each well.

6. Mix for 10 seconds. It is very important to mix completely.

7. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).

8. At the end of incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the biohazard container.

9. Hand washing. Fill the wells with a minimum of 300 µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.

10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

11. Machine washing ensure that 300 µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

12. Dispense 100 µl substrate solution into each well and mix gently for 5 seconds.

13. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).

14. Stop the reaction by adding 10µl stop solution to each well.

15. Gently mix for 30 seconds to ensure that the blue colour changes completely to yellow colour.

16. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450 nm filter.

ASSAY VALIDITY

Assay was validated as per product

OD of Cal. A = < 0.75

OD of Cal. F = > 1.5

CALCULATION OF RESULTS

Calculate the mean absorbance value (A 450) for each set of standards and test samples. Construct a standard curve by plotting the mean absorbance from each standard against its concentration in ηg/ml on graph paper, with absorbance values on the y-axis and concentrations on the x-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of prolactin in ηg/ml from the standard curve.

The graph produced by the calibrators should be Hyperbolic in shape with the OD 450 by the calibrators proportional to their concentration.

REFERENCE RANGE

Male - 6 ηg/ml

Female - 15 ηg/ml

SENSITIVITY

The minimum detectable concentration of human prolactin by pathozyme prolactin is 2 ηg/ml. Concentrations of 4000 ηg/ml have been observed using pathozyme prolactin with no prozone (Hook) effect.

ESTIMATE OF SERUM ALKALINE PHOSPHATASE

EC 3.1.3.1, Orthophosphoric-monoester phosphohydrolase

Kit used - Erba kit

METHOD

Methodology Adaptation by Wilkinson et al., of the Bersey., Lowry et al.,

Principle

$$\text{P-Nitrophenyl phosphate} + H_20 \xrightarrow[Mg^{2+}]{ALP} \text{Nitrophenol} + \text{phosphate}$$

The rate of formation of phosphate by the action of alkaline phosphatase on 4 - nitrophenol at 37°C can then be measured at 405 nm.

ASSAY PARAMETERS

Mode	Kinetic
Wavelength (nm)	405
Sample Volume (µl)	10 / 20
Reagent Volume (µl)	500 / 1000

Lag time (Sec)	60
Kinetic interval (Sec)	60
No. of Readings	3
Kinetic Factor	2713
Reaction Temperature °C	37
Reaction Direction	Increasing
Linearity Low (IU/L)	0
Linearity High (IU/L)	1000
Absorbance Limit (max)	0.800
Units	IU / L

Procedure

Pipette	Volume
Working Reagent	1000 µl
Sample	20 µl

Mix and read absorbance at 30, 60, 90, 120 seconds at 405 nm.

CALCULATION

Activity of alkaline phosphatase at 37°C IU/L =Δ A/min. x Factor (2713)

REFERENCE RANGE

15 - 112 1U/L 37°C

ESTIMATION OF SERUM CALCIUM

Kit used - Erba kit

METHOD

Arsenazo III method.

Principle

Arsenazo III combines with calcium ions at pH 6.5 to form a coloured chromophore the absorbance of which is measured at 650 nm (630 - 660 nm) and is proportional to calcium concentration.

Reagents : I Calcium Arsenazo Reagent

Arsenazo III	0.20 mmol/l
Imidozole buffer (pH 6.5 ± 0.1)	100 mmol/l

Reagents : II Calcium Standard

Calcium Salt	10 mg/dl

ASSAY PARAMETERS

Mode	Kinetic
Wavelength (nm)	630
Sample Volume (µl)	10 / 20
Reagent Volume (µl)	500 / 1000

Incubation Time (Mins)	1
Incubation Temp (°C)	37
Linearity Low (mg/dl)	0
Linearity High (mg/dl)	16
Concentration of Standard (mg/dl)	10
Absorbance Limit (Max)	1.2
Units	mg/dl

Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Sample**
Reagent 1	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl

Mix well read the absorbance of each tube against blank at 630 nm after 1 minute.

CALCULATION

$$\text{Calcium mg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

REFERENCE RANGE

8.4 - 10.49 - 11 mg/dl

ESTIMATION OF SERUM PHOSPHORUS

Kit used - Erba kit

METHOD

Modification by Wang et al., of Daly and Ertingshausen's method

PRINCIPLE

Inorganic phosphorus combines with ammonium molybdate in the presence of strong acids to form phosphomolybdate. The formation of phosphomolybdate is measured at 340 nm and is directly proportional to the concentration of inorganic phosphorus present.

Reagent I Phosphorus Reagent

Ammonium molybdate	0.43mmol
Sulphuric acid	213mmol
Surfactant	-

Reagent 2 Phosphorus Standard

Inorganic Phosphate	5 mg/dl (1.613 mmol/L)

Assay Parameters

Mode	Endpoint
Wavelength 1 (nm)	340
Sample Volume (µl)	10/20
Reagent Volume (µl)	500 / 1000
Incubation Time (Mins)	5
Incubation Temp. (°C)	37
Linearity Low (mg/dl)	0
Linearity High (mg/dl)	15
Concentration of Standard (mg/dl)	5
Absorbance Limit (Max)	0.4
Units	mg/dl

Assay Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Sample**
Reagent 1	1000µl	1000µl	1000µl
Distilled Water	20µl	-	-
Standard 2	-	20µl	-
Sample	-	-	20µl

Mix well and incubate at 37°C for 5 minutes. Read the absorbance of standard and each sample at 340 nm against blank .

CALCULATION

$$\text{Serum phosphorus mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Standard mg/dl}$$

REFERENCE RANGE

2.5 - 4.5 mg/dl

RESULTS

The biochemical parameters, namely s.alkaline phosphatase, s.calcium, s.phosphorus, s.prolactin levels of the 124 subjects who formed the study group are listed in Master Table 1 along with their respective BMD which has been calculated. Serial No.1 to 34 of this table gives the results in apparently normal subjects who form the control group. Serial No. 35 to 94 indicates the results in subjects of the osteopenic group and serial No.95 to 124 indicates the same in subjects of the osteoporotic group. The results of the subjects belonging to the different groups enumerated above are segregated separately in Tables II,III and IV respectively. The mean and standard deviation for each parameter of these tables are calculated and shown below in the respective Tables.

Bar diagrams depicting the mean values of the parameters in the different group of study along with their standard deviation have been shown in figure 8(a) to (e) . To determine whether the levels of s.prolactin or any other biochemical parameter of study varied between male and female controls, the males in this group have been segregated in Table II(a) and the mean and standard deviation for the parameters have been calculated and shown in the table. Like wise the females of the control group have been depicted in Table II (b). The obtained mean and standard deviation of the biochemical parameters in Table II (a) and II (b) have been compared with each other in Table No. V to arrive at the statistical significance between the levels of male and female controls. As statistical significance has not been obtained for any parameter in this table the mean levels of the parameters obtained from all the subjects of the control group irrespective of sex is taken as the reference range for the study. Hence the reference range for the parameters is obtained from Table II.

The reference range so obtained is compared with the mean levels of the parameters obtained from the osteopenic group and osteoporotic group in Table No. VI and VII respectively to find out whether there is any variation in the level of the parameters in the pathological groups from that of the control group and if so whether there is any statistical significant for the difference. Similarly the difference in the level of the parameters between the two pathological groups is shown in Table VIII.

The statistical significance of the study parameters in the comparison table is obtained from the corresponding `P' value, which is arrived at using the students `t' test.

The correlation of s.prolactin with the other parameters of the study is shown in Table IX where the correlation has been arrived at using the Karl Pearson correlation coefficient.

The statistical evaluation of sensitivity and specificity between osteoporosis versus control and osteopenic group are shown in Table X to arrive at the appropriate cut off level.

Scatter diagram showing s.prolactin levels and BMD in different groups are shown in figure 9(a) controls, 9(b) osteopenic group, 9(c) osteoporotic group respectively.

Box and whisker plot showing BMD and s.prolactin in different groups are shown in figure 10(a) and 10(b) respectively.

Correlation between s.prolactin and BMD is shown as bar diagram in figure 11(a) and as a line diagram in 11(b). Levels of s.prolactin in the 3 different groups along with the cut off point are shown in Figure 12.

DISCUSSION

Analysis of the results obtained in this study starts with scrutinizing the reference range obtained. The reference range of s.prolactin which is 10.08 ± 6.82 ηg/ml though higher than 6-15 ηg/ml quoted in the kit methodology adopted for its determination is within the reference range of 3-23 ηg/ml given by W.B. Saunders Co.[143]

The reference range of serum calcium is 9.71 ± 0.731 mg/dl. This is within the reference range of 8.4 to 10.4 mg/dl of the Arsenazo III kit methodology which was used to estimate s.calcium and is well within the reference range of 8.6 - 10.2 mg/dl given in standard text books for s.calcium.

The reference range for s.phosphorus is 3.91 ± 0.459. This reference range is within the reference range of 2.5 - 4.5 mg/dl of the kit methodology adopted for its study and that of standard text books.

The reference range of s.alkaline phosphatase is 101.71 ± 14.69 IU/L. This level is within the reference range of 53-141 IU/L of s.alkaline phosphatase specified in standard textbooks for geriatric age group and that of the kit methodology where the range is 15 - 112 IU/L.

The reference range of - 0.2 ± 0.77 obtained for BMD is within the reference range of ± 1 SD of the `T' score for the young adult specified by the ultrasound method undertaken for its analysis.

Hence the mean levels of the analysed biochemical parametes namely 10.08 ± 6.82 for s.prolactin, 9.71 ± 0.731 for s.calcium, 3.91 ± 0.459 for

s.phosphorus, 101.71 ± 14.69 for s. alkaline phosphatase and -0.2 ± 0.77 for BMD obtained from apparently normal geriatric individuals are accepted as valid reference range for the study.

Comparison of the levels of the study parameters obtained in osteopenia with the above reference range Table VI reveals a significant decrease of s.calcium, [p value: 0.03] significant increase of s.alkaline phosphatase, [p value : 0.03] and a significant decrease in BMD [p value : 0.01].

The significant decrease of s.calcium observed in this table can be explained in the following lines:-

It is a well-documented fact that age impairs both calcium absorption and renal calcium conservation[143]. Therefore even the control level obtained in the age group above 60 namely 9.71 mg/dl of s.calcium can be a value lower than that which would have been obtained if the age group between 30 to 60 years had been analysed. In the osteopenic elders this impairment in calcium absorption and renal conservation is more predominant than in the control elders leading to its significant decrease. It has also been reviewed that lowering of s.calcium level will trigger the parathyroid to secrete more parathormone where by the level of calcium will be restored as the hormone increases calcium absorption in the intestine, increase renal calcium conservation and promotes bone resorption. The effect of PTH on bone to maintain calcium homeostasis by resorption of bone calcium leads to a decrease in BMD and thereby leads to osteopenia in this group. However when the mean level of s.calcium in osteopenia is analysed its level of 9.085 is found to be still within the reference range of 8.979 - 9.841 obtained for s.calcium in

controls and as well within the reference range in standard text books and methodology employed. Therefore the statistically significant decrease of s.calcium in osteopenia can be ignored.

