"A COMPARATIVE STUDY TO CORRELATE THE LEVELS OF SERUM ZINC, CALCIUM, PHOSPHOROUS AND ALKALINE PHOSPHATASE IN POST MENOPAUSAL WOMEN WITH OSTEOPOROSIS: DIABETICS VERSUS NON-DIABETICS".

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Endorsement by the HOD, Principal/Head of the Institution

This is to certify that the dissertation entitled **"A Comparative Study To Correlate The Levels Of Serum Zinc, Calcium, Phosphorous And Alkaline Phosphatase In Post Menopausal Women With Osteoporosis: Diabetics Versus Non-Diabetics"** is a bonafide research work done by **REG. NO : BC0110002.**

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LIST OF ABBREVIATIONS USED

DDD	-	defined daily dosages
BMD	-	Bone Mineral Density
DEXA	-	Dual Energy X-Ray Absorptiometry
Zn	-	Zinc
Ca	-	calcium
P	-	Phosphorous
Pi	-	Inorganic phosphate
ALP	-	Alkaline Phosphatase
L	-	Litre
g/cm^2	-	gram per centimetre square
µg/dL	-	Microgram per decilitre
mg/dL	-	Milligram per decilitre
mmol/L		Millimole per litre
g/cm^2	-	Gram per centimetre square
r	-	Correlation coefficient
CI	-	Confidence interval
p	-	Probability value
SPSS	-	Statistical package for social sciences
g or gm	-	Gram
hrs	-	hours
T1DM-	-	Type 1 Diabetes Mellitus
T2DM	-	Type 2 Diabetes Mellitus
IDDM	-	Insulin Dependent Diabetes Mellitus

NIDDM	-	Non Insulin Dependent Diabetes Mellitus
WHO	-	World Health Organization
NOF	-	National Osteoporosis Foundation
SD	-	Standard Deviation
PTH	-	Parathyroid Harmone
BMI	-	Body Mass Index
HRT	-	Hormone Replacement Therapy
PPARγ	-	Peroxisome proliferator -activated receptor
PICP	-	Procollagen C-terminal peptide
25(OH)D	-	25 Hydroxy Vitamin D
$1,25(OH)_2D$	-	1, 25- Dihydroxy Vitamin D
OC	-	Osteocalcin
DPD	-	deoxypyridinoline
IOF	-	International Osteoporosis Foundation
IGF-1	-	Insulin Like Growth Factor 1
VEGF	-	Vascular endothelial growth factor
TSH	-	Thyroid-stimulating hormone
AAS	-	Atomic absorption spectrometry
AGEs	-	Advanced glycation end products
SHBG	-	Sex Hormone Binding Globulin
RANKL	-	receptor activator of nuclear factor- k B ligand
RANK	-	receptor activator of nuclear factor- k B
OPG	-	Osteoprotegerin

ABSTRACT

Background and Objectives:

Osteoporosis in postmenopausal women is a public health problem associated with significant morbidity and mortality. Patients with Diabetes mellitus are at increased risk of osteoporosis related fractures. Low BMD has been consistently observed in T1DM, but it is not so in T2DM which is associated with normal or higher BMD levels. Zinc is an essential trace element having a role in collagen metabolism and its levels are shown to be altered in DM. This study was undertaken to compare the levels of serum zinc with other bone parameters like serum calcium, phosphorous and alkaline phosphatase between diabetic and non diabetic postmenopausal women to see whether these parameters are altered in diabetes and if they influence BMD in postmenopausal women with diabetic osteoporosis.

Materials and methods

30 diabetic and 30 age matched non-diabetic DEXA confirmed cases of postmenopausal women with osteoporosis were included in the study. Serum zinc, calcium, phosphorous and alkaline phosphatase levels were estimated in diabetic women with osteoporosis and the levels were compared with non diabetic women. Serum zinc was analysed in AAS and other parameters using standard biochemical methods. Mean and Standard Deviation of the parameters of the two groups were computed and compared by unpaired student's *t* test. Relationship between the variables was measured by Karl Pearson's correlation co-efficient. A statistical significance is set at 5% level of significance ($p<0.05$).

Results

All the 30 diabetic women belonged to type 2 category. The mean serum zinc, calcium, phosphorous and alkaline phosphatase levels for diabetics were 62.4±13.35 µg/dL, 8.22±1.13 mg/dL, 4.53±0.83 mg/dL and 92.47±22.65 IU/L and for non diabetics 68.2±13.86 µg/dL, 8.4±1.27 mg/dL, 4.32±0.92 mg/dL and 87.23±14.66 IU/L respectively. The mean serum zinc and calcium levels were lower and mean serum phosphorous and ALP levels were higher in case of diabetics as compared to non diabetics but there was no significant difference between the values in the two groups ($P>0.05$). Diabetics had a significantly lower mean age at menopause (46±1.78 years) compared to non diabetics (47.20±1.42 years) $p<0.01$. Diabetics had a higher mean BMD (0.83±0.06 g/cm^2) than non- Diabetics(0.79±0.09 g/cm^2) but it was not statistically significant ($p=0.0661$). However the t-scores were significantly higher in case of diabetic(-2.84±0.42) women compared to non diabetics(-3.22±0.74) $p<0.05$. BMD in diabetics showed a significant positive correlation with BMI ($p<0.05$), Zinc ($p<0.001$) and calcium ($p<0.05$) while it showed a significant positive correlation with Zn ($p<0.01$) and Ca ($p<0.001$) and a significant negative correlation with P ($p<0.05$) and ALP ($p<0.001$) in non- diabetics. Diabetics had a significantly higher weight ($p<0.001$) and BMI ($p<0.05$) than non-diabetics. BMI of diabetics showed a significant positive correlation with zinc ($p<0.05$).

Interpretation and conclusion:

Our study suggests that serum zinc, calcium, phosphorous and alkaline phosphatase are not significantly altered in type 2 diabetic postmenopausal women with osteoporosis as compared to non diabetic women with postmenopausal osteoporosis when overt nephropathy is excluded. Type 2 DM mellitus women may

be at risk of early menopause. High BMI in type 2 DM may contribute to high BMD and may be a protective factor against zincuria and hypercalciurea as well.

Medications with oral hypoglycaemic drugs and/or insulin may reduce the zincuria and hypercalciurea that may accompany diabetes. However to what extent it can prevent diabetes related fractures cannot be told as fractures in type 2 DM are attributable to poor bone quality rather than BMD. More studies are required in this arena on the role of zinc in postmenopausal women with Diabetic Osteoporosis.

Key words: osteoporosis, menopause, diabetes, serum zinc, serum calcium, serum phosphorous, serum alkaline phosphatase.

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INTRODUCTION

Osteoporosis, which literally means "porous bone", is a disease in which the density and quality of bone are reduced. The loss of bone occurs "silently" and progressively with often no symptoms until the first fracture occurs. It is the commonest metabolic bone disease in clinical practice and is a major public health problem as commonly it is underdiagnosed.[1] Osteoporosis is defined as a disease characterised by low bone mass and micro architectural deterioration of bone tissue leading to increased bone fragility and therefore to an increase in fracture risk.[2] Dual X-ray absorptiometry (DXA) is considered the method of choice for measurement of low bone mineral density (BMD) associated with osteoporosis.[3]

30-50% of women and 15-30% of men have lifetime risk for osteoporosis and osteoporosis related fractures all over the world .[4] Conservative estimates suggest that 20% of Indian women and about 10-15 % of Indian men would be osteoporotic by 2015. The total affected Indian population would, therefore, be around 25 million.[5] Osteoporosis is predominantly a disease of the women.[1] In their lifetime, women lose approximately 50% of their trabecular bone and 30% of all postmenopausal women eventually will have osteoporotic fractures.[6]

75% of total bone loss seen during the postmenopausal period is due to estrogen deficiency (52-66%) and aging (34-48%).[7] The risk of nutritional disturbances, in particular trace element and vitamin deficiencies, is high during menopause. The elements have catalytic functions in organic bone matrix synthesis.[2]Zinc (Zn) is an essential mineral that is a component of more than 200 enzymes and is known as to be necessary for normal collagen synthesis and

mineralization of bone.[8] Calcium ions, inorganic orthophosphate ions and hydroxyl ions are the principal chemical constituents of mineral phase of bone.[9] Zinc acts as a cofactor for alkaline phosphatase (ALP), bone forming metalloenzyme which induces the increase in DNA synthesis stimulating bone growth.[10] Reduced serum or plasma zinc concentrations and increased urinary zinc excretion have been reported in women with osteoporosis.[11]

Although osteoporosis traditionally has not been listed as a complication of diabetes, patients with Diabetes are among those at increased risk for this disease.[12] Glucose is the principal energy source for osteoclasts and hyperglycemia leads to non enzymatic glycosylation of various bone proteins including type I collagen, which may impair bone quality.[13] While the relationship between type 1 diabetes mellitus and osteoporosis has been well documented in literature, its presence in type 2 diabetes mellitus has not been well established. Studies have shown normal or higher BMD values in type 2 DM.[14] Zinc plays an important role for insulin action with several investigators showing the perturbation of zinc metabolism in diabetics.[15] It has not been clearly elucidated whether zinc deficiency is a consequence of hyperglycemia or alternatively, whether zinc deficiency contributes to the pathogenesis of diabetes.[16] Morever zinc nutritional deficiency is a global health problem. Half of world's population does not get enough zinc from their food programs especially in developing countries.[17]

Biochemical bone markers are non-invasive, less expensive diagnostic tools for diagnosis and treatment follow-up of metabolic bone diseases and can be repeated often. Clinical questions that may be answered by these markers are in relation to diagnosing osteoporosis, identifying "fast bone losers" and patients at high risk of

fracture, selecting the best treatment for osteoporosis, and providing an early indication of the response to treatment.[18]

Studies have shown increased risk of fractures and bone abnormalities in DM patients. While the risk is associated with low BMD in type 1DM, it is not so in type 2DM where studies have reported normal or high BMD values. [6, 12, 13, 14, 19, 20] Zinc is an essential trace element having an important role in collagen metabolism and its levels are altered in osteoporosis.[21, 22, 23] These effects are further enhanced by the presence of zincuria and hypercalciurea in diabetes.[11, 24, 25] Studies have been done on serum zinc levels and other bone parameters between diabetics regardless of being menopausal and normal controls or between osteoporotics and normal controls. There has been no study which has compared these parameters between diabetic and non-diabetic postmenopausal women with osteoporosis. Hence this study is undertaken to compare the levels of serum zinc, calcium, phosphorous and alkaline phosphatase between diabetic and non diabetic postmenopausal women with osteoporosis to see if these parameters are altered in diabetes and if they influence bone mineral density in diabetic osteoporosis.

AIM AND OBJECTIVES

AIM

This study is undertaken to compare the levels of serum zinc with other bone parameters like serum calcium, phosphorous and alkaline phosphatase between diabetic and non diabetic postmenopausal women to see if these parameters are altered in diabetes and if they influence bone mineral density in diabetic osteoporosis.

OBJECTIVES

1. To estimate serum Zinc, Calcium, Phosphorous and Alkaline Phosphatase levels in Post Menopausal women with Osteoporosis.
2. To compare the above parameters between Diabetic and Non- Diabetic Post Menopausal women with Osteoporosis.

REVIEW OF LITERATURE

BONE STRUCTURE AND METABOLISM

Bone is a dynamic tissue that is remodeled constantly throughout life. Bone is generally classified into two types. Cortical bone is a dense and strong bone found primarily in the shaft of long bones. Trabecular bone is more porous or weak and typically occurs at the ends of long bones and within the interior of vertebrae and flat bones.[26] Osteogenesis, the process of bone formation, involves 3 main steps: 1) the production of the extracellular organic matrix – osteoid; 2) the mineralization of the matrix to form bone; and 3) bone remodeling by resorption and deposition. The cellular activities of osteoblasts, osteocytes, and osteoclasts are necessary for this process. Current research indicates a role for zinc in every step of bone metabolism.[10]

Osteoid is the unmineralized organic matrix secreted by osteoblasts. It is composed of 90% type I collagen and 10% ground substance. The mineralization of osteoid by inorganic mineral salts provides bone with its strength and rigidity. The inorganic content of bone consists primarily of calcium phosphate and calcium carbonate, with small quantities of magnesium, fluoride, and sodium.[27] The ionic forms of calcium and phosphorus combine to form calcium phosphate. First as dicalcium phosphate and on through sequential synthetic reactions to become tricalcium phosphate or hydroxyapatite. During the process of bone hardening or aging, the Ca:P ratio gradually increases from 1:1 to 1.67.[28]

Bone Remodeling

In a typical remodeling cycle, resorption takes 7–10 days, whereas formation requires 2–3 months. Overall; 10% of bone is replaced each year.[18] After reaching

peak bone mass at the age of 25 to 30 years, remodeling is associated with an imbalance between formation and resorption, resulting in a mean bone loss. Key determinants of the rate of bone remodeling are parathyroid hormone (PTH), vitamin D, and sex hormones.[26]

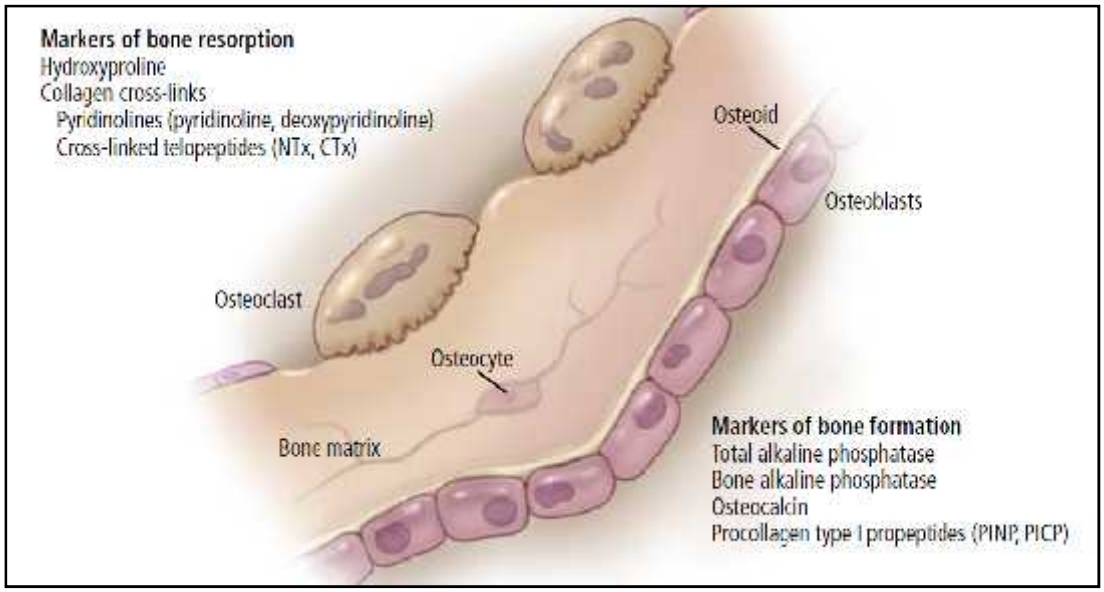
At the cellular level, bone remodeling is a complex interplay in which osteoblasts, osteoclasts, and osteocytes work together. Basically, osteoclasts resorb bone and osteoblasts replace bone by forming an osteoid protein matrix that subsequently mineralizes, whereas osteocytes and their canalicular network serve as sensors to adjust bone response to mechanical stimuli. On their surface, osteoblasts constitutively express the receptor activator of nuclear factor- k B ligand (RANKL). When binding to its receptor (receptor activator of nuclear factor- k B [RANK]) on the surface of preosteoclast cells, the latter differentiate into mature and activated osteoclasts. Additionally, osteoblasts but also stromal cells secrete a soluble decoy receptor, osteoprotegerin (OPG), which blocks the RANK/RANKL interaction, thereby acting as a physiologic regulator of bone turnover.[29]

Imbalance between RANKL and OPG results in excessive activity of osteoclasts and is considered a major cause of osteoporosis.[30] Another pathway that is less well understood is the Wnt/ b -catenin signaling cascade. Wnt signaling activates osteoblasts and bone formation, whereas reduced Wnt signaling may lead to osteoporosis.[31]

Bone markers can be divided into markers of bone formation derived from osteoblasts and markers of bone resorption representing degraded products of osteoclastic activity. Alkaline phosphatase is a marker for osteoblasts and its cellular levels correlate with rates of bone formation. Bone turnover is a function of age and

levels of bone markers reflect the state of bone remodeling at any given point of time. Their primary use is for monitoring excessive bone remodelling and the response to treatment of osteoporosis.[32]

Figure 1.[33] Markers of bone formation and resorption



ZINC

Zinc (Zn) is an essential mineral that is a component of more than 200 enzymes and is known as to be necessary for normal collagen synthesis and mineralization of bone. It is an essential cofactor for enzymes involved in synthesis of various bone matrix constituents, and plays a particularly important role in the regulation of bone deposition and resorption.[10]

HISTORY OF ZINC[34]

The importance of zinc was first documented for Aspergillus niger (Raulin, 1869). It took over 75 years to realize that zinc is also an essential trace element for rats (Todd et al., 1935), and an additional 30 years went by before it was recognized that this was also true for humans (Prasad et al., 1963; Sandstead et al., 1967).

Following the initial observation that zinc is required for the catalytic activity of carbonic anhydrase (Keilin and Mann, 1940), it became clear that zinc is a component of more than 300 enzymes from all six enzyme classes (Vallee and Falchuk, 1993). Bioinformatic estimates report that 10% of the human proteome contain zinc binding motives (Andreini et al., 2006). Zinc has diverse biological functions in enzymatic catalysis (Auld, 2001), redox regulation (Maret, 2006), cellular signal transduction (Beyersmann and Haase, 2001), the immune system (Wellinghausen et al., 1997), and neurons (Frederickson et al., 2005).

PREVALENCE OF ZINC DEFICIENCY[34]

The prevalence of zinc deficiency is estimated to be high, with billions of people at risk, in particular in the developing world (Maret and Sandstead, 2006). In the United States, the Third National Health and Nutrition Survey showed that zinc uptake decreases with age and only 42.5% of the participants who were 71 years or older had an adequate zinc intake (Briefel et al., 2000).

ROLE OF ZINC IN BONE METABOLISM

Zinc acts as a cofactor, stimulating protein synthesis needed for organic matrix formation. It acts as a cofactor for alkaline phosphatase, bone forming metalloenzyme. Recent studies indicate that zinc supplementation can have direct stimulatory effects on alkaline phosphatase and osteocalcin.[10]

Zinc status may play a substantial role in indicators of bone mineralization like bone mineral content, bone mineral density, height and weight. In a 2-year French study of 139 healthy premenarcheal girls, zinc status was significantly related with bone mineral density increase, suggesting that during puberty onset, zinc is important

to normal growth and bone mineralization. In another study, a group of 396 men, age 45-92, men in the lowest plasma zinc quartile had significantly lower bone mineral density. These results were independent of age or body mass index.[10]

Zinc increases the synthesis of insulinlike growth factor 1 (IGF-1) which is critical for the regulation of bone formation, resorption and calcium homeostasis. Decreased IGF-1 lead to more rapid loss of calcium from bone with aging and the subsequent development of osteoporosis. Study by Devine and colleagues in 119 postmenopausal women indicated that zinc intake strongly correlates with IGF-1 blood levels.[10]

Zinc stimulates proliferation of osteoblasts, inhibits osteoclasts and prevents PTH-induced bone resorption. Aminoacyl-tRNAsynthetase, which is the first step in protein biosynthesis, is directly activated by zinc in osteoblasts.[10]

ROLE OF ESTROGEN IN BONE METABOLISM

Oestrogen is an important physiological regulator of osteoblast activity. It has a direct stimulatory effect on bone DNA, promoting the proliferative ability of bone protein content. It plays a major role in bone remodelling.[10] Marrow cells as well as bone cells express Estrogen receptors α and β. Loss of estrogen increases production of RANKL and may reduce production of osteoprogerin, increasing osteoclast recruitment. Estrogen increases production of IGF-1, TGF-β and procollagen synthesis, increases osteoblast longitivity and decreases the lifespan and activity of osteoclasts. Its deficiency would hence result in bone loss.[35, 36, 37]

ROLE OF ZINC AND OESTROGEN IN BONE REMODELING

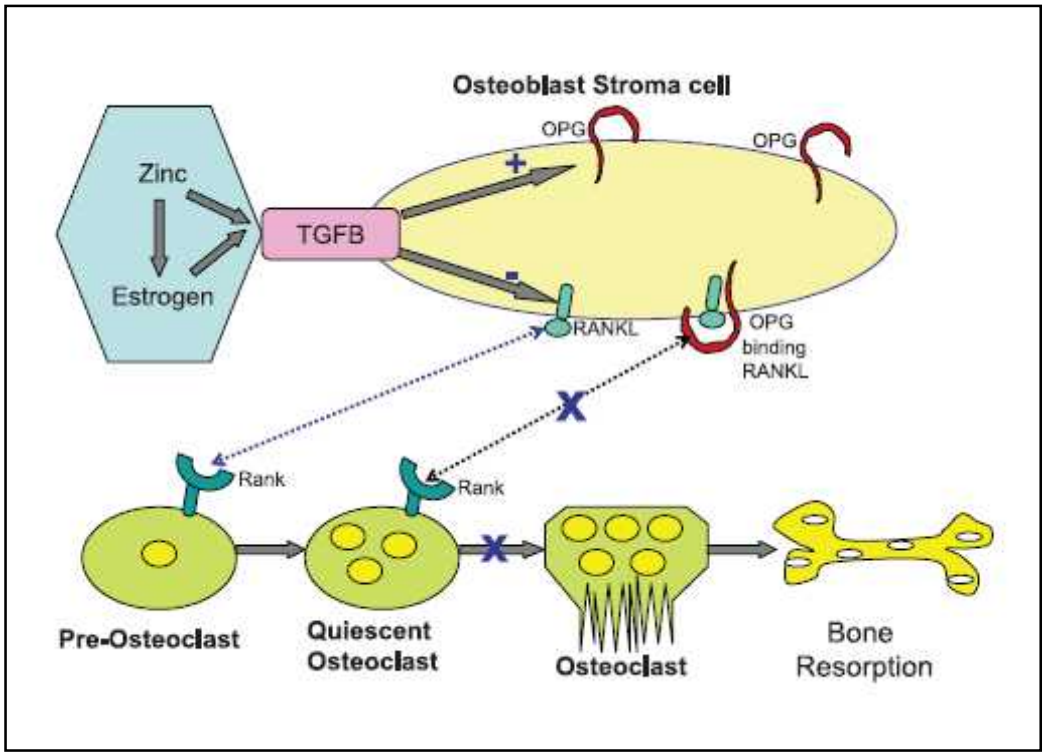


Figure 2:[10] Effect of zinc coupled with estrogen on osteoclast and osteoblast signaling.

INTERACTION OF ZINC WITH OESTROGEN

Zinc stimulates bone protein synthesis directly or by enhancing the effect of estrogen to increase DNA content. Menopausal woman have a high level of SHBG(Sex hormone binding globulin). Zinc binding to SHBG has been shown to reduce its affinity for estrogens and hence may increase the bioavailability of estrogen. Some studies have shown that estrogen decreases the amount of zinc loss from bone via urinary zinc excretion.[10] Herzberg et al[38] showed significant decrease in urinary zinc excretion in postmenopausal osteoporotic women after 3 months of estrogen replacement therapy.

OSTEOPOROSIS

DEFINITION

Osteoporosis is defined as a disease characterised by low bone mass and micro architectural deterioration of bone tissue leading to increased bone fragility and therefore to an increase in fracture risk.[2] The World Health Organization (WHO) operationally defines osteoporosis as a bone density that falls 2.5 standard deviations (SD) below the mean for young healthy adults of the same gender—also referred to as a T-score of –2.5.[35]

EPIDEMIOLOGY OF OSTEOPOROSIS

Incidence and prevalence of osteoporosis

1 in 3 women over 50 and 1 in 2 over 60 will suffer a fracture due to osteoporosis.[39] Approximately 11% of men and 27% of women aged 60 years or over are osteoporotic.[40] The highest risk of hip fractures are seen in Norway, Sweden, Iceland, Denmark and the USA. Approximately 1.6 million hip fractures occur each year worldwide. 1 out of 4 hip fractures occur in Asia and Latin America. The annual incidence rate of osteoporotic fractures in women is greater than the combined incidence rates of heart attack, stroke and breast cancer.[39] Based on 2001 census, approximately 163 million Indians are above the age of 50; this number is expected to increase to 230 million by 2015. Even conservative estimates suggest that of these, 20 per cent of women and about 10-15 per cent of men would be osteoporotic. The total affected population would, therefore, be around 25 million. If the lower bone density is shown to confer a greater risk of fracture, as is expected, the figure can increase to 50 million.[5] In most Western countries, while the peak incidence of osteoporosis

occurs at about 70-80 years of age, in India it may afflict those 10-20 years younger, at age 50-60.[1]

Burden of osteoporosis

According to WHO, osteoporosis is second only to cardiovascular disease as a global healthcare problem. International Osteoporosis Foundation (IOF) estimates that the annual direct cost of treating osteoporosis fractures of people in the workplace in the USA, Canada and Europe alone is approximately USD48 billion. The worldwide cost burden of osteoporosis is forecast to increase to USD131.5 billion by 2050. [39]

HISTORY[41]

Osteoporosis has haunted women since the dawn of history— Egyptian mummies from 4,000 years ago have been found with the telltale dowager's hump.

In eighteenth century English surgeon John Hunter discovered that as new bone is laid down in the body, old bone is destroyed, or resorbed. This process is now known as remodeling. Osteoporosis was first recognized by an English surgeon named Astley Cooper, during the early eighteen hundreds. He noticed that older individuals were at an increased risk of fracture due to lower bone density. In the 1830s by the French pathologist Jean Georges Chretien Frederic Martin Lobstein, noticed that some patients' bones were riddled with larger than normal holes, and he coined the term osteoporosis (porous bone).

In 1940 Albright proposed his revolutionary hypothesis: Estrogen triggers the buildup of calcium reserves in bone, from which calcium can be released into the bloodstream during pregnancy and lactation to serve the needs of the fetus and newborn. The sharp reduction in estrogen that occurs with menopause causes a loss of

bone. Albright named the resulting condition postmenopausal osteoporosis. He also showed that regular injections of estrogen reversed this calcium imbalance. At about the same time, Albright's mentor, the Viennese pathologist Jacob Erdheim, noted that the parathyroid glands—pea-sized glands in the neck—were enlarged in three patients with a condition known as osteomalacia. Erdheim removed the parathyroid glands in rats, following which their teeth lost calcium. In 1925 James B. Collip independently isolated the same active extract and showed that it boosted the level of calcium in the blood. The active compound was named parathyroid hormone.

In the 1960s, Herbert Fleisch discovered the potential usefulness of pyrophosphates in treating various bone disorders, including osteoporosis. Some of these pyrophosphate-mimicking drugs, known as bisphosphonates were shown to suppress bone resorption. Two of these drugs, alendronate (Fosamax) and risedronate (Actonel), have been on the market since 1996 and 2000, respectively, as treatments for postmenopausal osteoporosis.

MECHANISMS OF OSTEOPOROSIS

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, or (3) inadequate replacement of lost bone as a result of decreased bone formation.[36]

1. Inadequate Peak Bone Mass and Strength

Peak bone mass is the bone tissue and the outcome of the skeletal maturation.[42] While the major determinants of peak bone mass are genetic, major factors like nutrition, particularly of calcium, physical activity, and intercurrent

illnesses also play an important role. Estrogen also plays a critical role in regulating bone remodeling and in determining the time of epiphyseal closure.[36] A recent study from India shows that peak bone mineral density is attained in healthy males at the age of 25 years and in females at 28 years.[43]

2. Increased Resorption

Peak bone mass is probably achieved in the third decade. The time required for resorption is much shorter than that for formation; hence, any increase in the number of resorption sites will result not only in decreased bone mass, but also in microarchitectural changes.[36]

3. Decreased Bone Formation

Skeletal bone mass increases during puberty and young adult life, even though the rate of resorption is high. With age, the amount of bone formed decreases due to an age-related decline in skeletal growth factors, decreased Growth hormone and IGF-1 levels.[36]

NUTRITION

Calcium nutrition

Peak bone mass may be impaired by inadequate calcium intake during growth among other nutritional factors (calories, protein, and other minerals), thereby leading to increased risk of osteoporosis later in life. Insufficient calcium intake in adults leads to secondary hyperparathyroidism and an increase in the rate of bone remodeling to maintain normal serum calcium levels.[35]

Vitamin D

Vitamin D insufficiency is more prevalent in the elderly; those living in northern latitudes; individuals with poor nutrition, malabsorption, or chronic liver or renal disease. Dark-skinned individuals are also at high risk.[35]

Other dietary factors

Zn stimulates bone protein synthesis and bone formation in tissue cultures. An analysis of NHANES III data found that 35%–45% of adults aged 60 years or older had zinc intakes below the estimated average requirement of 6.8 mg/day for elderly females.[44] Low protein, phosphate and magnesium intake may also contributes to bone loss.[36,5] There is a positive association between vitamin C, needed for collagen metabolism, and bone mass. Vitamin K may be required for bone metabolism while excess vitamin A may cause bone loss. Elevated serum homocysteine, may also be a risk factor.[45] Keramat et al showed pure vegetarianism and red meat consumption more than 4 times a week to be risk factors for osteoporosis.[46] Regular consumption of milk, almonds and fruits act as protective factors.[5]

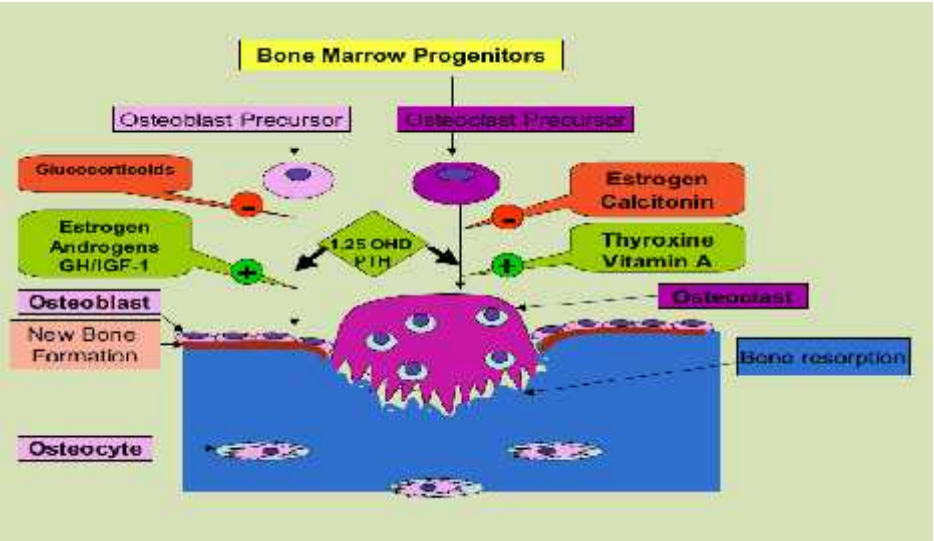


Figure 3:[32] Factors affecting the development and functions of osteoblasts and osteoclasts.