The significant increase in SAP observed in this table is due to increase of osteoblastic action in bones due to the decrease in BMD. In the present study we find that though a significant increase of SAP is obtained in osteopenia its mean level of 101.71 IU/L is within the reference range of 53-141 IU/L obtained for SAP in the study and that of the reference range quoted in standard text books. Therefore the increase in this parameter can also be ignored.

The mean levels of -1.58 for BMD in the osteopenic group studied satisfies the `T' score classification by being well within -1 to -2.5 SD BMD that has to be obtained for osteopenia. This proves that the subjects selected in this group are osteopenic. The reasons for the decrease in BMD to a level lower than in the elderly controls has already been putfourth.

S.prolactin even though has marginally increased in the osteopenic group than that of its control level has not shown any statistical significance in this table. On the other hand the BMD has decreased from its reference level to a statistically significant degree (p -.01). In the elderly it has been reviewed that there is a rise in s.prolactin at the rate of 5.3% per year due to loss of hypothalamic pituitary regulatory function that occurs with aging. The increase in prolactin has been ascribed to an age-related decline in dopamine, the neurotransmitter responsible for inhibition of prolactin secretion [163]. But in this osteopenic group of the study whose age group is similar to that of controls we find that decrease of BMD is not associated with a relevant statistical increase

of prolactin. When the levels in osteoporosis are compared with the reference range (Table VII) highly significant increase of SAP (p value : 0.001) and s.prolactin (p value 0.001) are observed against a highly significant decrease in BMD (p value 0.001). BMD of -2.7 SD obtained in the study of osteoporotic group satisfies the `T' score norms because this value is > -2.5 SD of BMD which pertains to osteoporosis in the above scoring. This proves that the subjects selected for this study group are osteoporotic.

The highly significant increase of SAP and s.prolactin can be attributed to the same reasons discussed in the osteopenic group. While increase in osteoblastic activity is the cause for increase in SAP, decline of dopamine in the elders is said to be the cause for hyperprolactinemia. But as the mean level of SAP in osteoporosis is still within the reference range of 87.02 to 116.40 IU/L of the study and that in standard text books and methodology adopted, this increase is ignored. Hyperprolactinemia in the osteoporotic group of the study correlates well with the finding of John Bernard who has said that the metabolic manifestations of hyperprolactinemia include decrease BMD[99]. As per Klibanski. A and Neer .R this association can be due to the direct action of prolactin on calcium mobilization through prolactin receptors which are independent of vitamin D and parathormone and that the hyperprolactinemic subjects have an increased risk of developing osteoporosis.

Comparison of the levels in osteopenia and osteoporosis (Table VIII) reveals highly significant increase of s.prolactin (p value .001) against a highly significant decrease (p value .001) of BMD in osteoporosis from its level in osteopenia which finding is similar to that obtained between osteoporosis and

controls. The significant decrease of BMD in osteoporosis from that in osteopenia is understandable as this condition is a more severe form of decreased BMD than osteopenia. This is also made evident by the higher `T' scoring of the osteoporotic group of the study than osteopenia.

Similarly the highly significant increase in s.prolactin levels of this group over that of the osteopenic group and as well over that of controls can be the relevant cause for the greater deterioration of BMD in osteoporosis. So it is presumed that greater the increase of s.prolactin level higher is the intensity of decrease in BMD. At this point it should be noted that there is absence of any significant elevation of s.prolactin in osteopenia from the reference range. Hence it can be said from this study that impairment of calcium homeostasis which is the hall mark of aging is the basic cause for decrease in BMD.

Further it has been stated that a net effect of the changes that occur as age advances increases the circulating level of PTH by 30% between 30 and 80 years of age. Therefore in elders when s.calcium level is maintained predominantly by resorption of bone calcium rather than intestinal calcium absorption or renal calcium conservation being increased by PTH it results in significant decrease in BMD the grading of which are osteopenia and osteoporosis. In this study it is clear that in elders when there is associated hyperprolactinemia (osteoporotic subjects) there is more bone resorption than in those elders where its level is not elevated. (Osteopenic subjects). In the osteopenic subjects the decrease in BMD is attributed only to the action of PTH. Therefore it is inferred that the excess bone resorption due to hyperprolactinemia leads to severe decrease of BMD resulting in osteoporosis.

Analysis by the Karl Pearson correlation coefficient of s.prolactin with the other parameters of the study revealed in Table IX shows the following :-

1. Correlation of prolactin with calcium shows a negative fair correlation in control and a fair correlation in osteopenic group.

2. Correlation of prolactin with BMD shows a negative fair correlation in osteopenic and osteoporotic group and a negative fair correlation in the overall group.

As s.prolactin in the osteoporotic group has increased statistically above the reference range of the study and that in the osteopenic group, attempt is made to establish a cut off level between the latter groups and that of osteoporosis. For this line graph has been plotted with the levels of s.prolactin in the 124 subjects as per their grouping in Figure 12 various cut off levels have been selected and the sensitivity, specificity, positive predictive value and negative predictive value for these values have been calculated and shown in Table X. The most appropriate level between osteoporosis and the osteopenic and control group is found to be 11 ηg/ml which has a sensitivity of 86.6%, specificity of 52.12% positive predictive value 36.619, negative predictive value 92.452. The cut off level is shown in Figure 12 as intermittent lines. Hence s.prolactin level of 11 ηg/ml or more indicates osteoporosis.

CONCLUSION

From the discussion held so far on the results obtained from the blood samples of the 124 subjects analysed classified into the control, osteopenic and osteoporotic groups, the following inferences are made.

* Reference range for s.prolactin is 10.08 ηg/ml for the study.

* S.prolactin level is increased above the reference - range in osteopenia and osteoporosis but only in osteoporosis the elevation is statistically significant, the degree of elevation being highly significant in this condition.

* The cut off level of s.prolactin to determine osteoporosis is 11 ηg/ml.

* S.prolactin's correlation with s.calcium is negative fair in the osteopenic group, negative poor in osteoporotic group; the over all correlation is negative fair.

* S.prolactin's correlation with s.phosphorus is negative poor in osteopenic group, negative poor in osteoporotic group; the over all correlation is negative poor.

* S.prolactin's correlation with s.alkaline phosphatase is poor in both osteopenic and osteoporotic group; the over all correlation is poor.

* S.prolactin's correlation with BMD is negative fair in both osteopenic and osteoporotic group; the over all correlation is negative fair.

SCOPE FOR FURTHER STUDY

* To determine s.prolactin level in normal adult group and compare the level with geriatric group.

* To determine the parameters along with parathormone and vitamin D.

* To determine the level of s.prolactin in relation to other physiological conditions.

* To determine the level of s.prolactin and other parameters in conditions like chronic renal failure, polycystic ovarian disease and cirrhosis, where the level of s.prolactin is said to be raised.

ABBREVIATIONS

BMD	Bone mineral density
BMC	Bone mineral content
SD	Standard deviation
DEXA	Dual energy x-ray absorptiometry
PTH	Parathyroid hormone
TRH	Thyrotropin - releasing hormone
GnRH	Gonadotropin releasing hormone
ACTH	Adrenocorticotropic hormone
ELISA	Enzyme linked immuno sorbent assay
SAP	Serum Alkaline phosphatase

BIBLIOGRAPHY

1. Normal Bone Anatomy - Structure and Function of bone by Arthus W. Fetter.

2. Carl A Burtis, Edward R-Ashwood, Dand E.Bruns Tietz Text book of Clinical Chemistry and molecular Diagnostics 4th Edition Chapter 49 Pg. 1891.

3. Histology Inderbir Singh.

4. Robert K. Murray, Daryl K. Granner, Peter A. Mayesm *et al* Haper's Biochemistry 25th edition pg.708.

5. David B. Endres, Ph.D, and Robert K. Rude M.D., Teitz. Mineral and Bone Metabolism Chapter 49.

6. Bettica, P.Bevilacqua M. Short term variations in Bone Remodeling Markers. Bone Remodeling J.Clin Endocirnol. Metab 82, 3034 - 3039.

7. Osteoporosis Suzanne L. Quinn M.D. Jan 1999.

8. Ganong WF. Review of Medical Physiology 17th ed.

9. Review of Medical Physiology 17th ed Appleton Lange 1995.

10. Owen M. Lineage of estrogenic cells and their relationship to the stromal system In : Peck WA ed, Bone and mineral research Amsterdam : Elsevier, 1985 : 1 - 25.

11. Maleval L, Modrowski D, Gupta Ak, et al Cellular expression of bone - related proteins during in vitro osteogenesis in rat bone marrow stromal cell cultures. J.Cell Physiol 1994; 158 : 555 - 572 (Medline).

12. Marks SC, Jr. Walker DG. Mammalian Osteoporosis - a model for studying cellular and humeral factors in bone resorption. In : Bourne GH, ed. The biochemistry and physicology of bone 2nd ed. Vol.4. New York : Academic Press, 1976 : 227 - 301.

13. Serke S, Sauberlich S, Abe Y et al. analysis of CD 34 - positive hemopoietic progenitor cells from normal human adult peripheral blood : flow - Cytometrical studies and in - vitro colony. (CFU - GM, BFU - E) anays. Ann Hematol 1991; 62 : 45 - 53 (Medline).

14. Mundy G.R, Local Factors regulating osteoclast function. In : Rigkin BR, Gay CV, ads. Biology and Physiology of osteoclast. Boca Raton, Fla. CRL Press, 1992 : 171 - 85.

15. Horowitz MC, Jilka RL. Colony Stimulating factors and bone remodeling. In : Gowen M.ed. Cytokines and bone metabolism Boca Raton, fla. CRL Press, 1992 : 185 - 227.

16. Girasole G, Passeri G, Jilka RL, et al. Interleukin - II : a new cytokine critical for osteoclast development . J. Clin Invest, 1994; 93 : 1516 - 1524 (Med line).

17. Demulder A, Suggs SV, Zsebo KM, *et al.* Effects of stem cell factor on osteoclast - like cell formation in long - term human marrow cultures. J. Bone Miner Res 1992; 7 : 1337 - 1344. (Med Line).