RISK FACTORS FOR OSTEOPOROSIS

NON MODIFIABLE RISK FACTORS

Genetic factors

Peak bone mass is lower in individuals who have a family history of osteoporosis.[7] Genetic determinants are responsible for up to 85% of the variation in peak bone mass, bone turnover and fracture risk. Polymorphisms of candidate genes, including vitamin D and estrogen receptors, collagen, cytokines, neurotransmitters, and growth regulators , may be responsible.[36]

Body Habitus

Low body mass index (BMI) (less than 20) is a strong independent risk factor for osteoporosis and fracture. During the Framingham Osteoporosis Study, women who gained weight also gained BMD and vice versa.[47]10% weight loss can induce about 2% bone loss. The impact is higher in case of intense and rapid weight loss compared to moderate decrement in weight and for longer periods.[48]

Aging.

With aging, the balance between resorption and formation is offset such that more bone is removed than replaced. There is decreased ability to aromatize androgens to estrogens resulting in estrogen deficiency and hence bone loss. Ageing also results in reduced renal calcium conservation efficiency, decreased vitamin D production and its renal activation.[7] Aging also leads to accumulation of AGEs interfering with osteoblast differentiation and production of collagen and Osteocalcin. Their formation is further increased in Diabetes.[49]

Age related osteoporosis is also shown to be accompanied by an increase in marrow adipose tissue. Osteoblasts and adipocytes share a common progenitor mesenchymal stem cells. Peroxisome proliferator -activated receptor gamma (PPARγ) is a transcription factor involved in adipocyte differentiation. PPARγ induces adepogenesis over osteoblastogenesis in pluripotent cells.[14]

ABO blood types

ABO blood types may be a significant contributory factor in the development of osteoporosis in postmenopausal women. Choi et al. showed the prevalence of Osteoporosis to be high in blood type AB and to be significantly lower in O blood type.[50]

Gender

Young men have bone mass levels approximately 5% to 10% higher than that of young women. Sex differences in peak bone mass may be related in part to sex differences in periosteal bone apposition at puberty, with higher androgen levels resulting in greater periosteal apposition in adolescent boys and estrogen inhibiting this apposition in adolescent girls.[45]

Ethnicity

Differences in VDR gene polymorphism in different races and ethnic differences in body weight could account for differences in bone mass.[5] Indians have a lower bone density than their North American and European counterparts.[51] Caucasian and Asian women are at highest risk.[5] Osteoporotic fractures usually occur 10-20 years earlier in Indian/Pakistani women as compared with western Caucasian

counterparts. It could be due to lower BMD and shorter hip axis in Indian/Pakistani women.[52]

MODIFIABLE RISK FACTORS

Physical activity

Inactivity, such as prolonged bed rest or paralysis, results in significant bone loss. Athletes have higher bone mass than the general population. Fracture risk is lower in rural communities and in countries where physical activity is maintained into old age. More active individuals are less likely to fall and are more capable of protecting themselves upon falling, thereby reducing fracture risk.[35]

Menstrual function

Previous studies have shown late menarche, early menopause and amenorrhoea as risk factors for osteoporosis.[46]

Pregnancy and lactation

Pregnancy and lactation increase calcium demand for fetal skeletal development and milk production, respectively, and bone serves to supply calcium during these reproductive periods. Wongdee et al. showed that osteoclastic bone resorption is enhanced at trabecular sites from mid-pregnancy to late lactation. Maternal BMD is usually restored within 12 months post-weaning. However, some breastfeeding mothers manifest a long-term sequel known as pregnancy/lactation-induced osteoporosis, which features back pain, height loss and/or vertebral fracture.[19]

Parity

A study done on postmenopausal Saudi Arabian women showed that women who had borne >6 children were less osteoporotic and of low fracture risk as

compared to those women who had <5 children.[53] High parity saves ova and explains a delay in menopause. Some studies show that menopause occurs 1 year later in women with 5 or more pregnancies.[54]

Low birth weight

Studies in Britain and Sweden have provided evidence that weight in infancy is a detriment of bone mass in adulthood. In one study 153 women born in Bath were followed up to age 21 years. There was a statistically significant relationship between weight at 1 year and bone mineral content at 21 years; These relationships were seen to persist in to later life as demonstrated by a study in men and women born in Hertfordshire.[55]

Cigarette consumption

Chronic cigarette consumption causes toxic effects on osteoblasts and modifies estrogen metabolism. On average, cigarette smokers reach menopause 1–2 years earlier than the general population. Cigarette smoking also produces secondary effects that can modulate skeletal status, including intercurrent respiratory and other illnesses, frailty, decreased exercise, poor nutrition, and the need for additional medications (e.g., glucocorticoids for lung disease).[35]

Alcohol consumption

Alcohol has a direct, antiproliferative effect on osteoblasts and causes dose dependent suppression of osteocalcin levels. In the Nurses' Health Study cohort (age 35-64 years), alcohol intake (more than 25 g or one drink per day) was associated with increased risk of hip fracture and forearm fracture when compared with non-drinkers.[47]

Secondary osteoporosis

Drugs

Glucocorticoids reduce the activity of osteoblasts while Osteoclasts are more active during the early phase of glucocorticoid therapy. Glucocorticoids decrease intestinal absorption of calcium and increase urinary calcium loss. Glucocorticoids may reduce estrogen levels in women by decreasing pituitary secretion of FSH and LH.[47]

Other medications that have adverse effects on the skeleton include antiepileptics, excessive thyroid hormone, chemotherapy, and gonadotropin-releasing hormone antagonists.[45]

Diseases

These include endocrine diseases like Diabetes mellitus[36], hyperthyroidism, hyperparathyroidism, rheumatologic conditions (such as rheumatoid arthritis and systemic lupus), chronic lung disease, malabsorption (celiac disease or inflammatory bowel disease), eating disorders, neurologic diseases (Parkinson's disease, multiple sclerosis, and spinal cord injury), hematologic/oncologic diseases (most notably multiple myeloma), and organ transplantation.[45]

Bone loss associated with these diseases could be due to medication use, nutritional factors, the diminished ability to perform physical activity and factors that directly affect bone remodeling.[45]

Post menopausal women

75% of total bone loss is seen during the postmenopausal period due to estrogen deficiency (52-66%) and aging (34-48%). Bone loss in early postmenopausal

women is more rapid due to more rapid osteoporosis, and 30% of total body bone mass is lost in the first 15-20 yr after the onset of menopause.[7]

OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

The increased rate of bone resorption immediately after menopause clearly indicates a hormonal influence on bone density in women. The most likely explanation for this increased resorption is the drop in ovarian estrogen production that accompanies menopause.

Estrogen deficiency causes bone loss by

(1) activation of new bone remodeling sites, and

(2) exaggeration of the imbalance between bone formation and resorption.[35]

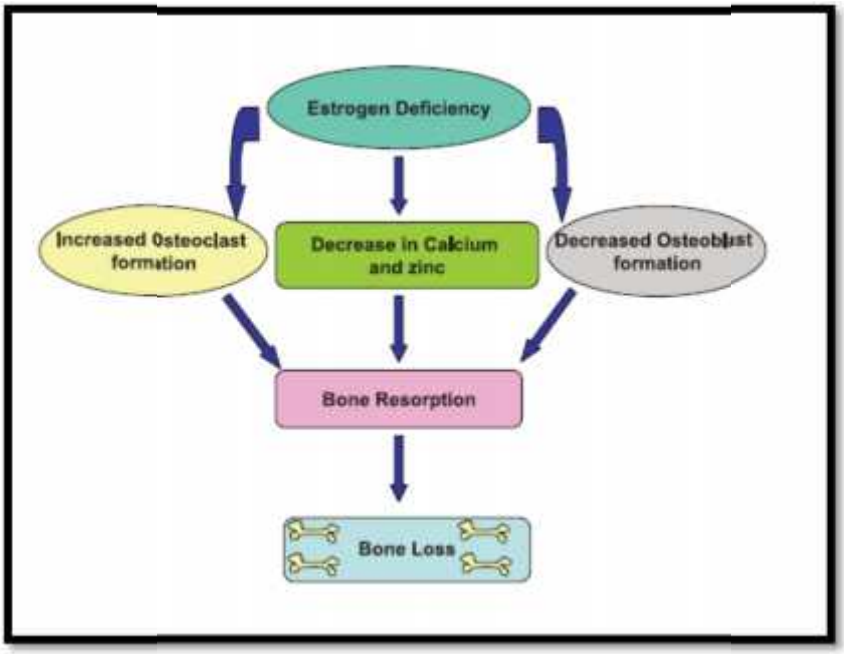


Figure 4.[10] The effect of estrogen deficiency on postmenopausal women.

The average age of menopause for Indian women is 47.5 years with an average life expectancy of 71 years.[56] This is lower than those found in developed countries(51 years). Ethnic diversity seems to be the most important explanation for

the difference of age at menopause in diverse populations.[54] Premature menopause, either spontaneous or surgical due to bilateral oophorectomy, represents an established risk factor for osteoporosis and associated hip fractures.[57] Smoking is associated with early menopause. High parity saves ova and some studies show that menopause occurs 1 year later in women with 5 or more pregnancies.[53,54] Diabetes may be associated with early menopause.[58, 59]

Morbidity And Mortality In Postmenopausal Osteoporosis

Hip fractures, elicit a devastating toll, resulting in higher cost, disability, and mortality than all other osteoporotic fracture types combined. Vertebral fractures cause substantial pain, loss of height and exaggerated thoracic kyphosis. Thoracic fractures may restrict lung function and cause digestive problems. Osteoporotic fractures take a psychological toll as well. Pain, loss of mobility, changed body image, and loss of independence can have a strong impact on self-esteem and mood.[60]

DIABETES MELLITUS

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

The two broad categories of DM are designated type 1 and type 2

- Type 1 diabetes is the result of complete or near-total insulin deficiency.
- Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production.[35]

CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS[35]

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for DM

- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/L (200 mg/dL) *or*
- Fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) *or*
- Two-hour plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral
- glucose tolerance test

Table 1:[35] Spectrum of glucose homeostasis and diabetes mellitus (DM).

Type of Diabetes	Normal glucose tolerance	Hyperglycemia		
		Pre-diabetes	Diabetes Mellitus	
		Impaired fasting glucose or impaired glucose tolerance	Not insulin requiring	Insulin required for control / Insulin required for survival
Type 1				
Type 2				
Other specific types				
Gestational Diabetes				
Time (years)				
FPG	<5.6 mmol/L (100 mg/dL)	5.6–6.9 mmol/L (100–125 mg/dL)	≥7.0 mmol/L (126 mg/dL)	
2-h PG	<7.8 mmol/L (140 mg/dL)	7.8–11.1 mmol/L (140–199 mg/dL)	≥11.1 mmol/L (200 mg/dL)	

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken.[61] Diabetes is estimated to afflict about 170 million people worldwide and this represents about 2% of the world's population.[25]

ASSOCIATION OF DIABETES WITH OSTEOPORORSIS

Besides having a role in glucose and lipid metabolism, it is thought that insulin also has an anabolic effect on bone. There are conflicting results on bone involvement in patients with diabetes mellitus due to the pathogenesis complexity of the condition. Bone loss is one of the chronic complications of diabetic patients. Both type 1 and type 2 diabetes have been associated with the higher risk of fractures. While low bone BMD is consistently observed in Type 1 diabetes, the relationship is less clear in type 2 diabetes with some studies showing modestly increased or unchanged BMD. However clinical trials uniformly support the fact that new bone formation, bone microarchitecture and thus bone quality are altered in both types of Diabetes.[14]

PATHO PHYSIOLOGY OF INCREASED FRACTURE RISKS IN DIABETICS

Hyperglycemia

Hyperglycemia has adverse effects on bone metabolism in both poorly controlled type 1 and type 2 DM. Glucose is the principal energy source for osteoclasts and dose-dependently enhances avian osteoclast activity in vitro.

Hyperglycemia also leads to nonenzymatic glycosylation of various bone proteins including type I collagen, which may impair bone quality.[13]

Insulin

Insulin has an anabolic effect on bone.[13] Insulin like growth factor-1(IGF-1), structurally similar to insulin plays an important role in childhood growth and has anabolic effects in adults. Osteoblasts have receptors for both insulin and IGF-1. A positive correlation has been found between insulin, IGF-1and BMD. In the untreated insulin deficient state decreased bone strength, deficit in mineralized surface area, decrement in the rate of mineral opposition, decreased osteoid surface, decreased osteoblast activity and fewer osteoclasts have been observed.[14]

Advanced glycation end products(AGEs)

Hyperglycemia generates high concentration of AGEs in collagen that decreases bone strength, promote osteoblast apoptosis and increase osteoclast mediated bone resorption.[14]

Phosphocalcic balance

Hypercalciurea is a potential risk factor for osteoporosis in poorly controlled type 1 and type 2 diabetes but glycemic control can reduce hypercalciurea.[14]

Adipokines

Adipokines are soluble factors released from adipocytes. They have mixed effects on human bone metabolism.