18. Grays Anatomy 39th ed. pg. 87 Susan Stranding.

19. Medical Physiology 11th ed. Guyton and Hall.

20. Mario Skugor, M.D., Angelo Licata M.D. Disease Management project osteoporosis - July 1 2004.

21. Bettica P, Moro, Robins. Bone resorption markers. Clin Chem 1992; 38 : 2313 - 18.

22. Robins SP. Biochemical markers of bone metabolism CPD. Bulletin clin Biochem 1999; 1 : 116 - 21.

23. Dempster DW, Lindsay R. Pathogenesis of osteoporosis Lancet 1993; 341 : 797 - 801.

24. Salamone LM, Caulay JA, Zmuda J, et al. Apolipoprotein E gene polymorphism and bone loss : estrogen status modifies the influence of apolipoprotein E on bone loss. J. Bone Miner Res. 2000; 15 : 308 - 314.

25. Salmen T, Heikkinen AM, Mahonen A, et al. Early postmenopausal bone loss is associated with pru II estrogen receptor gene polymorphism in Finnish women: effect of hormone replacement therapy. J.Bone Miner Res. 2000; 15 : 315 - 321.

26. Harris SS, Patel MS, Cole DEC, et al. Associations of the collagen type I alpha I sp-I polymorphism with five year rates of bone loss in older adults. Calcif Tissue Int. 2000; 66 : 268 - 271.

27. The Merck Manual of diagnosis and therapy section 5. Musculoskeletal and connective Tissue Disorders. Chapter 57. Osteoporosis.

28. S.A. Brown, C.J. Rosen, Osteoporosis, Med.Clin. N. Am. 87 (2003) 1039 - 1063.

29. Gina S, Wei, et al, Osteoporosis management in the new millenium. Prim Care Clin office Pract 30 (2003) 711 - 741.

30. Eddy DM, Johnston CC, Lemmings SR, et al. Osteoporosis cost effectiveness analysis and review of the evidence for prevention, diagnosis and treatment. The basis for a guideline for the medical management of osteoporosis.

31. Johnson CC, Jr.Melton LJ III, Lindsay R et al. Clinical indications for bone mass measurements. J. Bone Miner Res. 1989; 4 (Suppl 2) : 1 - 28.

32. Kanis JA, Melton LJ, Christiansen C, et al. Perspective ; the diagnosis of osteoporosis. J. Bone. Miner. Res. 1994; 9 : 1137 - 1142.

33. World Health organization Technical report series 843 : Assessment of fracture risk and its application to screening for post menopausal osteoporosis. Geneva, Switzerland: World Health Organization; 1994.

34. National Osteoporotic Foundation, 1996 and 2015 Osteoporosis Prevalence Figures. Jan 1997. Women's Health Matters 1998 : 2 (3) : 1.

35. Harper KD, Weber T.J. Secondary Osteoporosis. Diagnostic Considerations. Endocrinol Metab Clin. North Am. 1998; 27 (2) : 325 - 48.

36. Who are candidates for prevention and treatment for esteoporosis? osteoporos Int 1997; 7 : 106.

37. Kanis KA. Osteoporosis and its consequences. Osteoporosis. Blackwell Science Ltd. P. 18 (1993 Data).

38. Parfitt AM. The two stage concept of bone loss revisited. Triangle 1992; 31 : 99 - 110.

39. Gallagher JC, Goldgar D, Moy A. Total bone calcium in normal women; effect of age and menopause status. J. Bone Miner. Res. 1987; 2 : 491 - 496.

40. Heaney RP. Estrogen - calcium interactions in the post menopause : a quantitative description. Bone Miner 1990; 11 : 67 - 84.

41. Nordin BE, Need AG, Bridges A, et al. Relative contribution of years since menopause, age and weight to vertebral density in post menopausal women. J. Clin Endocrinol Metab, 1992 : 74 : 20 - 23.

42. Eastell R, Delmas PD, Hodgson SF, et al. Bone formation rate in older normal women : Concurrent assessment with bone histomorphometry, calcium kinetics, and biochemical markers. J. Clin Endocrinol Metab 1988; 67 : 741 - 748.

43. Parfitt AM, Bone - forming cells in clinical conditions. In : All BK, ed. The Osteoblast and osteocyte Vol.1 of Bone. Boca Raton, Fla : Telford Press / CRC Press, 1990 : 351 - 429.

44. Osteoporosis by Balu Sankaran Pg. 4.

45. Holick MF, Kvane SM. Introduction to bone and mineral metabolism. Bone structure and metabolism. In : Braunwald E, Fauci AS, Kasper DL, et al Harrison's principles of internal medicine. New York : Mc Graw Hill : 2001 p. 2192 - 205.

46. Frost HM. Bone dyunsmics in metabolic bone disease J. Bone Joint Surg Am. 1966 : 48 : 1192 - 203.

47. Young N, Formica C, Szmukler G, et al. Bone density at weight - bearing and non weight bearing sites in balket dancers; the effects of exercise hypogonadism, and body weight. J.Clin Endocrinol Metab 1994; 78 : 449 - 454.

48. Arisaka O, Arisaka M, Nakayama Y, et al. Effect of testosterone on bone density and bone metabolism in adolescent male hypogonadism. Metabolism 1995 : 44 : 419 - 423.

49. Barett - Connor E, Mueller J, von Muhlen DG, et al. Low levels of estradiol are associated with vertebral fractures in older men, but not women. The Rancho Bernardo Study.J. Clin Endocrinal Metab 2000 : 85 : 219 - 223.

50. Longeope C, Baker RS, Hui SL, et al. Androgen and estrogen dynamics in women with vertebral crush fractures. Maturites, 1985 ; 6 : 308 - 318.

51. Kenny AM, Prestwood KM, Marcello KD, et al. Determinants of bone density in health older men with low testosterone level. J Gerontol A. Biol Sci Med. Sci 2000; 55 : M 492 - M 497.

52. Stone K, Bauer DC, Balck DM, et al. Hormonal Predictors of bone loss in elderly women : a prospective study. The study of osteoporotic fractures Research Group. J. Bone Miners Res. 1998; 13 : 1167 - 1174.

53. Khosla S, Atkinson EJ, Melton LJ III, et al. Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover women : a population - based study. J. Clin Endocrinol Metab 1997 : 82 : 1522 - 1527.

54. Abrahamsen B, Bonnevie - Nielson. V, Ebbesen EN et al. Cytokines and bone loss in a 5 year longitudinal study : hormon replacement therapy suppresses

serum soluble interleukin - 6 receptor and increase interleukin 1 receptor antagonists : The Danish osteoporosis prevention study. J.Bone Miner Res. 2000; 15 : 1545 - 1554.

55. Abrahamsen B, Shalhoub V, Larson EK, et al. Cytokine RNA levels in transiliac bone biopsies from healthy early postmenopasual women. Bone 2000; 26 : 137 - 145.

56. Bismar H, Diel I, Ziegler R, et al. Increased cytokine secretion by human bone marrow cells after menopause of discontinuation of estrogen replacement J.Clin. endocrinol Metab 1995 ; 80 : 3351 - 3355.

57. Kassem M, Khosla S, Spelsberg TC, et al. Cytokine production in the bone marrow micro environment, failure to demonstrate estrogen regulation in early post menopausal women. J. Clin Endocrinol Metab 1996 : 81 : 513 - 518.

58. Tiegs RD, body JJ, Wahner HW, et al. Calcitonin secretion in postmenopausal osteoporosis. W Engl. J.Med. 1985 : 312 - 1097 - 1100.

59. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. N. Engl. J.Med. 1995; 332 - : 767 - 773.

60. Melton LJ, Ardila E, Crowson CS, et al. Fractures following thyroidectomy in women a population based Cohort study. Bone 2000; 27 : 695 - 700.

61. Rosen CJ, Donahue LR, Insulin like growth factors and bone : the osteoporosis connection revisiting. Proc Soc Exp Biol Med. 1988; 219 : 1-7

62. National osteoporosis foundation, physician's Guide to prevention and Treatment of osteoporosis.

63. Osteoporosis, Williams Text book of Endocrinology. By Lawrence G. Raisz, Barbara E. Kream and Joseph A. Lorenzo, pg. 1389.

64. Definition of bone mineral density. www.n.of Org/ phys guide / glossary. htm.

65. Melton LJ, III Khosla S, Achenbach SJ, et al. Effects of body size and skeletal site on the estimated prevalence of osteoporosis in women and men. Osteoporosis Int 2000; 11 : 977 - 983.

66. Lee S.Simon, Osteoporosis Clin Geriatr Med 21 (2005) 603 - 629.

67. Sheryl H. Follin, Laura B. Hansen, Current Approaches to the prevention and treatment of postmenopausal osteoporosis, Am. J. Health - syst Pharm 60 (9) : 883 - 901, 2003.

68. Gina S.Wei, et al, osteoporosis management in the new millennium. Prim Care Clin Office Pract 30 (2003) 771 -741.

69. Michael R.Mc Clung, MD Bisphosphates Endocrinol. Metab Clin N Am; 32 (2003) 253 - 271.

70. Stuart L. Silverman, Calcitonin. Endocrinol Metab Clin N Am. 32 (2003) 273 - 284.

71. Osteoporosis Part I Evaluation and Assessment by Jeannette. South Paul, Col, MC, USA. March 1, 2001 American Family Physician.

72. Schlechte, J, Sherman B, Martin R. Bone density in amenorrheic women with or without hyperprolactinemia. J Clin Endocrinol Metab 1983; 56; 1120 - 1123.

73. Klibanski A, Neer R, Beitins I, et al. Decreased bone density in hyperprolactinemia women. N Engl J. Med 1981; 303 : 1511 - 1514.

74. Kalibanski et al. Decreased bone density in hyperprolactinemia women N.Engl J.Med. 1980; 303 (26).

75. Koppelmann. M, Kurtz D, Morrish A, et al Vertebral body bone mineral content in hyperprolactinemia women. J. Clin Endocrinol Metab, 1984, 59 : 1050 - 1053.

76. Kovacs, C, Chik C Hyperprolactinemia caused by lactation and pituitary adenomas is associated with altered serum calcium, phosphate, parathyroid hormone and parathyroid hormone - related peptide levels. j.Clin Endocrinol Metab, 1995; 80; 3036 - 3042.

77. Beck BR, Shoemaker MR, Osteoporosis : Understanding key risk factors and therapeutic options. The physician and sports medicine. Feb 2000.

78. Nattiv A. Osteoporosis : It's prevention, recognition and management. Family Practice Recertification, Feb 1998.

79. Licata AA. Update on osteoporosis strategies for prevention and treatment women's health in Primary Care March 1999.

80. Consensus Development Conference : Diagnosis, prophylaxis and treatment of osteoporosis. Am.J. Med. 1993.

81. Physicians guide to prevention and treatment of Osteoporosis. National Osteoporosis Foundation, 1999.

82. Deal CL. Osteoporosis : Prevention, diagnosis and management Am.J.Med. 1997 : 102.

83. Evans RA, Marel GM, Lancaster EK, et al. Bone Mass is low in relatives of osteoporosis patients Ann. Intern Med. 1988 : 109.