***Leptin*:** A few studies in non diabetics have shown that leptin has a positive correlation with BMD. It may also reduce osteoclastogenesis. Whereas negative correlation with BMD has been shown in Diabetics in some studies.[14]

***Adiponectin*:** Adiponetin receptors are present on both osteoblasts and osteoclasts. It suppressed osteogenesis in cultured osteoprogenitor cells. But in the presence of insulin this suppression was blunted. Adiponectin levels negatively correlate with BMD.

***Resistin*:** Resistin stimulated osteoclastogenesis and enhanced preosteoblastic proliferation without affecting osteoblastic differentiation.[14]

Amylin

Amylin is an osteotropic factor secreted by pancreatic β cells and absent in Type 1 DM. In a rat model of T1DM in which streptozotocin selectively destroys pancreatic β cells, the administration of amylin maintained bone mass, inhibited biochemical markers of bone resorption, and elevated biochemical markers of bone formation.[13]

Peroxisome proliferator activated receptor gamma

Peroxisome proliferator -activated receptor gamma(PPARγ) is a transcription factor involved in adipocyte differentiation. PPARγ induces adepogenesis over osteoblastogenesis in pluripotent cells. Increased PPARγ expression has been detected in type 1 diabetic mice contributing to bone loss due to fewer mature osteoblasts and more adipose accumilation. Thiozolidonediones given in Diabetes activate PPARγ.[14]

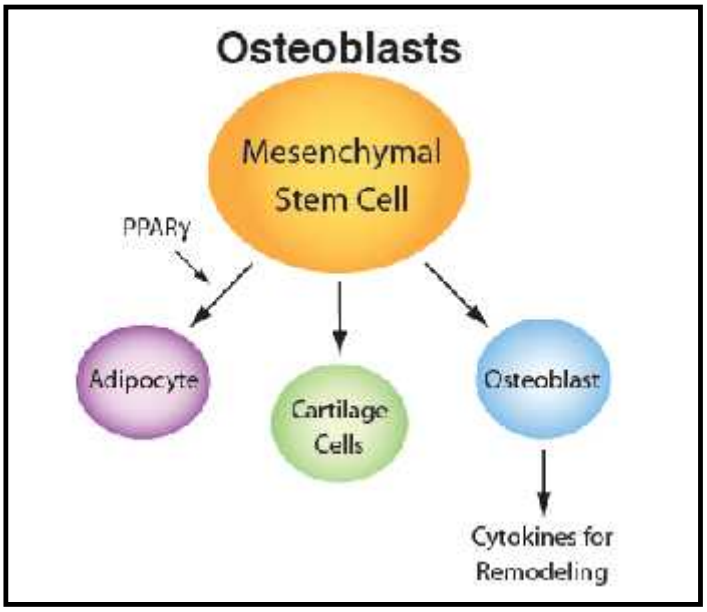


Figure 5:[62] Osteoblasts, Cartilage, and Adipocytes are derived from Marrow Mesenchymal Stem Cells.

Decreased bone mineral density and peak bone mass

Low BMD in type 1 DM may be due to insufficient skeletal mineralization during puberty. A one year follow up prospective study that teenagers with type 1 DM have smaller bone mass and bone size despite normal growth and maturation and the BMD correlated inversely with HBA1c levels.[14]

Diabetic complications

Retinopathy prevents exercise and therefore linked to low muscle strength. Campos Pastor et al demonstrated that patients with retinopathy were at greater risk of osteopenia and osteoporosis.

Nephropathy- Causen et al showed that BMD was low in patients with an increased albumin excretion rate. Further hydroxyproline excretion is increased in those with diabetes and microalbuminuria. Diabetics are also at greater risk of 25 hydroxy vitamin D deficiency correlating with low BMD.

Neuropathy- In type 1 DM patients, Rix et al demonstrated that neuropathy is an independent risk factor for low BMD.

Peripheral vascular disease- was negatively associated with femoral neck BMD in type 1 DM females.[14]

Bone turnover in Diabetes

Studies have shown that diabetics have normal or low serum osteocalcin levels. Dobnig et al. reported serum PTH and osteocalcin to be significantly lower in type 2 Diabetes. Kemink et al. reported that patients with low BMD also had low alkaline phosphatase levels in diabetes. In a study by Isaia et al. with postmenopausal diabetic patients markers of bone resorption like urinary calcium, hydroxyproline and telopeptides were higher than controls. Decreased bone formation with normal bone resorption corresponds to a state of low bone turnover in Diabetes. Hence fractures take longer to heal in Diabetes. In type 2 Diabetes low bone turnover slows bone loss and hence corresponds to higher bone mass seen in these patients. However it could increase bone fragility independent of BMD through the accumulation of fatigue damage.[14]

Propensity to fall

Diabetic have propensity to fall due to their impaired vision, impaired proprioception due to polyneuropathy and/or frequent nocturia contributing to increased risk of fractures.[14]

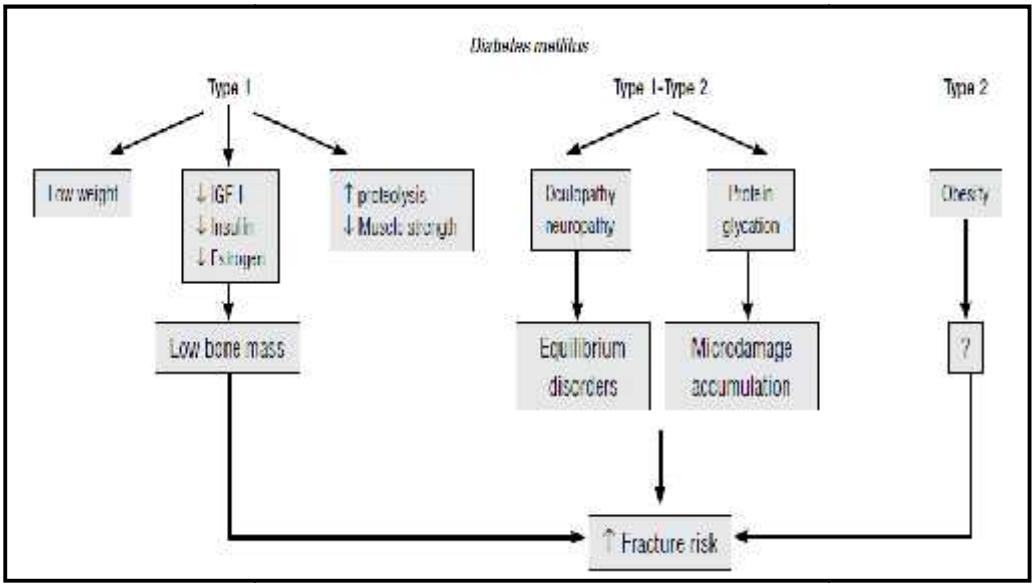
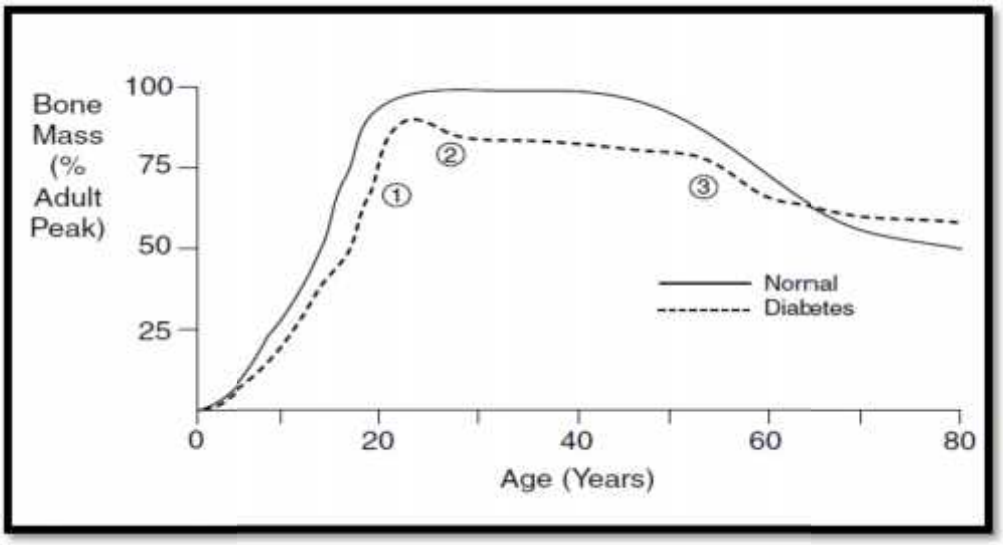


Figure 6:[48] Pathophysiology of the increased fracture risk in type 1 and type 2 diabetes mellitus.



Graph 1.[63] Effects of diabetes on BMD at different times of life. (1) Initial adolescent accumulation of bone is diminished. (2) lower plateau with continued loss associated with hypercalciuria in early adult life (3) later onset and retardation of age-related bone loss Depending on the age of onset, stages could overlap.

PERSPECTIVES ON THE PREVENTION OF DM-INDUCED OSTEOPOROSIS

1. Effective glycemic control

Since most detrimental effects of DM on bone emanate from hyperglycemia and its consequences (e.g. AGE production and impaired vascularization) effective glycemic control is of paramount importance.[19]

2. Appropriate use of antidiabetic agents

Recombinant insulin therapy-

Besides lowering plasma glucose levels and promoting anabolic bone function, insulin also enhances production of proteoglycans, and hence might also protect against osteoarthritis in overweighed DM patients. It also alleviates microangiopathy and restores microcirculation in diabetic bone. Injection of bone marrow stem cells treated with pancreatic extract into streptozotocin-induced DM rats not only normalized plasma glucose, but also elevated production of VEGF, IGF-1 and basic fibroblast growth factor (bFGF), all of which have anti-apoptotic and angiogenic effects.[19]

Antidiabetic drugs

i. Metformin and sulfonylureas

In a large pharmaco-epidemiological case-control study, use of metformin and sulphonylureas was associated with a decreased risk of any fracture. Use of more than 150 defined daily dosages (DDD) of metformin was associated with a 19% reduced risk of any fracture (OR 0.81, 95% CI, 0.71-0.94), and use of more than 400 DDD of a sulfonylurea drug was associated with an 18% reduced risk of any fracture

(95% CI, 0.75-0.90).[64] ADOPT study showed decreased risk of hip fracture in users of sulphonylureas but not in users of metformin. However, lower plasma osteocalcin levels have been found in T2DM patients treated with oral sulphonylureas indicating that treatment with sulphonylureas may not completely counter the diabetes related deficient osteoblastic function/decreased number of osteoblasts.[64]

ii. **Glimepiride** has been shown to stimulate proliferation and differentiation of primary rat osteoblasts in vitro.[19]

iii. Herbal preparations

Cinnamon bark extract, has been found to increase serum insulin levels and improve insulin sensitivity in adipose tissue. It helps reduce fat accumulation in bone marrow and indirectly facilitates bone formation.[19]

iv. Thiazolidinediones

Thiazolidinediones such as rosiglitazone, should be used with caution especially in postmenopausal DM patients. They may decrease bone formation and BMD, while increasing bone resorption, as indicated by the reduced synthesis of alkaline phosphatase, osteocalcin, and procollagen type N-terminal propeptide.[19]

v. Pentoxifylline

Pentoxifylline, which increase blood flow and osteoblast activity, might be promising as anti-osteoporotic agent in both DM and non-DM patients.[19]

ZINC HOMEOSTASIS

Zinc (Zn) is an essential mineral that is a component of more than 200 enzymes and is known as to be necessary for normal collagen synthesis and mineralization of bone. It is an essential cofactor for enzymes involved in synthesis of

various bone matrix constituents, and plays a particularly important role in the regulation of bone deposition and resorption.[10]

Zinc Absorption

The absorption of zinc in the intestinal takes place via active transport as well as a passive diffusion process. Zinc absorption at brush border occurs via carrier-mediated mechanism by zinc transporter named hZIP. Downregulation of this transporter protein will reduce fractional zinc absorption. Dietary fiber is considered as inhibitor to zinc absorption, mainly due to its high phytate contents which bind the mineral into an insoluble complex.[65] In the presence of zinc deficiency, absorption of copper is enhanced resulting in elevated serum copper level and an elevated serum copper/ zinc ratio. Thus, measurement of the serum copper level may be a helpful auxiliary test in the diagnosis of zinc deficiency.[21]

Effect of Calcium on Zinc Absorption

A high concentration of calcium in the lumen may depress passive reabsorption of zinc. It is now suggested that postmenopausal woman on estrogen therapy take both calcium and zinc supplements due to the inhibitory effect that increased luminal calcium has on zinc absorption.[10]

Factors influencing serum zinc levels

Since the intracellular level of zinc is higher than its serum level, serum zinc level may not faithfully reflect the nutritional state of an individual. It must be borne in mind that the serum zinc level may fall at the lower end of the normal range even in the presence of zinc deficiency.[21] Serum zinc concentrations are generally 5% to15 %

higher than plasma zinc because of osmotic fluid shifts from the blood cells when various anticoagulants are used.[66]

Table 2:[21] Factors influencing serum zinc levels

Condition	Change
Fasting	Increase
Food ingestion	Decrease (2–3 hours later)
Stress	Increase
Ingestion of marine products	Increase (oyster, etc.)
Neonates and infants	Decrease
Pregnancy	Decrease (gradually)
Drugs	
Glucocorticoids	Decrease
Thiazides	Increase
Loop diuretics	Increase
Disulfirams	Increase
Clofibrates	Decrease
Oral contraceptive pills	Decrease

Urinary discharge of Zn is increased in postmenopausal women with osteoporosis and the degree of urinary zinc excretion correlates with the severity of osteoporosis.[21,22] Lindeman et al. reported decreased plasma Zn levels with advancing age in both men and women. Ozmen et al states hyperzincuria is potentially a valuable biochemical marker of osteoporosis and may identify high turn-over bone loss in postmenopausal osteoporotic patients.[67]

Influence of diabetes on zinc

Physical–chemical relationship exists between insulin and zinc.[68] Changes in body zinc status affects the production, storage and secretion of insulin. Several investigators have shown the perturbation of zinc metabolism in diabetics. It has been

suggested that hyperzincuria and impaired absorption are major causes of zinc deficiency among diabetics. Alterations in trace element levels has been attributed to hyperglycemia and increased protein glycoslation in Diabetes. Hyperglycemia has been postulated to interfere with the active transport of zinc back into the tubular cells.[69] There is increased production of metallothionein in diabetics. Metallothionein acts as an inhibitor of Zn transport. This decrease in gastrointestinal absorption, coupled with hyperzincuria, could account for significant loss of intracellular Zn.[69]

CALCIUM AND PHOSPHOROUS HOMEOSTASIS

Calcium

Serum calcium levels are normally tightly controlled within a narrow range, usually 8.5 to 10.5 mg/dL. However, the serum calcium level is a poor reflection of overall total body calcium, because serum levels are only 0.1% to 0.2% of extracellular calcium, which, in turn, is only 1% of total body calcium. The remainder of total body calcium is stored in bone. Ionized calcium is physiologically active, whereas the nonionized calcium is bound to albumin or anions such as citrate, bicarbonate, and Pi. In the presence of hypoalbuminemia, there is a relative increase in the ionized calcium relative to the total calcium, thus total serum calcium may underestimate the physiologically active (ionized) serum calcium. Serum levels of ionized calcium are maintained in the normal range by inducing increases in the secretion of PTH. PTH acts to increase bone resorption, increase renal calcium reabsorption and increase the conversion of 25(OH)D to 1,25$(OH)_2$D in the kidney, thereby increasing gastrointestinal calcium absorption. In individuals with normal homeostatic mechanisms, these interactions of PTH and vitamin D metabolites at

target organs, including the kidney, maintain the serum ionized calcium level within the normal range to ensure proper cellular function.[70]

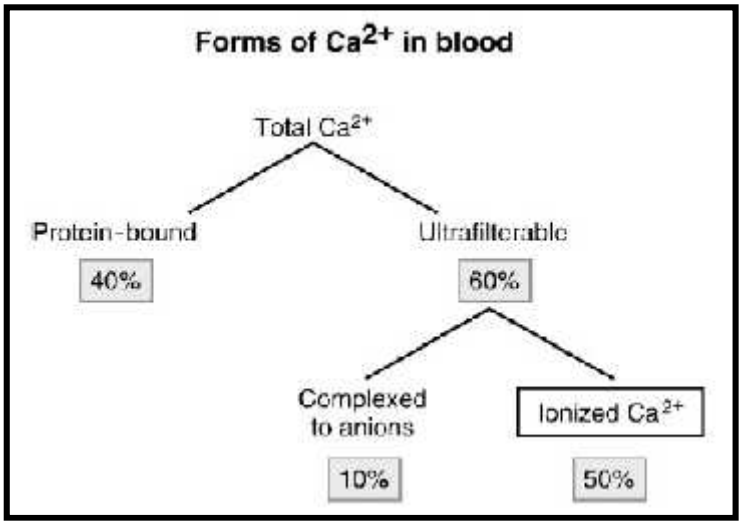


Figure 7:[71] Forms of calcium in blood

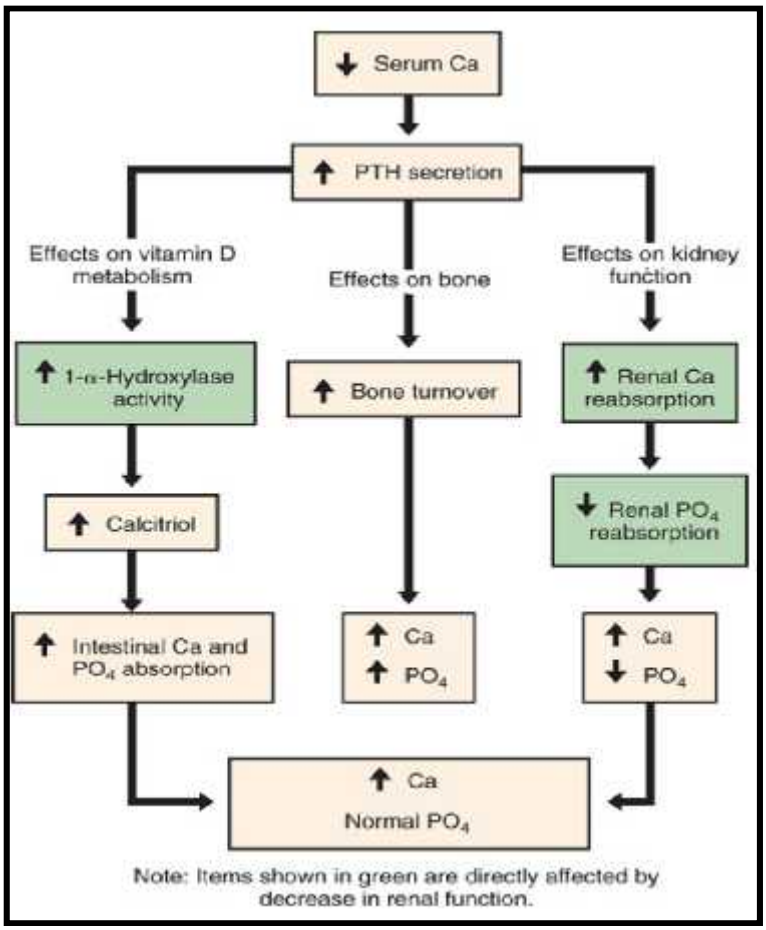


Figure 8:[70] Regulation of calcium and phosphorous

PHOSPHORUS

Inorganic phosphorus is critical for numerous physiologic functions including skeletal development, mineral metabolism, cell membrane phospholipid content and function, cell signaling, platelet aggregation, and energy transfer through mitochondrial metabolism. Normal homeostasis maintains serum phosphorous concentrations between 2.5 and 4.5 mg/dL (0.81 and 1.45 mmol/L). Levels are highest in infants and decrease throughout growth, reaching adult levels in the late teens. Total adult body stores of phosphorus are approximately 700 g, of which 85% is contained in bone in the form of hydroxyapatite. Of the remainder, 14% is intracellular, and only 1% is extracellular. Of this extracellular phosphorus, 70% is organic (phosphate) and contained within phospholipids, and 30% is inorganic. The inorganic fraction is 15% protein bound, and the remaining 85% is either complexed with sodium, magnesium, or calcium or circulates as the free monohydrogen or dihydrogen forms. It is this inorganic fraction that is freely circulating and measured. At a pH of 7.4, it is in a ratio of about 4: 1 HPO_4^{-2} to $H_2PO_4^{-1}$. Thus, serum measurements reflect only a minor fraction of total body phosphorus and, therefore, do not accurately reflect total body stores in the setting of the abnormal homeostasis. The term phosphate means the inorganic freely available form (HPO_4^{-2} and $H_2PO_4^{-1}$). There are three organs involved in Pi: intestine, kidney, and bone. The major hormones controlling Pi levels are vitamin D and PTH. More recently, there is increasing evidence for an important role of a group of circulating factors called phosphatonins in the regulation of serum Pi.[70]

Between 60% and 70% of dietary phosphate is absorbed by the gastrointestinal tract, a process stimulated by calcitriol and, to a much lesser extent,

PTH. Most inorganic phosphate is freely filtered by the glomerulus. Approximately 70% to 80% of the filtered load is reabsorbed in the proximal tubule, the remainder in the distal tubule. Phosphate excretion is increased by increased plasma phosphate concentration, PTH, volume expansion, metabolic acidosis, glucocorticoids and calcitonin.[70]

ALKALINE PHOSPHATASE

The normal serum alkaline phosphatase consists of many distinct isoenzymes found in the liver, bone, placenta, and, less commonly, small intestine. Patients over age 60 can have a mildly elevated alkaline phosphatase (1–11/2 times normal), while individuals with blood types O and B can have an elevation of the serum alkaline phosphatase after eating a fatty meal due to the influx of intestinal alkaline phosphatase into the blood. It is also nonpathologically elevated in children and adolescents undergoing rapid bone growth, because of bone alkaline phosphatase, and late in normal pregnancies due to the influx of placental alkaline phosphatase. [35]

ALP is a marker of bone formation and can increase even with normal ageing.[51] It plays an important role in osteoid formation and mineralisation. Alkaline phosphatase is a zinc dependent metalloenzyme. Hence Zinc deficiency results in low levels of serum alkaline phosphatase, Alkaline phosphatase levels decline with age, and lower than expected levels should raise the possibility of zinc deficiency.[72] Due to its long half life alkaline phosphatase levels are not affected by diurnal variation.[18]

Zinc in osteoporosis

Mutlu[23] studied serum zinc levels in 120 postmenopausal women. 3 groups-normal, osteopenic and osteoporotic women of 40 each were made. They found that

the mean serum zinc levels in osteoporotic women were significantly lower than either osteopenic or normal women. Further osteopenics had lower serum zinc levels than normal postmenopausal women. They concluded by saying that zinc supplementation may have beneficial effects on bone mineral density.

A study[73] evaluated serum zinc levels in 134 postmenopausal women with osteoporosis and 68 postmenopausal women without osteoporosis(control group). Serum zinc levels were significantly lower in postmenopausal women with osteoporosis with $p<0.0001$. E2 deprivation period was longer and Serum levels of E2 were significantly lower in the osteoporotic group as compared to the control group ($p<0.0001$). Osteoporotic women showed lower OPG levels, high sRANKL levels and a low OPG/sRANKL ratio.

In contrast study by **Odabasi**[2] showed no significant difference between postmenopausal women with osteoporosis and control groups, both in plasma and in red blood concentrations, for zinc. They said that serum zinc concentrations are notoriously unreliable since they can be affected by several factors unrelated to the body levels, such as medication, including hormone-replacement therapy, diuretics and laxatives. This unreliability is reflected by the inconsistencies in the literature regarding plasma levels of these minerals in the elderly.[2]

Zinc in Diabetes

Masood et al investigated serum zinc levels in type 2 DM patients and the effect of age, gender, glycemic control and duration of diabetes on the serum levels. Serum zinc levels were significantly lower in diabetic subjects than controls($p<0.001$). There was no association of age, gender, glycemic status and duration of diabetes on the serum concentration of these trace elements in type-2

diabetic patients.[25] Garg et al studied 30 diabetics and 20 non-diabetics with age/sex matching as controls. The study showed serum zinc level in diabetics is 40% lower than controls ($p<0.001$), however, the exact mechanism is still unclear. In conjunction with this result, Williams also showed that plasma zinc level in diabetics is 17% lower than in non-diabetics.[65] **Nsonwu et al[24]** studied the influence of age, gender and duration of diabetes on serum zinc levels. He found that in the diabetics, ageing and increasing duration of diabetes enhances the urinary loss of zinc and decreased serum zinc levels.

However Diwan et al[74] in their study showed no significant difference in the serum zinc levels between type 2 DM patients and controls. They concluded by saying that additional studies are needed to elucidate the mechanism of action of trace elements and their role in Diabetes. **Winokan et al**[65] studied serum zinc in Diabetes mellitus. The mean value for serum zinc level in 18 subjects with type 1 DM and 22 subjects with type 2 DM was within normal range 18± 0.9 mmol/L.

Calcium, Phosphorous and alkaline phosphatase in Post and premenopausal women

Relationship between serum Calcium, Phosphorous, ALP along with several other parameters between 50 premenopausal and 50 postmenopausal women was evaluated in a study using colorimetric methods. The mean BMI, Calcium levels were significantly higher in the postmenopausal group compared to premenopausal women. ($p<0.05$). No significant difference in the serum phosphorous and ALP levels between the two groups was found($p>0.05$).[75]

However **Vemuru M[51]** showed no significant difference in the serum calcium and phosphorus between the two groups($p<0.05$). Serum alkaline phosphatase showed

raised levels in pre-menopausal women compared to post-menopausal women. Significantly high BMD scores in pre-menopausal women compared to post-menopausal women ($p<0.01$) was seen.

Comparison of calcium, phosphorous and alkaline phosphatase in normal and hyperglycaemic postmenopausal women

Verma M[6] evaluated serum Ca, P and ALP levels in 40 type 2 DM postmenopausal women and 38 postmenopausal women without diabetes as controls. They found significant increase ($p < 0.001$) in the alkaline phosphatase level with significant decrease ($p < 0.001$) in the serum calcium and phosphorus level with an associated increase ($p < 0.001$) of urinary calcium and hydroxyproline levels in the study group. Blood sugar levels and HbA1c values were significantly high ($p < 0.001$) in the study group subjects. They concluded that the biochemical indices of bone turnover estimation show significantly increased bone activity in hyperglycemic postmenopausal women as compared to normal postmenopausal women.

AGE AT MENOPAUSE IN DIABETES

A study found that women with type 1 diabetes compared with non- diabetic sisters or unrelated control subjects were more likely to have an older age at menarche ($p< 0.001$), more menstrual irregularities before 30 years of age($p = 0.04$), and a younger age at menopause (41.6, 49.9, and 48.0 years, respectively, $p= 0.05$). This resulted in a 6-year reduction in the number of reproductive years ($p =0.05$) for women with type 1 diabetes. Multivariate analysis confirmed that type 1 diabetes (HR 1.98, $p=0.056$), menstrual irregularities by 30 years of age (HR 2.36, $p=0.01$), and unilateral oophorectomy (HR 9.76, $p < 0.0001$) were independent determinants of earlier menopause. They hypothesize that an earlier menopause, which resulted in a

17% decrease in reproductive years, is a major unstudied complication of type 1 diabetes.[58]

Similarly **Malacara et al[59]** observed an earlier mean age at menopause in Type 2 DM women compared to controls. The diabetic women had more central obesity and less peripheral fat.

However **Lopez et al** found no difference in the age of menopause between women without Diabetes and women with type 2 Diabetes mellitus who were 5-8 years since the diagnosis was made. However they had excluded patients with previous history of irregular mensus which is an important determinant of age at menopause. This criteria must have selected DM patients with milder or better controlled disease.[54]

BMD in diabetes

Tuominen et al[76] measured BMD and 56 type 1 and 68 type 2 diabetic patients and 498 nondiabetic community control subjects. All patients developed diabetes after the age of 30 years (i.e., after achievement of peak bone mass) and were treated with insulin. BMD values were significantly lower in type 1 diabetic patients than in type 2 diabetic patients or the control subjects. Past low-energy fractures were more common in type 1 diabetic women than in type 2 diabetic women. They concluded that the lower BMD in type 1 versus type 2 diabetic patients and control subjects probably results from more rapid bone loss after the onset of type 1 diabetes. Patients with type 1 DM should be evaluated for the risk of osteoporosis and related fractures and offered appropriate preventive measures.

Saeed et al[77] conducted a cross sectional study in which they compared BMD values in 60 T2DM postmenopausal women and 48 age matched controls. BMD was significantly greater in subjects with T2DM than controls($p<0.01$). Women with T2DM also had higher Body Mass Index (BMI) than the control group with $p<0.0001$. The difference in BMD between the two groups became non-significant after adjusting for the effect of BMI by multiple regression analysis. They concluded that postmenopausal women with type 2 Diabetes Mellitus apparently have higher BMD and slow bone turnover when compared with matched controls. This study did not provide evidence that T2DM per se affects bone mineral density in postmenopausal women.

METHODOLOGY

The present study was conducted in Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between Jan 2011 to Mar 2012

Study design

A Cross-sectional study.