84. Lonzer MD, Imric R, Rogers D, et al. Effects of heredity, age, weight, puberty, activity and calcium intake on bone mineral density in children. Clin. Pediatric, 1996 : 35.

85. Grisnik JA, Hodge A. Study in Progress.

86. NIH Consensus Development Panel of Optimal Calcium Intake. NIH Consenes conference : Optimal Calcium intake. JAMA. 1994; 272 : 1942 - 1948.

87. Parfit AM. Osteonal and hemi - osteonal remodeling : The spatial and temporal frame work for singal traffic in adult human bone J.Cell Biochem 1994 : 55 : 273 - 286.

88. Tannirandorn, P. Epstein, S. Drug, Induced Bone loss. Osteoporosis. International 2000; 11 : 637 - 659.

89. Favus M. (ed) Primer on Metabolic bone disease and disorders of mineral metabolism, 4th ed. Lippencott, Williams and wilkins : Philadelphia, 1999.

90. Hodgson, SF, Watts NB, Bilezikian JP, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis; 2001 ed, with selected updates for 2003. Endor Pract 2003; 9; 6 : 544 - 564.

91. Tresolini CP, Gold DT, Lee LS, eds. Working with patients to prevent, treat and manage osteoporosis : a curriculum guide for health professions 2nd ed. San Francisco. National Fund for Medical Education, 1998.

92. Osteoporosis in men suspect secondary diseases first. Angelo Licata MD. Cleveland Clinic Journal of Medicine volume 70 No.3 March 2003.

93. Crandall C. Laboratory workup for Osteoporosis : which tests are most cost effective? Post grad Med 2003; 114 (3) : 35 - 44.

94. Tannenbaum C, Clark J, Schwartzman K, et al. Yeild of laboratory testing to identify secondary contributors to osteoporosis in otherwise healthy women. J.Clin Endorcinol Metab 2002; 87 (10) : 4431 - 7.

95. Pathophysiology of Age - Related bone loss and osteoporosis. Sundeep. Khasle. Endocrinol Metab Clin N.Am. 34 (2005) 1015 - 1030.

96. Melmed S, Kleinberg D, Anterior Pituitary in Larsen P. Kornenberg H, Melmed et al , Williams Text books of endocrinology, Saunders, Philadelphia (2003) pp 177 - 279.

97. Greenspan SL, Oppen heim DS, Klibanski A, Importance of gonadal steroids to bone to mass in men with hyperprolactinemia hypogonadism Ann. Intern. MEd. (1989); 110 (7) pp 526 - 531.

98. Klibanski A, Biller BM, Rosenthal DI, et al. Effects of prolactin and estrogen deficiency in amenorrheic bone loss". J.Clin. Endocrinol Metab (1988) 67 (1) pp 124 - 130.

99. Clinical Chemistry John Bernard Henry MD. Pg. 329.

100. BM Biller, HB Baum, DI Rosenthal, et al. Progressive trabecular osteopenia in women with hyperprolactinemia amenorher. J. of Clin Endocrinol and Metab. Vol 75 692 - 697.

101. Klibanski A, Neer R, Beitins I, et al, 1980. Decreased bone density in hyperprolactinemic women. N. Engl. J.Med. 303 :1511 - 1514.

102. Cann C, MArtin M, Genant H, et al. 1984. Decreased spinal mineral content in amenorrheic women JAMA 251 : 626 - 629.

103. Koppelmann M, Kurtz D, Mornish K et al. 1984. Vertebral body bone mineral content in hyperprolactinemic women. J.Clin.Endocrinol Metab. 59 : 1050 - 1053.

104. Schlechte J, el-khoury G, Kathol M, et al, 1987. Forearm and vertebral bone mineral in treated and untreated hyperprolactinemic amenorrhea . J. Clin Endocrinol Metab 64 : 1021 - 1026.

105. Klibanski A, Biller BMK, Rosenthal DL, Saxe V. 1998. Effects of prolactin and estrogen deficiency in amenorrheic bone loss. J. Clin. Endocrinol Metab. 67 : 124 - 130.

106. Hyperprolactinemia - New Treatment October 2, 2005.

107. Hyperprolactinemia Donald W. Stenonberger MD. October 18, 2005.

108. Postmenopausal osteoporosis Clifford J. Rosen MD. The New Eng. J. of Med. 2005 : 353 : 595 - 603.

109. Neer RM, Arnud, CD, Zanchetta JR, et al. Effect of parathyroid hormone (1 - 34) on fractures and bone mineral density in postmenopausal women with osteoporosis N. Engl.J. Med. 2001; 344 : 1434 - 41.

110. Clinical use of serum and urine bone markers in the management of osteoporosis. Apurva K. Srivatsava etla. Current Medical Research Vol. 21 No.7 2005, 1015 - 1026.

111. Prolactinomas in adolescent persistant bone loss after 2 years of prolactin nermalization. Clinical Endocrinology, Vol. 52 pg. 319 Mar 2000.

112. Osteoporosis in men with hyperprolactinemic hypogonadism Greenspan SL, Neer RM, Ridgway EC, Klibanski A. Ann Inter MEd. 1986 June 104. (6) : 777 - 82.

113. Van Staa TP, Leufkens HG, Aben Haim, L, et al. Cooper - C. Use of oral corticosterioids and risk of fracture J Bone Miner RE. 2000 : 15 : 1993 - 1000.

114. Laan RF, Van Riel PL, Van de Putte LB et al. Low dose prednisolone Ann. Intern Med. 1993; 119 : 963 - 968.

115. Cohen A, Shane E. Osteoporosis after organ transplantation. 2003 : 14 : 617 - 630.

116. J.Schlechte, L. Walkner and M. Kathol. A longitudinal analysis of premenopausal bone loss in healthy women and women with hyperprolactinemia. J. of Clin Endo and Metab Vol. 75, 698 - 703.

117. Greenspan SL, Neer RM, Ridgway EC, Klibanski A. Osteoporosis in men with hyperprolactinemic hypogonadism . Ann. Intern Med. 1986 ; 104 : 777 - 782.

118. Joel S. Finkelstein, Anne Klibanski, Elizabeth H, et al. The N Eng. J. of Med. Vol. 331 : 1618 - 1623 1994 - 24.

119. Osteoporosis Am. I at Risk. Clark H. Cobb MD, Columbus, Georgia.

120. Osteoprosis. The silent Epidemic in women biochemical markers of bone turnover. J. of FMA Jan 2000 : 86 (1).

121. Bone Marker and Bone Density Responses to dopamine Agonist therapy. J. Clin Endocrinol Metab. 75 : 690 - 691.

122. Osteoporosis Screening by Eduardo J. Balbong MD. May, 2000.

123. Osteoporosis. The silent Epidemic Andrew. M.D, JAcksoville MEdicine / May, 2000.

124. Bone health and aging implication for menopause faryal S. Mirza, MD, Karen M. Prest wood MD. Endocrinol Metab Clin NAM 33 (2004) 741 - 759.

125. Freeman, Marc E, Bela KAnyicska, et al. Prolactin : structure, Function and Regulation of secretion. Physiol Rev. 8 : 1523 - 1631, 2000.

126. Veldman RG, Frolich M, Pincers SM, et al. Basal, pulsatile, entropric and 24 hour rhythmic features of secondary hyperprolactinemia due to functional pituitary stalk disconnection mimics tumoral hyperprolactinemia J. Clin Endocrinol Metab 2001 : 86 : 1562 - 1567.

127. Waldstreicher J, Duffy JF, Brown EN, et al. Gender Differences in the temporal organization of prolactin secretion evidence for a sleep independent circadian rhythm of circulating PRL - levels. J. Clin Endocrinol Metab. 1996; 81 : 1483 - 1487.

128. Diag S, Seron - Ferre M, Cardenas H et al. Circadian Variation of basal plasma prolactin, J. Clin Endocrinol Metab 1989; 68 : 946 - 955.

129. Liu JW, Ben Jonathan N. Prolactin - Releasing activity of neurohypophysial harmones : Structure - Function relationship. Endocrinology 1994; 134 : 114 - 118.

130. Horseman N.D Prolactin. In De Groot LJ, Jameson JL (ed). Endocrinology. Philedephia, WB Saunders, 2001 pp 209 - 220.

131. Kanyicske B, Lerant A, Freeman ME, Endocrinology 1998; 139 : 5164 - 5173.

132. Sarkar DK, Kim KH, Minami S. Mol Endorcinol 1992; 6 : 1825 - 1833.

133. Bredow S, Kacsoh B, Obal F Jr et al. Res. 1994; 660 : 301 - 308.

134. Ben - Jonathan N, Hnasko R Dipamine as a prolactin inhibitor, Endocr Rev. 2001; 22: 724 - 763.

135. Macloed RM, Fontham EH, Lehmeyer JE, Prolactin and growth hormone production. Neruendocrinology. 1970 : 6 : 283 - 294.

136. Voogt JL, Lee Y, Yang S, Arbogast L. Regulation of prolactin Secretion during pregnancy. Res. 2001; 133 : 173 - 185.

137. Milen kovic L, Parlow AF, McCann SM. Physiological significance of the negative short - loop feed ba k of prolactin. Neuroendocrinology, 1990; 52 : 389 - 392.

138. Cooper DS, Ridgway EC, Kliman B et al. Metabolic Clearance and production rates prolactin in man. J. Clin. Invest. 1979 : 64 : 1669 - 1680.

139. Veldheris JD, Johnson ML. Operating characteristics of the hypothalamus - pituitary - gonal axis. J.Clin Endocrinol Metab, 1988; 67 : 116 - 123.

140. Greenspan SL, Klibanski A, Rowe JW. Age atters pulsatile prolactin release ; influence of dopaminergic inhibition. Am.J.Physiol 1990; 258 : E 799 E 804.

141. Samvels MH, Henry P, Kelinschmidt DE masters BK, et al. Pulsatile glycoprotein hormone secretion in glycoprotein - producing pituitary tumour. J. Clin Endocrinol Metab 1991; 73 : 1281 - 1288.

142. Sassin JF, Frantz AG, Weitzman ED, Kapen S. Human prolactin. 24 - hour pattern with increased release during sleep. Science 1972 : 177 : 1205 - 1207.

143. Tietz N, ed. Clinical guide to laboratory tests, 3rd ed. Philadelphia : WB Saunders Co., 1995

144. Progress in the Management of hyperprolactinemia. The N. Eng. J. of Med. Vol.331 : 942 - 944, Oct, 6, 1994 : 14.

145. Webb CB, Thominet JL, Barowsky H, et al. Evidence for lactotroph dopamine resistance in idiopathic hyerprolactinemia. J. Clin. Endocrinol. Metab 1983 : 56 : 1089 - 1093.