Source of data

The present study comprises of clinically, diagnostically confirmed cases of Postmenopausal Osteoporosis both Diabetics and Non-Diabetics and attending the orthopaedic unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Sample size

30 Diabetic and 30 Non-Diabetic Post menopausal women with Osteoporosis in the age of group of 45 to 75 years

Sample Calculation

- According to previous hospital records the number of postmenopausal osteoporotic cases from Orthopaedic department confirmed by DEXA scan in hospital has been around 80 cases per year
- The number of Diabetic patients with DEXA confirmed Osteoporosis was unknown.

- For my study to be statistically significant I have taken a minimum sample size of 30 Diabetic and 30 Non-Diabetic Post Menopausal women with Osteoporosis.

SELECTION CRITERIA- The women were characterized as postmenopausal if they had not menstruated for at least 12 months

Inclusion criteria for both groups

1. Clinically, Diagnostically DEXA confirmed cases of Post Menopausal Osteoporosis
2. Age- 45-75 years
3. DEXA scan 'T' score < -2.5

Exclusion criteria for both groups

1. Surgical Menopause
2. Age<45 years and>75 years
3. Hypertensives
4. Those treated with Biphosphonates, Calcitonin, Anabolic Steroids, Harmone Replacement Therapy, Calcium, Vit D, Zinc previously at any point since the beginning of menopause.
5. Kidney Diseases
6. Smokers
7. Alcoholics

Criteria for diabetic subjects

Inclusion criteria

- Known cases of diabetes with atleast 1 month diabetes duration
- Either type 1 or type 2 category

Exclusion criteria

- patients with diabetic nephropathy
- patients taking Insulin preparations containing Zinc
- patients taking thiazolidinediones

Criteria for non diabetic women subjects

Inclusion criteria

- Fasting serum glucose less than 100 mg/dL

Exclusion criteria

- diabetes

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. University science instrument centre (USIC), Shivaji university, Kolhapur

SCHEDULING:

This study was carried out for a period of 14 months. It was undertaken during Jan 2011 to March 2012

Procedure

All the cases were evaluated and selected by simple random technique after fulfilling selection criteria. The cases of Osteoporosis reported to the Department of Orthopaedics, KLES Dr. Prabhakar Kore Hospital and Medical Research Center were screened. After finding the suitability as per selection criteria they were requested to participate in the study and briefed about the nature of the study and interventions used. A written informed consent was obtained (Annexure I). The consented patients were enrolled in the study, further descriptive data of the participants like name, age, sex, detailed history were obtained by interviewing the participants and were recorded on a predesigned and pretested proforma (Annexure II).

BMD Assessment

Bone mineral density of the patient was determined using whole body densitometer, DEXA Scan (Dual Energy X-Ray Absorptiometry) (GE Healthcare Lunar prodigy advance, scanner serial no. PA + 302343, software version – ENCORE 2008 version 12.2, Germany).

Two X-ray beams with differing energy levels are aimed at the patient's bones. When soft tissue absorption is subtracted out, the BMD can be determined from the absorption of each beam by bone. The amount of bone loss is calculated from the amount of energy that travels through the bone and is picked up by the detector. The test takes about 10 minutes and is done with the patient fully clothed. No special preparations are required. This test involves minimal radiation exposure. The information from this test enables to compare bone density of the patient to others of same age.[78]

A patient's BMD is given a T-score, which is derived by comparing it to an average score for a healthy 30-year-old of the same sex and race. The difference between the "normal young" score and the patient's score is referred to as a standard deviation (SD).[79]

T-score	What the score means
2.5 SD to -1 SD	Normal bone density
Between -1SD and -2.5SD	Osteopenia (low bone density)
Below -2.5SD	Osteoporosis

T-scores and BMD values of Lumbar spine were considered. Height and weight were measured at the time of DXA measurement and body mass index (BMI) was calculated as the weight divided by the square of the height (kg/m^2).

Photographs of DEXA measurement and evaluation in Annexure III.

Collection and storage of blood sample

5 ml of blood was collected in the fasting state in the morning before breakfast in a plain non vacuum tube and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of venipuncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes. Serum was pipetted out into sterile eppendorf tubes.

MATERIAL USED IN THE STUDY

1. Non vacuum plain tube.
2. 5 ml Disposable syringe.
3. Tourniquet.
4. Sterile container.
5. Automated pipettes and tips.

6. Deionised water.
7. Microcentrifuge tubes.
8. Gloves.

INSTRUMENTS USED IN THE STUDY

1. Centrifuge machine

2. Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300)

3. ERBA Chem 5 Semi-auto analyzer

METHODS OF ASSAY

- Serum Zinc—Atomic Absorption Spectrophotometer

  The below tests were done by ERBA reagent Kits in ERBA Chem 5 Semi-auto analyser

- Serum Calcium—OCPC Method, End Point[80]
- Serum phosphorous—Ammonium Molybdate Method, End Point[81]
- Serum Alkaline Phosphatase—IFCC method, kinetic[82]
- Serum Glucose- Trinder's Method, End Point/Fixed time[83]

ESTIMATION OF ZINC

Serum zinc estimation was done using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300), University science instrument centre (USIC), Shivaji University, Kolhapur.

All samples were stored in non vacuum sterile tubes at -20 ^{0}C till further analysis.

SAMPLE PREPARATION

Serum

Serum sample was diluted to 1:10 or 1:5 with deionized water. The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy.

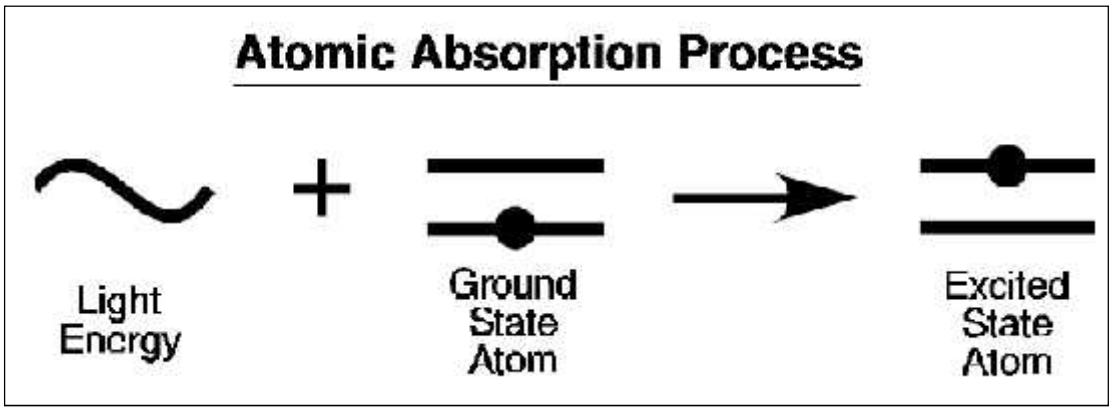


Figure 9: Atomic absorption process

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special

light sources and careful selection of wavelengths allow the specific determination of individual elements.

ATOMIC ABSORPTION INSTRUMENTATION

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest
2. An "absorption cell" in which atoms of the sample are produced
3. A monochromator for light dispersion
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics[66].

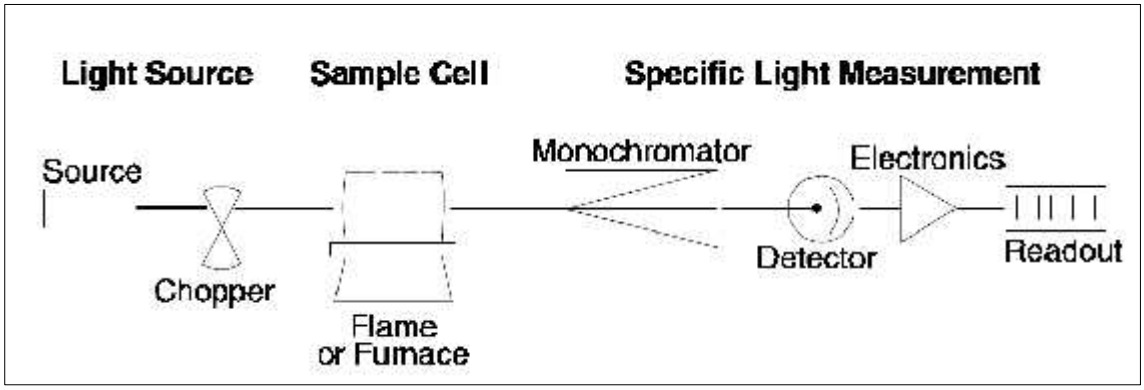


Figure 10: Schematic representation of flame Atomic absorption process.

PROCEDURE

Standard

Appropriate standards are prepared by diluting the zinc stock solution provided by the manufacturer.

Blank

Deionized water is used for blank solution.

ANALYSIS

Instrument was set in standard condition for zinc analysis.

Blank was aspirated first followed by a suitable standard and then the sample. Zinc standard was read after every five sample.

The readings were recorded in p.p.m. and then converted to µg/dL.

Photograph showing analysis of zinc in Annexure – III.

Analysis of serum calcium, phosphorous and alkaline phosphatase

Serum Calcium, phosphorous and alkaline phosphatase were analysed using Erba reagents Manual Chemistry kits in Lyopholised form for Semi Auto Analyzers from ERBA Diagnostics Manheim Gmbh and analysed in Erba- Chem 5 semi autoanalyser.

Photographs in Annexure – III.

STATISTICAL ANALYSIS:

The data was analysed by SPSS software. Mean and Standard Deviation of Serum Zinc, Calcium, Phosphorous and Alkaline Phosphatase of the two groups were computed and compared by unpaired student's *t* test. Results are expressed as Mean ± SD. Relationship between variables was measured by Karl Pearson's correlation co-efficient. A statistical significance is set at 5% level of significance ($p<0.05$).

RESULTS

A cross sectional study was done to compare serum zinc, calcium, phosphorous and alkaline phosphatase levels in 30 diabetic and 30 age matched non diabetic postmenopausal women with osteoporosis. We wanted to include both Type 1 and Type 2 Diabetic women. However the diabetic women who met the inclusion and exclusion in this study belonged to type 2 category.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 3 (Graph 2): Distribution of respondents (diabetics and non diabetics) according to age and age at menopause

The mean age of the diabetic group was 59.44 ± 7.42 years and of the non diabetic group 59.36 ± 7.44 years. As the two groups were age matched there was no significant difference in the mean age in both the groups($p= 0.9669$).

The mean age of menopause for diabetics 46(±1.78) and for non diabetics 47.20(±1.42). There was a significant difference in the mean age at menopause in both the groups ($p<0.01$). Diabetics attained menopause at an earlier age as compared to non diabetics.

Table 4 (Graph 3): Distribution of diabetic and non diabetic women according to height, weight and BMI

The mean height for diabetics 152.95 ± 5.29 cm and 151.03 ± 6.45 cm for non diabetics. There was no significant difference in the mean height in both the groups.

The mean weight 62.60 ±9.04 kg for diabetics and 53.73 ± 9.28 cm for non diabetic women. There as a significant difference in the mean weight($p<0.001$) in both the groups. Diabetics had a significantly higher mean weight then non diabetics.

The mean BMI for Diabetics was 26.79 ± 3.88 kg/m^2 and it was 23.56 ± 3.76 kg/m^2 for non diabetics. Diabetic group had significantly higher BMI than the Non-Diabetic group.

Table 5 (Graphs 4, 5): Comparison of Diabetics and non- diabetics with respect to BMD and T-score

Diabetics had a mean BMD of 0.83 ± 0.06 g/cm^2 and non- diabetics 0.79 ± 0.09 g/cm^2. Diabetics had a higher mean BMD than non- Diabetics but it was not statistically significant ($p=0.0661$). However the t-scores were significantly higher in case of diabetic women compared to non diabetics. The mean t-score for diabetic group was -2.84 ± 0.42 and for non diabetics -3.22 ± 0.74 ($p<0.05$).

Table 6 (Graphs 6, 7, 8, 9): Comparative analysis of serum zinc, calcium, phosphorous and alkaline phosphatase between diabetic and non diabetic postmenopausal women with osteoporosis.

The mean serum zinc, calcium, phosphorous and alkaline phosphatase levels for diabetics were 62.4 ± 13.35 µg/dL, 8.22 ± 1.13 mg/dL, 4.53 ± 0.83 mg/dL and 92.47 ± 22.65 IU/L and for non diabetics 68.2 ± 13.86 µg/dL, 8.4 ± 1.27 mg/dL, 4.32 ± 0.92 mg/dL and 87.23 ± 14.66 IU/L respectively. The mean serum zinc and calcium levels were lower in Diabetics but was not statistically significant($p=0.1042$ for serum zinc and $p=0.5564$ for calcium) while the mean serum phosphorous and ALP levels were higher in case of diabetics as compared to non diabetics but there was no

significant difference between the values in the 2 groups too. (p=0.349 for phosphorous and p=0.2924 for ALP)

Table 7: Correlation coefficient of BMI with BMD, serum zinc, calcium, phosphorous and alkaline phosphatase in diabetics

BMI of diabetics showed a significant positive correlation with BMD and zinc($P<0.05$). It correlated positively with calcium as well but was not significant. There was no correlation between BMI and Phosphorous. It correlated negatively with ALP but was not significant.

Table 8: Correlation coefficient of BMI with BMD, serum zinc, calcium, phosphorous and alkaline phosphatase in non-diabetics

BMI of non- diabetics correlated positively with BMD, zinc, calcium and ALP but were not significant while it showed a non-significant negative correlation with phosphorous ($P>0.05$).

Table 9: Correlation coefficient of Zinc with calcium, phosphorous and alkaline phosphatase in diabetics

Zinc did not correlate significantly with calcium, phosphorous and ALP. It showed positive correlation with calcium and phosphorous and negatively with ALP in diabetics ($p>0.05$).

Table 10: Correlation coefficient of Zinc with calcium, phosphorous and alkaline phosphatase in non diabetics

Zinc showed a non-significant positive correlation with calcium (p=0.05) and negatively with phosphorous and ALP in non diabetics ($p>0.05$).

Table-11 Correlation of BMD with Zn, Ca, P and ALP in diabetics

BMD showed a significant positive correlation with Zn($p<0.001$) and Ca($p<0.05$) and negative correlation with P and ALP but was not significant($p>0.05$).

Table-12 Correlation of BMD with Zn, Ca, P and ALP in non-diabetics

BMD showed significant positive correlation with Zn($p<0.01$), Ca($p<0.001$), P($p<0.05$) and ALP($p<0.001$).

Table 3: Distribution of respondents(diabetics and non diabetics)according to age, age at menopause

	Diabetic		Non Diabetic		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p value**
Age(years)	59.44	7.42	59.36	7.44	0.9669
Age at menopause(years)	46	1.78	47.2	1.42	0.0055*

*p<0.05

Table 4: Distribution of diabetic and non diabetic women according to height(cm), weight(kg) and BMI(kg/m$^{2)}$

	Diabetic		Non- Diabetic		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p- value**
Height(cm)	152.95	5.29	151.03	6.45	0.2133
Weight(kg)	62.6	9.04	53.73	9.28	0.0004*
BMI(kg/m^2)	26.79	3.88	23.56	3.76	0.0018*

*p<0.05

Table 5: Comparison of Diabetics and non- diabetics with respect to BMD and T-score

	DIABETIC		NON- DIABETIC		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**
BMD(g/cm^2)	0.83	0.06	0.79	0.09	0.0661
T-score	-2.84	0.42	-3.22	0.74	0.0177*

*p<0.05

Table 6: Comparative analysis of serum zinc, calcium, phosphorous and alkaline phosphatase between diabetic and non diabetic postmenopausal women with osteoporosis.

	Diabetic		Non- Diabetic		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p- value**
Zinc in µg/dL	62.4	13.35	68.2	13.86	0.1042
Calcium in mg/dL	8.22	1.13	8.4	1.27	0.5564
Phosphorous in mg/dL	4.53	0.83	4.32	0.92	0.349
ALP in IU/L	92.47	22.65	87.23	14.66	0.2924

Table 7: Correlation coefficient of BMI with BMD, serum zinc, calcium, phosphorous and alkaline phosphatase in diabetics

Diabetics	**Correlation coefficient between BMI with**	
	Correlation coefficient	**p-value**
BMD	0.427	0.019*
Zinc in µg/dL	0.419	0.021*
Calcium in mg/dL	0.316	0.089
Phosphorous in mg/dL	0	0.998
ALP in IU/L	-0.159	0.401

*p<0.05

Table 8: Correlation coefficient of BMI with BMD, serum zinc, calcium, phosphorous and alkaline phosphatase in non-diabetics

Non- Diabetics	Correlation coefficient between BMI with	
	Correlation coefficient	p-value
BMD	0.051	0.791
Zinc in µg/dL	0.122	0.522
Calcium in mg/dL	0.241	0.199
Phosphorous in mg/dL	-0.106	0.579
ALP in IU/L	0.176	0.353

Table 9: Correlation coefficient of Zinc with calcium, phosphorous and alkaline phosphatase in diabetics

Diabetics	Correlation coefficient between Zinc with	
	Correlation coefficient	p-value
Calcium in mg/dL	0.193	0.307
Phosphorous in mg/dL	0.035	0.854
ALP in IU/L	-0.133	0.483

Table 10: Correlation coefficient of Zinc with calcium, phosphorous and alkaline phosphatase in non-diabetics

Non- Diabetics	Correlation coefficient between Zinc with	
	Correlation coefficient	p-value
Calcium in mg/dL	0.361	0.05
Phosphorous in mg/dL	-0.101	0.596
ALP in IU/L	-0.269	0.151

Table-11 Correlation of BMD with Zn, Ca, P and ALP in diabetics

Diabetics	**Correlation coefficient between BMI with**	
	Correlation coefficient	**p-value**
Zinc in µg/dL	0.613	p<0.001*
Calcium in mg/dL	0.427	p<0.05*
Phosphorous in mg/dL	-0.056	0.769
ALP in IU/L	-0.027	0.887

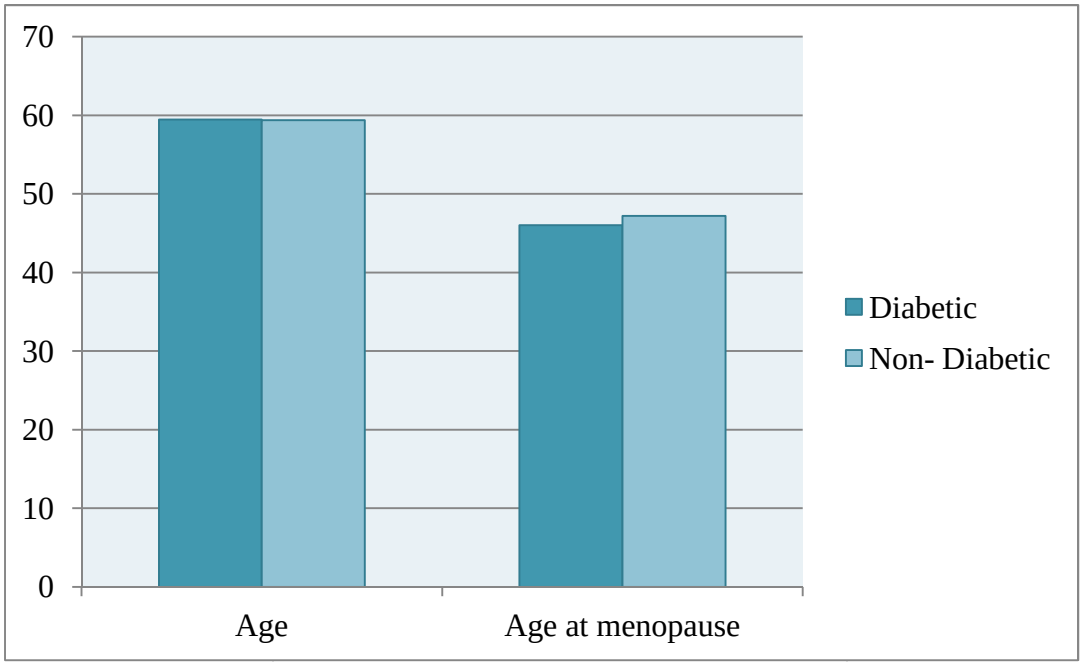
*p<0.05

Table 12 Correlation of BMD with Zn, Ca, P and ALP in non-diabetics

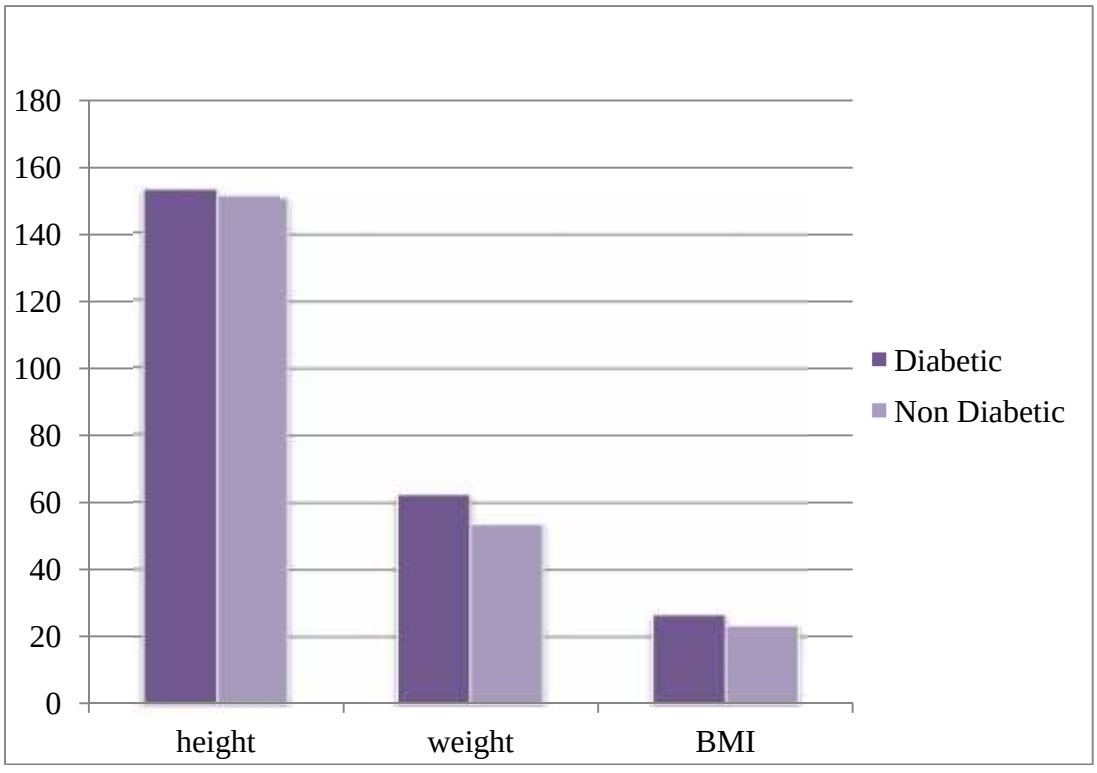
Non- Diabetics	**Correlation coefficient between BMI with**	
	Correlation coefficient	**p-value**
Zinc in µg/dL	0.544	p<0.002*
Calcium in mg/dL	0.778	p<0.001*
Phosphorous in mg/dL	-0.440	0.015*
ALP in IU/L	-0.658	p<0.001*

*p<0.05

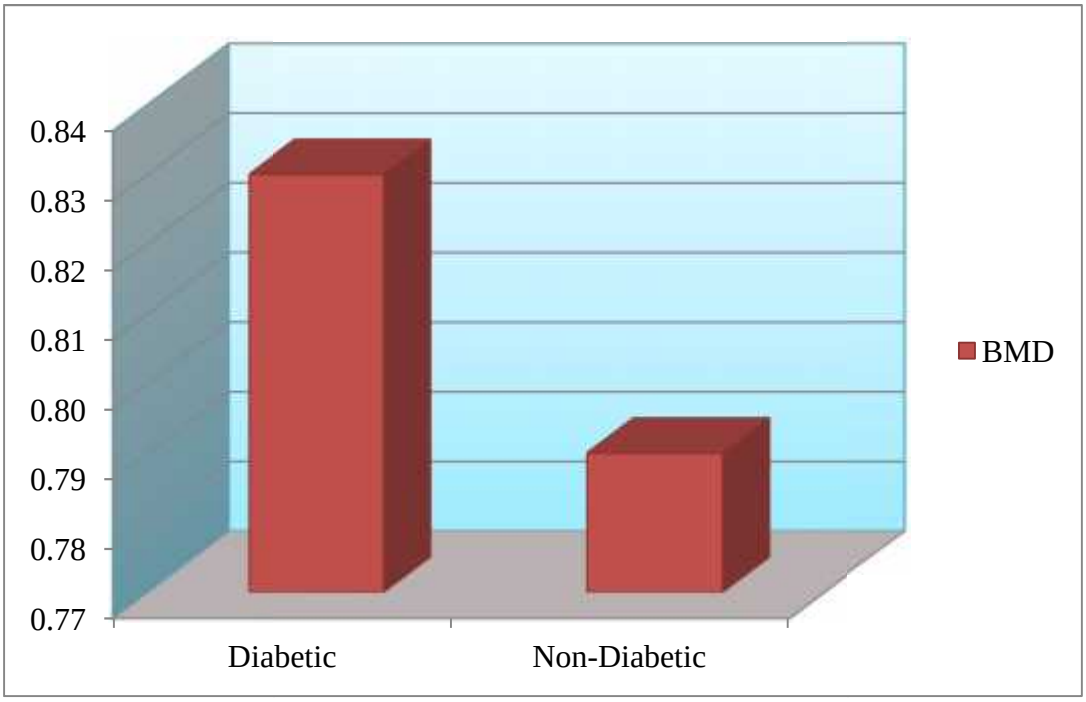
Graph 2: Distribution of respondents(diabetics and non diabetics)according to age, age at menopause



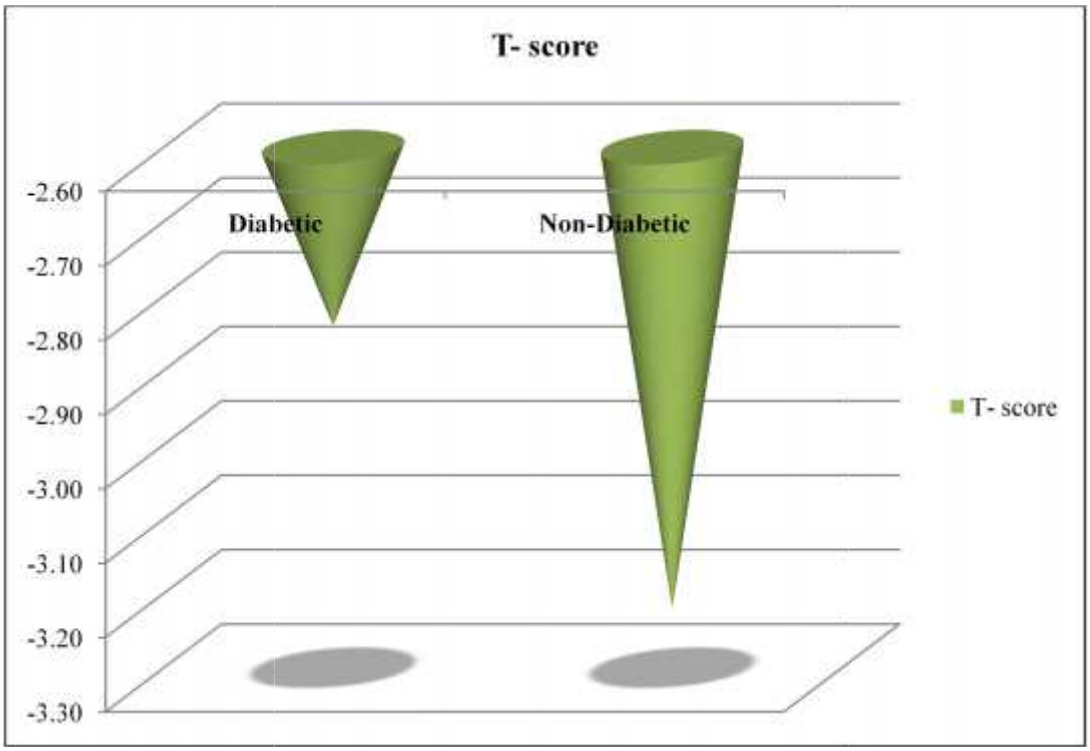
Graph 3: Distribution of diabetic and non diabetic women according to height, weight and BMI