146. Kleinberg DL. Pharmacologic therapies and surgical options in the treatment of hyperprolactinemia. Endocrinologist, 1997 : 7 (Suppl) : 379 - 384.

147. Shlomo Melmed and David Kleinberg. Williams Text book of Endocrinology 10th Edition, pg.206.

148. The class Article : American Academy of Orthopaedic Surgeons and is reprinted with permission from Steindler A. Osteoporosis. 1956 : 13 ; 167 - 174.

149. O.Sahap Atik, M.D., M. Murad Uslu, M.D., Fatih. Eksioglu et al., Etiology of senile osteoporosis. Number 443 pp.25 - 37, 2006. Lippincott Williams and Wilkins.

150. Cefalu CA. Is bone mineral density predictive of fracture risk reduction? Curr. Med. Res. Opin. 2004; 20, 341 - 349.

151. Ettinger MP. Aging bone and Osteoporosis; Strategies for preventing fractures in the elderly, Arch. Intern. Med. 2003; 163 : 2237 - 2246.

152. Kanis JA. Diagnosis of Osteoporosis and assessment of fracture risk Lancet. 2002, 359 : 1929 - 1936.

153. Koh L.K, Ng DC. Osteoporosis risk factor assessment and bone densitometry : Current status and future trends. Ann. Acad. Med. Singapore, 2002; 31 : 37 - 42.

154. McCalden. RW, McGeough JA, Court - Brown CM. Age - related changes in, compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. J. Bone. Joint Surg. 1997; 79A, 421 - 427.

155. Bohic S, Rey C, Legrand A, Sfiti H, et al., : Characterization of trabceular rate bone mineral. Bone 2000; 26 : 341 - 348.

156. Boivin G, Deloyfree P. Perrat B, et al., Strontrium distributionand interactions with bone mineral in monkey iliac bone - after strontium salt administration. J. Bone Miner. Rao, 196: 11 : 1302 - 1311.

157. Gadeleta. SJ, Boskey AL, Paschalis E et al., : A physical, chemical and mechanical study of lumbar vertebrae from normal, Ovariectomized and nandrolone treated monkeys bone, 2000 : 27; 541 - 550.

158. Huang RY, Miller LM, Carlson CS, et al. : Characterization of bone mineral componsition in the proximal tibia of cynomolgus moneksy, Bone : 2002 ; 30 : 492 - 497.

159. Huang R.Y. Miller CS, Chence Mr. In situ chemistry of Osteoporosis revealed by synchrotron infured microspectroscopy bone, 2003 : 33 - 514 - 521.

160. Monier - Faugere Mc, Geng., Arnala I et al., : Effects on bone morphometry and mineral properties. J. Bone Miner. Res. 1999 : 14 : 1768 - 1778.

161. Paschalis EP, Betts F, Dicarlo E, et al., Microspectroscopic analysis of human iliac crest biopsies from untreated osteoporotic bone calcif tissues Int. 1997; 61 : 487 - 492.

162. Paschalis. EP, Bun DB, et al., : Bone mineral and collagen quality in humeri of ovariectomized lynomolgus monkeys given rh PTH for 18 months. J. Bonhe Miner Res. 2003: 18 : 769 - 775.

163. Age Trends in the level of S.testosterone and other hormones by Vekemans.M, Robyn C, Henry Fieldman J of Endocrinol 1975.

TABLE - 1

COMPOSITION OF BONE

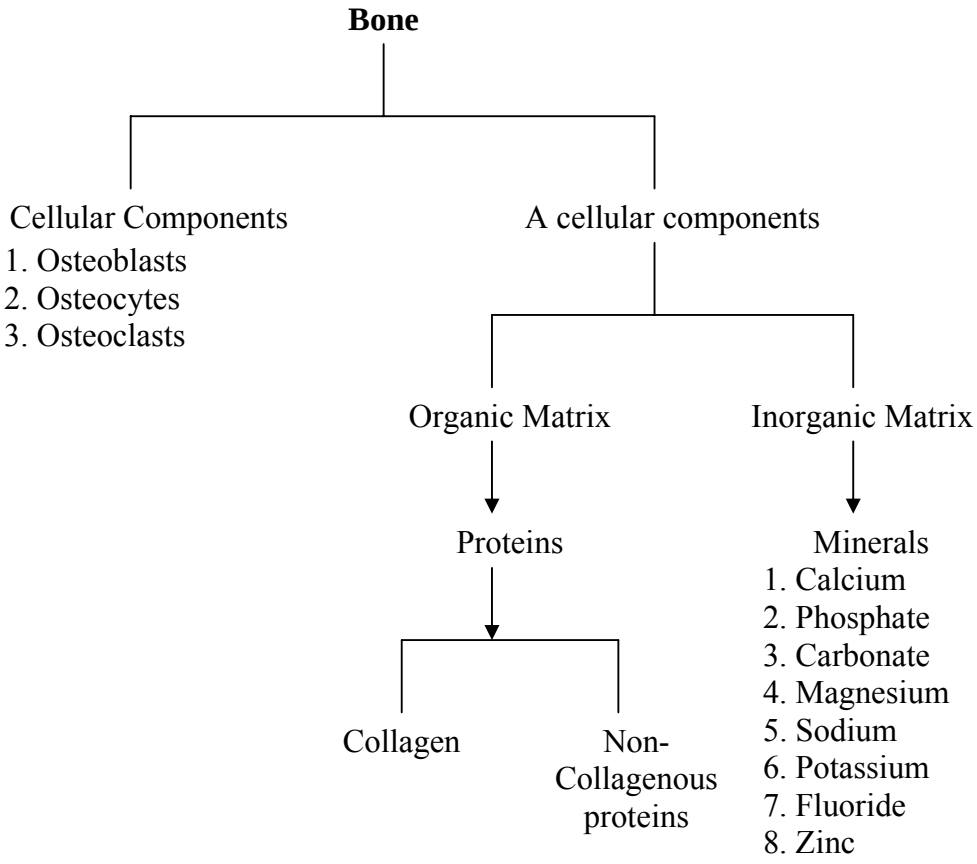


TABLE - 2

THE PRINCIPLE PROTEINS FOUND IN BONES[4]

Proteins	
Collagens	**Non Collagen**
1. Collagen type I (Constitutes 90-95% of organic materials)	1. Mixture of various plasma proteins
2. Collagen type V Minor component	2. Proteoglycans Chondroitin sulfate proteoglycans I (biglycan) Chondroitin sulfate proteoglycan II (decorin) Chondroitin sulfate proteoglycan III (Bone specific)
	3. Bone SPARC protein - Osteonectin. (Secreted protein acidic and rich in cysteine) not bone specific
	4. Osteocalcin (Bone Gla protein Bone Specific)
	5. Osteopontin - not bone specific
	6. Bone sialoprotein - Bone specific
	7. Bone morphogenetic protein (BMPS)

TABLE - 3

FACTORS AFFECTING OSTEOBLASTS AND OSTEOCLASTS[8,9,27,87]

Stimulate Osteoblasts	Inhibit Osteoblasts
Parathyroid hormone 1,25 - Dihydroxy Cholecalciferol Thyroid hormones Growth hormone, Insulin like growth factor- Prostaglandins E_2 Transforming growth factor β Estrogens Androgens	Corticosteroids
Stimulate Osteoclasts	**Inhibit Osteoclasts**
Parathyroid hormone 1,25 - Dihydroxy Cholecalciferol Interleukin - 1,3, 6,11 Tumour necrosis factor Transforming growth factors α Granulocyte macrophage colony stimulating factor Macrophage colony stimulating factor Leukemia inhibitory factor Stem cell factor 10,11,12,18	Calcitonin Estrogens (by inhibiting IL-6 production) Transforming growth factor β Interferon α Prostaglandins E_2

TABLE - 4

BIOCHEMICAL MARKERS OF BONE METABOLISM

Markers of bone formation - serum

1. Carboxy - terminal propeptide of type I collagen
2. Amino - terminal propeptide of type I collagen
3. Serum Alkaline phosphatase - shows increased osteoblast activity.
4. Bone specific alkaline phosphatase - more accurate.
5. Osteocalcin (bone Gla protein) marker of bone synthesis.

Markers of bone resorption - serum

1. C- telopeptide of collagen cross - links
2. N - telopeptide of collagen cross - links
3. Tartarate - resistant acid phosphatase
4. Bone sialoprotein
5. Hydroxy proline

Markers of bone resorption - urine

1. Hydroxy proline
2. Hydroxy lysine
3. N - telopeptide of collagen cross links.
4. C-telopeptide of collagen cross links
5. Total and free pyridinoline
6. Total and free deoxy pyridinoline

TABLE - 5

FACTORS AFFECTING PEAK BONE MASS

1. Polymorphism of genes
2. Vitamin D
3. Estrogen receptors
4. Collagen
5. Cytokines
6. Apolipoprotein E
7. Growth factors

TABLE - 6

DIAGNOSTIC CATEGORIES OF OSTEOPOROSIS

BASED ON MEASUREMENT

Category	Definition
Normal	A value for BMD or BMC $\pm$ ISD of the young adult reference mean
Osteopenia	A value for BMD or BMC > ISD and < 2.5 SD lower than the young adult mean
Osteoporosis	A value for BMD or BMC > 2.5 SD lower than the young adult mean
Severe Osteoporosis	A value for BMD or BMC > 2.5 SD lower than the young adult mean in the presence of one or more fragility fractures

FIGURE - 3

DIAGNOSIS BASED ON BMD

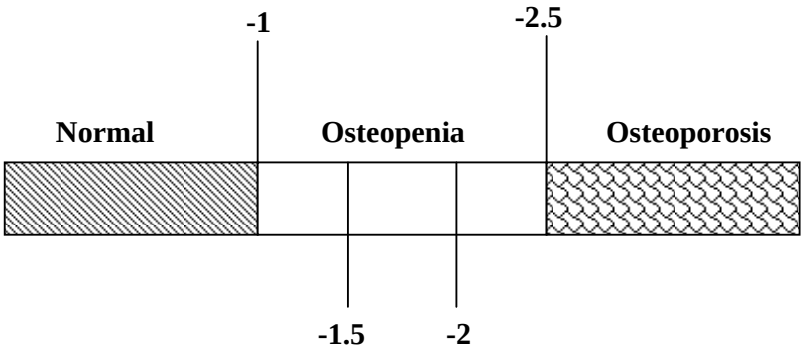


TABLE - 7

RISK FACTORS FOR OSTEOPOROTIC

FRACTURES

Sl.No.	Non Modifiable	Potentially Modifiable
1.	Personal H/o fracture	Current cigarette smoking
2.	H/o fracture in first degree relatives	Low body weight less than 127 lbs
3.	Caucasion race	Estrogen deficiency
4.	Advanced age	Early menopause <45 years or bilateral ovariectomy
5.	Gender	Prolonged premenopausal amenorrhea > 1 yr Low calcium intake Alcoholism Caffeine Impaired eye sight despite adequate correction Recurrent falls Inadequate physical activity Poor health / frailty

TABLE - 8

SECONDARY OSTEOPOROSIS

1. Endocrine Disorders
- Hyperparathyroidism
- Cushings syndrome
- Hypogonadism
- Prolactinoma
- Diabetes mellitus
- Acromegaly
- Pregnancy and lactation

2. Hematopoietic disorders
- Plasma cell dyscrasias; multiple myeloma and macroglobulinemia
- Systemic mastocytosis
- Leukemias and lymphomas
- Sickle cell disease and thalassemia minor
- Lipidosis; Gauchers disease
- Myeloproliferative disorders; polycythemia

3. Connective tissue disorders
- Osteogenesis imperfecta
- Ehlers - Danlos syndrome
- Marfans syndrome
- Homocystinuria and lysinuria
- Menkes syndrome
- Scurvy

4. Drug - induced disorders
- Glucocorticoids
- Heparin
- Anticonvulsants
- Methotrexate, cyclosporin
- Luteinising hormone - releasing hormone LHRH agonist or
- Antogenist therapy
- Aluminium containing antacids

5. Immobilisation

6. Renal Disease
- Chronic renal failure
- Renal tubular acidosis

7. Nutritional and Gastrointestinal Disorders
- Malabsorption
- Total parental nutrition
- Gastrectomy
- Hepatobiliary disease

8. Miscellaneous
- Familial dysautonomia
- Reflex sympathetic dystrophy

TABLE - 9

ADDITIONAL BIOCHEMICAL MARKERS IN BLOOD

1.	Increased creatinine level	Renal disease
2.	Increased hepatic transaminase level	Hepatic disease
3.	Increased calcium level	Primary hyper parathyroidism or malignancy
4.	Decreased calcium level	Malabsorption Vitamin D deficiency
5.	Decreased phosphorus level	Osteomalacia
6.	Increased alkaline phosphatase	Liver disease, Pagets disease, Fracture
7.	Increase ESR	Multiple myeloma
8.	Decreased albumin level	Malnutrition
9.	Decreased TSH level	Hyperthyroidism
10.	Increased testosterone level in men Decreased estrogen level in women	Hypogonadism
11.	Increased parathyroid hormone	Hyperparathyroidism
12.	Decreased 2,5 di hydroxy calciferol	Vitamin D deficiency
13.	Increased serum Iron Increased ferritin	Hemochromatosis
14.	Increased serum prolactin	Prolactinoma
15.	Increased serum IGF-I	Acromegaly

TABLE - 10

MANAGEMENT

1. Life style modifications
   - Exercise
   - Caffeine intake to be reduced
   - Smoking to be stopped
   - Alcohol to be stopped
   - Sodium intake to be reduced
2. Prevention of fall
3. External hip protectors
4. Intake of Calcium and Vitamin D3[68] to be increased
5. Bisphosphonate[69]
6. Calcitonin[70]
7. Estrogen
8. Estrogen receptor modulators - Raloxifene, Tamoxifene
9. Parathyroid hormone

TABLE - 11

DISTRIBUTION OF PROLACTIN RECEPTORS

Central Nervous System	Choroid plexus Amygdala Thalamus Hypothalamus Cerebral cortex Mid brain Olfactory bulb.
Peripheral Organs	Pituitary Gland Heart Lung Thymus Spleen Liver Pancreas Kidney Adrenal glands Uterus Skeletal muscle Skin

TABLE - 12

REPRESENTATIVE VALUES USING IMMUNORADIO METRIC ASSAY

		µg/L
Cord Blood		45 - 539
Children Tanner Stage		
1	Male	< 10
	Female	3.6 - 12
2 - 3	Male	< 6.1
	Female	2.6 - 18
4 - 5	Male	2.8 - 11
	Female	3.2 - 20
Adults	Male	3.0 - 14.7
	Female	3.8 - 23.0
Pregnancy, third Trimester		95 - 473

TABLE - 13

LOCAL REGULATORS OF PROLACTIN SECRETION

	Stimulators	**Inhibitors**
1.	Galanin	
2.	Vasoactive intestinal Polypeptide - VIP	Prolactin
3.	Endothelin - like peptides	Acetyl Choline
4.	Angio tensin II	Transforming Growth Factor β
5.	Epidermal Growth Factor	Calcitonin
6.	Basic Fibroblast Growth Factor	
7.	Luteinising hormone - releasing hormone	
8.	Cytokine - 1L-6	

TABLE - 14

REGULATORY CONTROL OF PROLACTIN

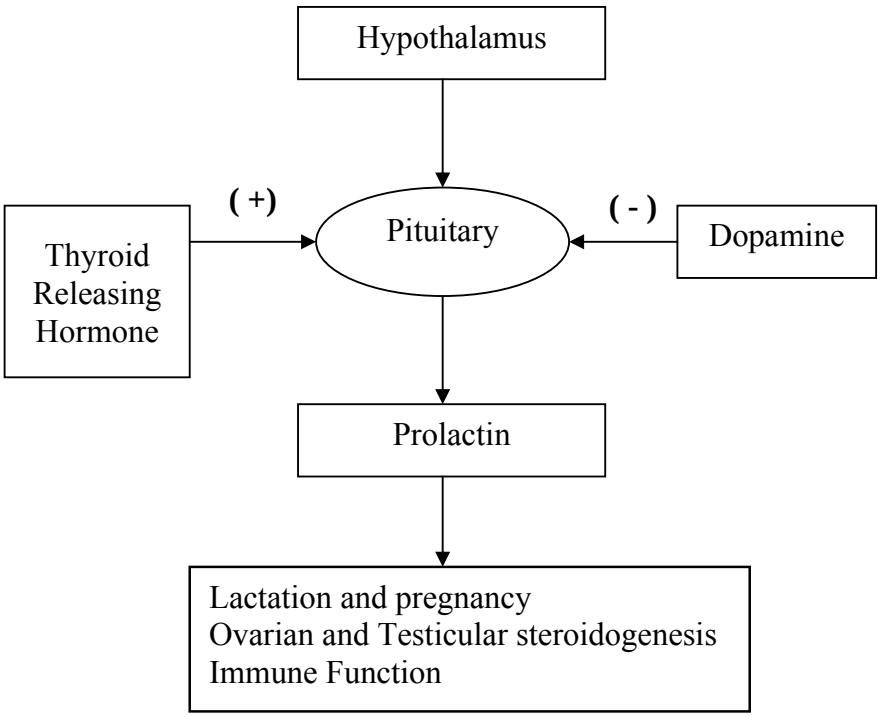


TABLE - 15

PROLACTIN STIMULATOR AND INHIBITORS

Prolactin Stimulators	Prolactin Inhibitors
Estrogens	Dopamine
Hypothyroidism	Hypo-osmolality
Oxytocin	Endothelin - 1
Serotonin	
Opiates	
Anti depressants	
H_2 blockers - histamine	
Phenothiazines	
Neuro epileptics	
Suckling	
Stress	
Exercise	
Pregnancy	
Head Injury	
ACTH	
GHRH	
GnRH	
Plasma hyperosmolality	

TABLE - 16

CLINICAL FEATURES OF HYPERPROLACTINEMIA

1.	Amenorrhea - 75% of women
2.	Oligomenorrhoea
3.	Primary amenorrhoea
4.	Infertility - 33% of women
5.	Decreased libido
6.	Impotence
7.	Premature ejaculation
8.	Erectile dysfunction
9.	Oligo spermia
10.	Galactorrhoea - 10% of women and 99% of men
11.	Osteoporosis

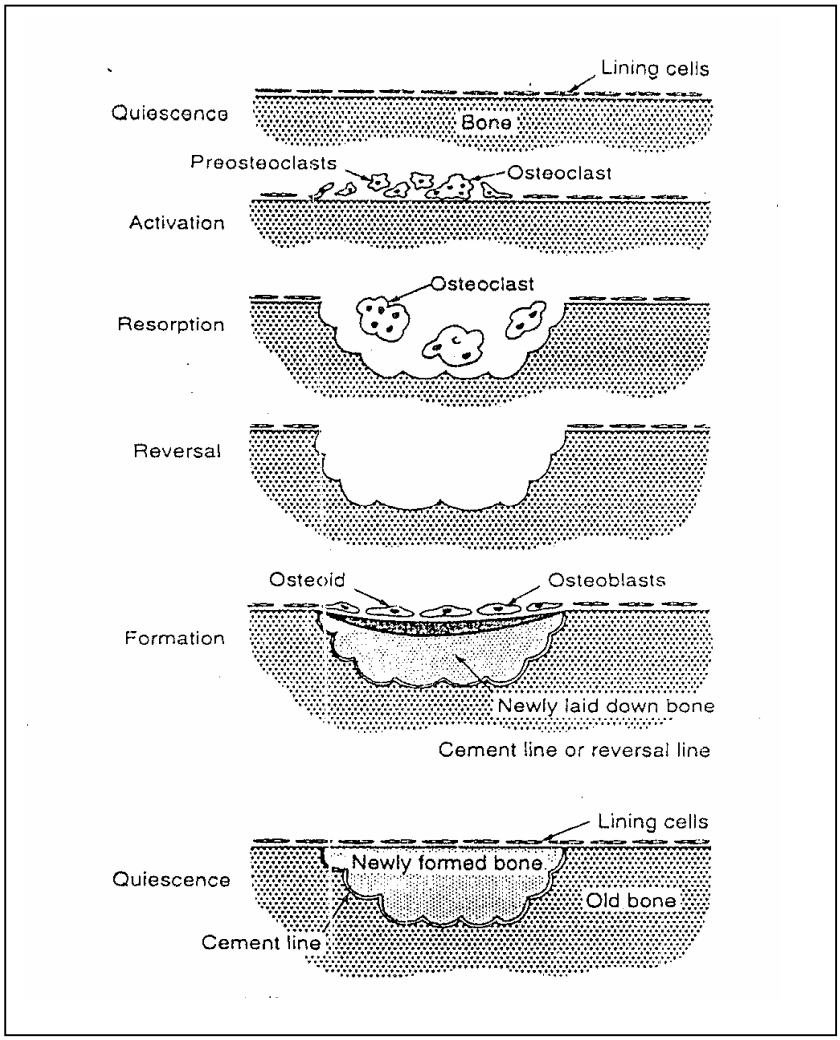
TABLE - 17

ETIOLOGY OF HYPERPROLACTINEMIA[147]

I.	Physiologic	:	Pregnancy
			Lactation
			Stress
			Sleep
			Coitus
			Exercise
II.	Pathologic	:	Hypothalamic pituitary stalk damage
			Tumours
			Craniopharyngioma
			Supra sellar pituitary mass extension
			Meningioma
			Granulomas
			Irradiation
			Trauma
			Pituitary Stalk Section
			Supra Sellar Surgery
III.	Pituitary	:	Prolactinoma
			Acromegaly
			Macroadenoma
			Idiopathic
			Plurihormonal adenoma
			Lymphocystic hypophysitis or parasellar mass
			Macroprolactinemia
			Surgery
			Trauma
IV.	Systemic Disorders	:	Chronic Renal failure
			Polycystic Ovarian disease
			Cirrhosis
			Pseudo cysis
			Epileptic seizures
			Cranial radiation
			Chest-neurogenic chest wall trauma, surgery, herpes zoster.
V.	Pharmacologic	:	Neuropeptides
			Thyrotropin releasing hormones
			PRL - releasing peptide
VI.	Drug Induced	:	Dopamine receptor blockers
	Hyper secretion		Phenothiazines, chlorpromazine, perphenazine
			Butyro phenones; haloperidols
			Thioxanthenes
			Metoclopramide
VII.	Dopamine		Methyl dopa
	Synthesis inhibitor		Reserpine
VIII.	Anti Depressants		Tricylic antidepressants

FIGURE - 1

BONE REMODELLING



A diagrammatic representation of the normal remodelling sequence in adult bone. (After Riggs and Melton, 1988).

FIGURE - 6

REGULATION OF THE HYPOTHALAMIC - PITUITARY - PROLACTIN AXIS

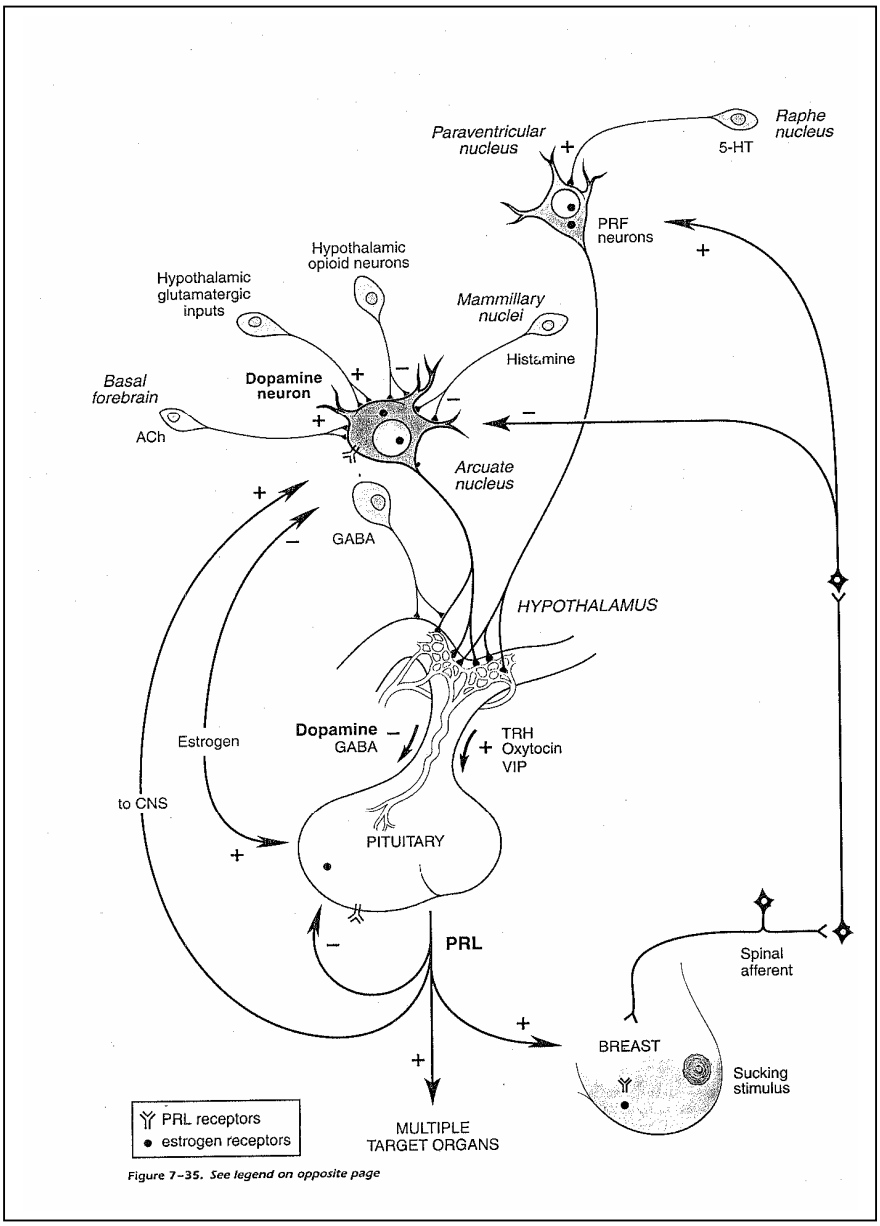


Figure 7–35. *See legend on opposite page*

COURTESY : NEUROENDOCRINOLOGY BY ROGER .D.CONE, MALCOLM J. LOW.

FIGURE - 7
RISK FACTORS FOR OSTEOPOROSIS

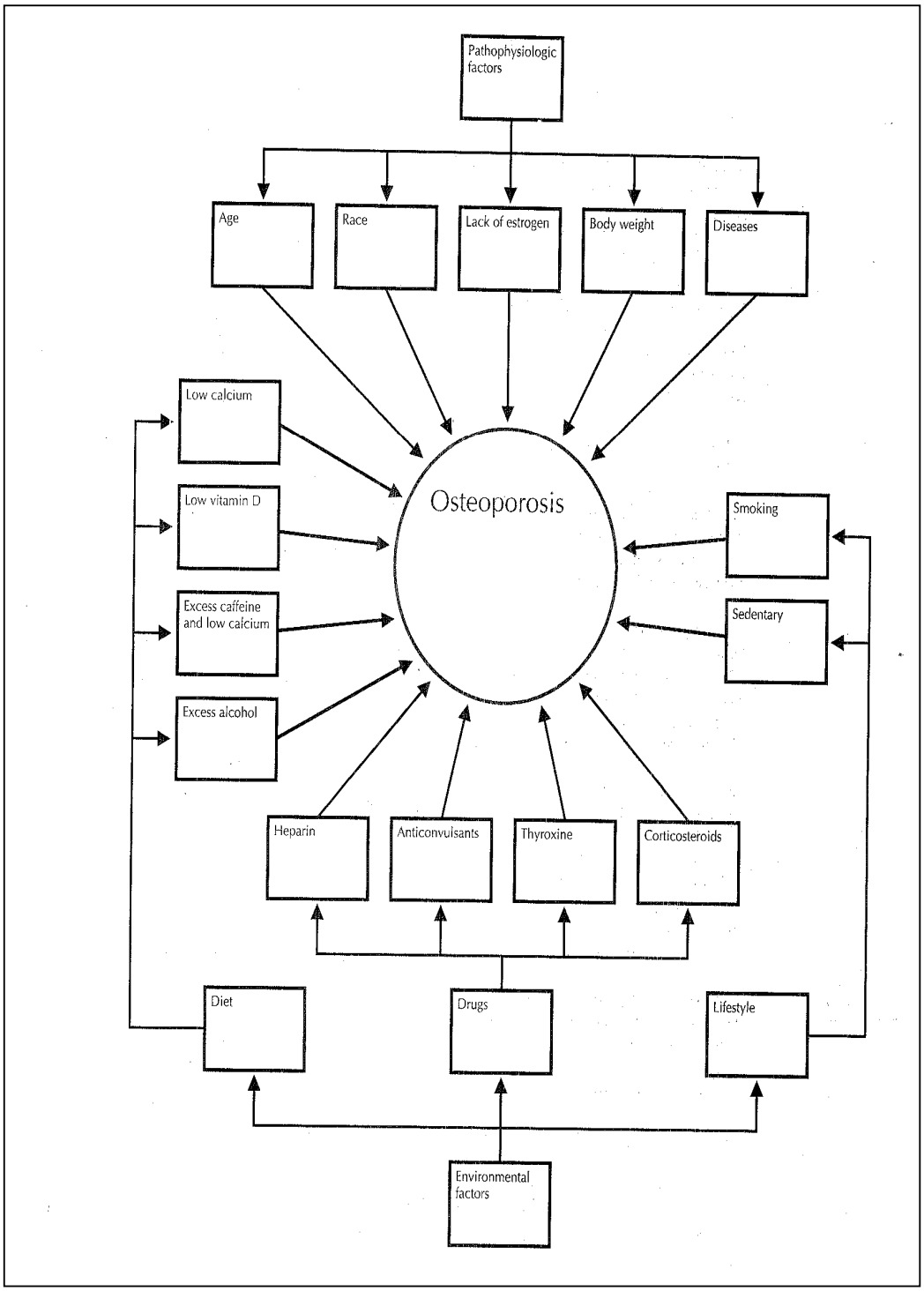
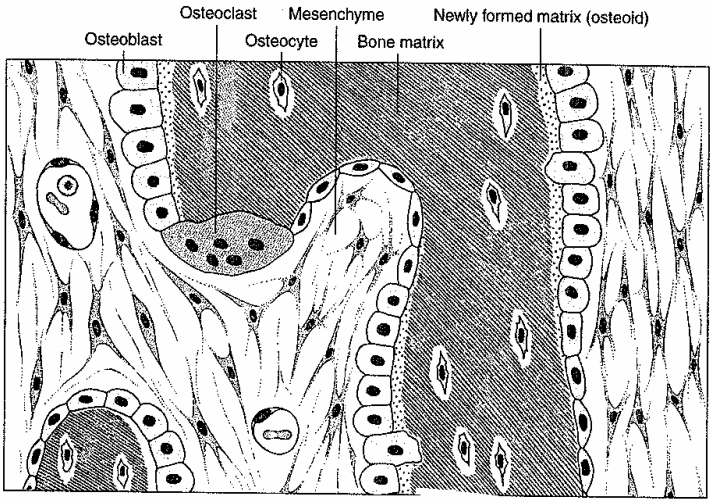
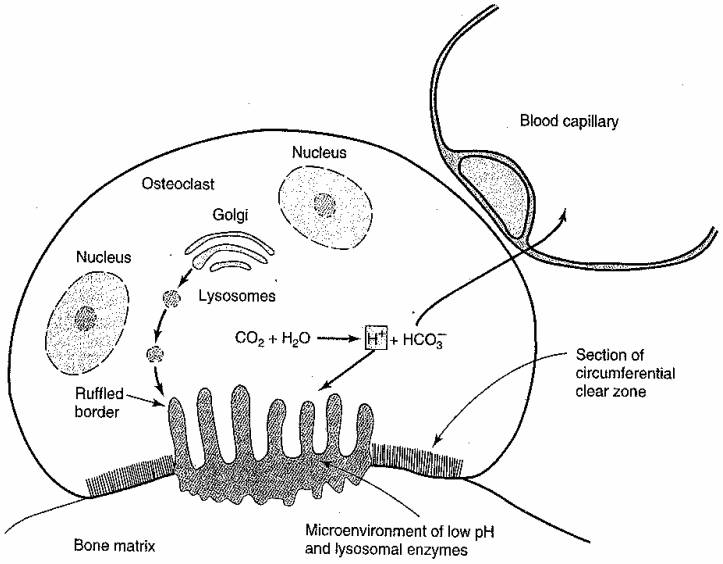


FIGURE - 2

MAJOR CELLS PRESENT IN MEMBRANOUS BONE



ROLE OF OSTEOCLAST IN BONE RESORPTION



COURTESY : HARPER'S BIOCHEMISTRY, 25th EDITION

FIGURE - 4

PROLACTIN - AMINO ACID SEQUENCE AND SECONDARY STRUCTURE

Domain Description	
Number of Fragments	1
Number of Monomers	199
Fragments	Fragment 1: On Chain A from Residue 1 to Residue 199

Sequence and Secondary Structure

Key: = extended strand, = turn, TCKNGQTNCY = disulfide bond
= alpha helix, = 310 helix, = pi helix,
Greyed out residues have no structural information

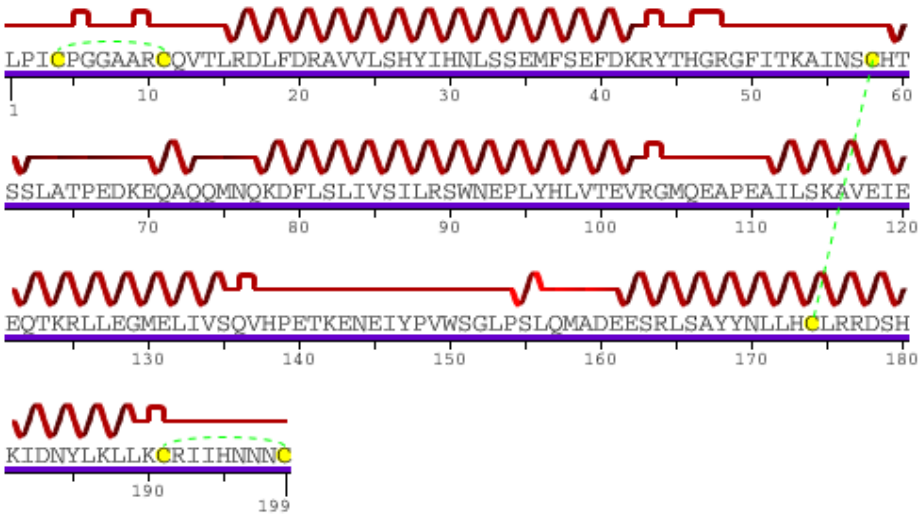


FIGURE - 5

THREE DIMENSIONAL STRUCTURE OF PROLACTIN

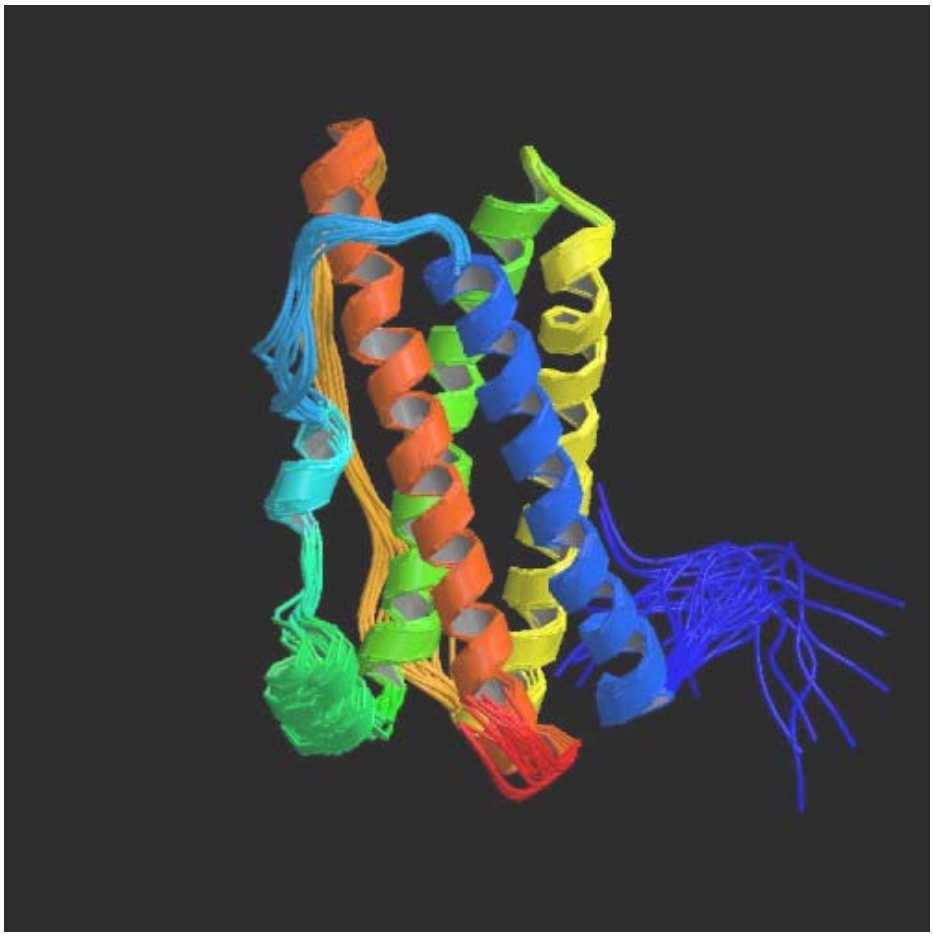
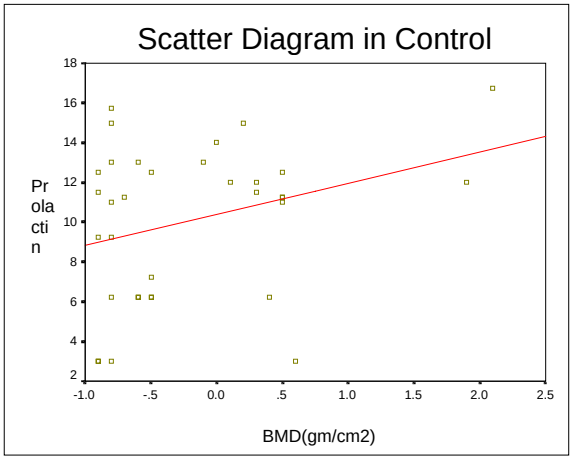


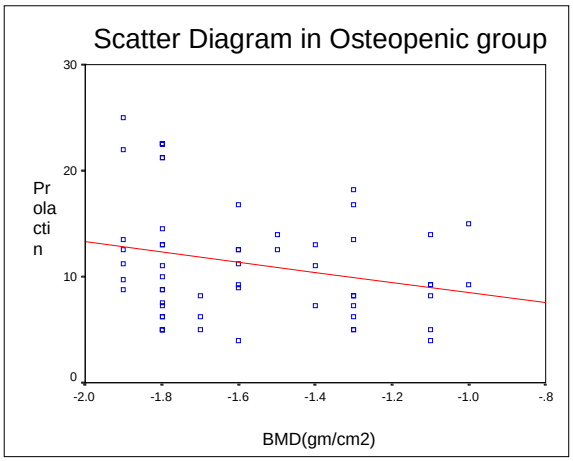
FIGURE - 3(a)

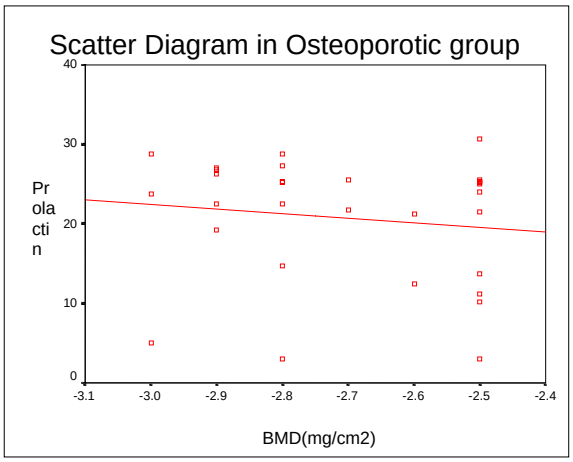
BONE DENSITOMETRY
ULTRASOUND EVALUATION OF BONE

CORRELATION BETWEEN PROLACTIN AND BMD IN DIFFERENT GROUPS

Figure - 9 (a,b,c)







BOX AND WHISKER PLOT

Figure - 10 (a)

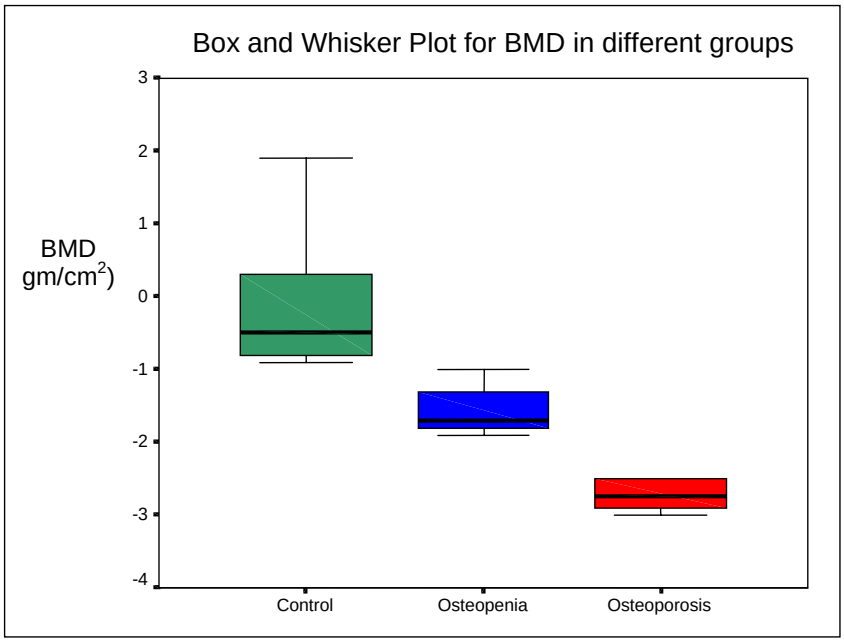


Figure - 10 (b)