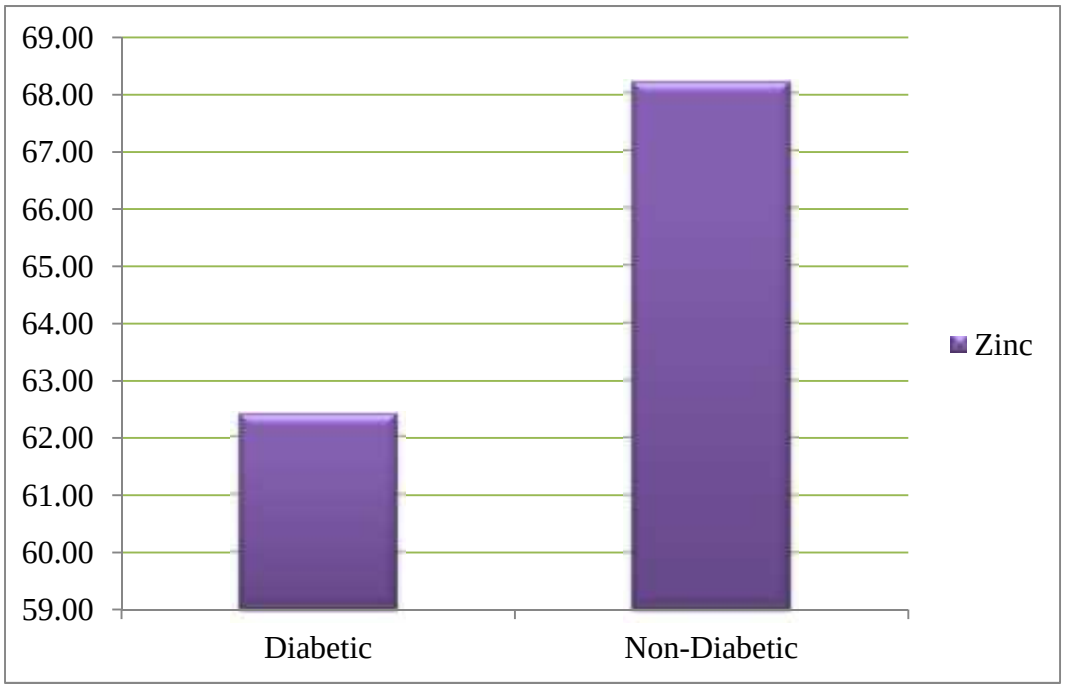
Graph 4: Comparison of BMD levels between diabetic and non diabetic postmenopausal women with osteoporosis



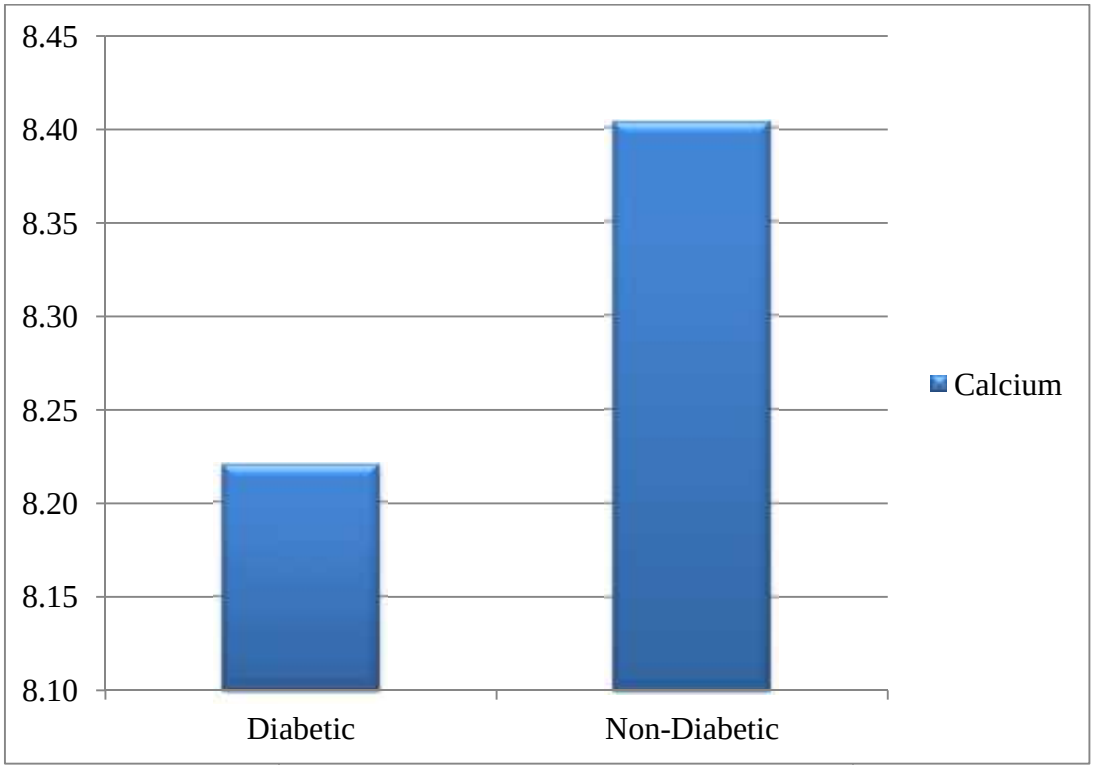
Graph 5: Comparison of T- scores between diabetic and non diabetic postmenopausal women with osteoporosis



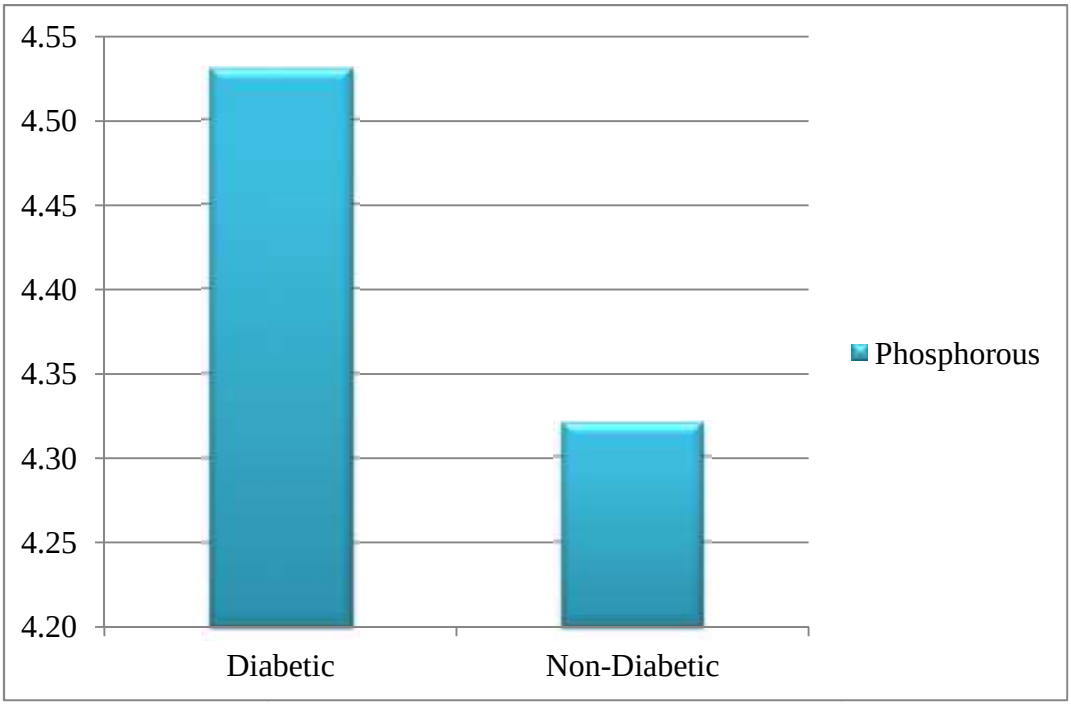
Graph 6: Comparison of mean serum zinc levels between diabetic and non-diabetic postmenopausal women with osteoporosis



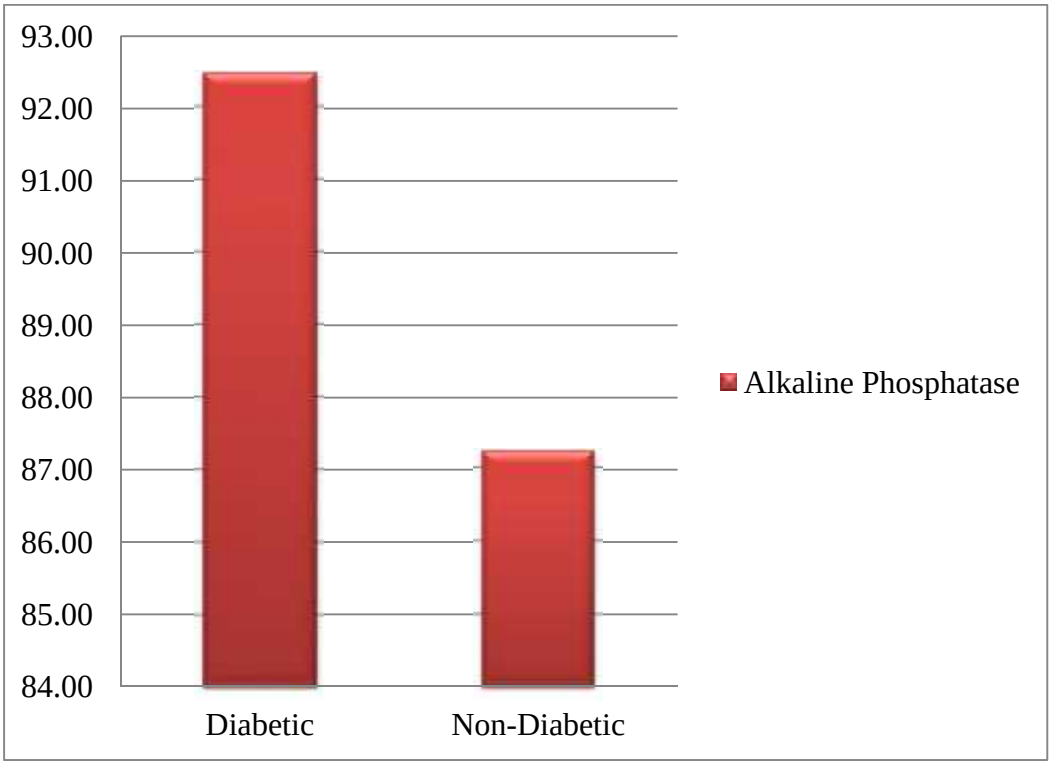
Graph 7: Comparison of mean serum Calcium levels between diabetic and non-diabetic postmenopausal women with osteoporosis



Graph 8: Comparison of mean serum phosphorous levels between diabetic and non-diabetic postmenopausal women with osteoporosis



Graph 9: Comparison of mean serum alkaline phosphatase levels between diabetic and non-diabetic postmenopausal women with osteoporosis



DISCUSSION

Serum zinc, calcium, phosphorous and alkaline phosphatase levels in 30 diabetic postmenopausal women with osteoporosis were estimated and the levels were compared with 30 age matched non diabetic postmenopausal women with osteoporosis. All 30 diabetic patients belonged to type 2 category. There was no significant difference in these biochemical parameters between the two groups ($p>0.05$).

Urinary discharge of Zn has been shown to be increased in postmenopausal osteoporotic women and the degree of zincuria correlates with the severity of osteoporosis.[21, 22] Zincuria as a feature of diabetes has been demonstrated by several studies.[69,84] **Walter[84]** noted a six fold increase in urinary zinc excretion in the diabetic subjects which included type 1 and type 2 compared with controls. Urinary zinc did not correlate significantly with plasma glucose concentrations, urinary glucose concentrations, HbA1c, proteinuria, or diuretic use. Age, sex and obesity was not a significant predictor for serum zinc levels in their study. Whether the high urine zinc losses are offset by increased absorption and/or decreased endogenous fecal loss remains unknown.

Our study showed no significant difference in the mean serum zinc levels between the two groups though they were lower in Diabetics. This is in accordance with **Zargar et al[85]** and **Diwan et al[74]** who similarly found no significant difference in the serum zinc levels between type 2 Diabetics and controls. In contrast **Emeribe[86]** found significantly higher serum zinc levels in type 2 diabetics compared to controls which they thought could be due to the imbalanced rate of absorption to excretion of zinc.

All diabetic patients in our study were on regular oral hypoglycaemic drugs with four of then taking insulin as well. Only insulin preparations containing zinc were excluded. Besides having anabolic effect on bone, insulin may reduce hyperzincuria accompanying diabetes.[69] Insulin or oral hypoglycaemic drugs which increase insulin secretion[69] might have been responsible for the insignificant difference in the serum zinc levels between diabetic women as compared to the non-diabetics in our study. None of the patients were taking thiazolidinediones. These drugs activate PPARγ which induces adepogenesis over osteoblastogenesis in pluripotent cells.[14]

In this study low serum calcium levels and high phosphorous and alkaline phosphatase levels in diabetic postmenopausal women with osteoporosis than non-diabetics were found but were not statistically significant. However evaluation by **Verma M[6]** in mild type 2 DM postmenopausal women and postmenopausal women without diabetes as controls found significant increase ($p < 0.001$) in the alkaline phosphatase levels with significant decrease ($p < 0.001$) in the serum calcium and phosphorus level with an associated increase ($p < 0.001$) of urinary calcium. A study showed that bone turnover is remarkably lower in type 2 DM compared to healthy postmenopausal women.[6] Poorly controlled NIDDM patients have relative hypercalciuria probably caused by osmotic diuresis associated with glycosuria. This could lead to negative calcium balance which might result in accelerated bone resorption and loss of bone. Metabolic control results in decrease in urinary calcium which correlates with the decrease in urinary glucose excretion.[6] Our study excluded diabetic patients with nephropathy. Hence they must have not had significant hypercalciurea. Morever they were on medications with oral hypoglycaemic drugs

with four of them taking insulin as well might have been a protective factor against hypercalciurea leading to no significant differences in the levels between the diabetic and the non diabetic group.

Cakatay U[87] found similar alkaline phosphatase levels between type 2 DM patients and controls. No statistically significant difference in the serum calcium and phosphorus levels was found as well. In spite of the decrease in the OC levels in the diabetic group no difference was observed in DPD levels, was attributed to the fact that only the formation phase was affected in diabetes, while the resorption phase remained unaltered.

In contrast **Levy J**[88] found significantly increased calcium levels in diabetics ($p<0.001$) compared to controls and similar phosphorous levels between the 2 groups. The difference in plasma Ca was not influenced by age, sex, or mode of treatment.

Zinc did not correlate significantly with calcium, phosphorous and ALP in both the groups. It showed positive correlation with calcium and phosphorous and negatively with ALP in diabetics and positively with calcium and negatively with phosphorous and ALP in non diabetics. Low Zinc levels lead to decrease in the synthesis of IGF-1 which is critical for the regulation of bone formation, resorption and calcium homeostasis. Lower amounts of circulating IGF-1 lead to more rapid loss of calcium from bone with aging and subsequently osteoporosis.[10] Though the correlation was not significant lower zinc levels might have marginally lead to the lower calcium levels that have been observed in this study in diabetic women.

Women with type 2 diabetes attained menopause at an earlier age compared to non diabetic participants in this study and it was significant ($p<0.01$). This is in accordance with **Malacara JM**.[59] DM is presumptively associated with autoimmune

damage in the ovary. Another possible mechanism for the effect of DM on menopause is the interaction of insulin resistance and diabetes with ovarian alterations such as polycystic ovarian disease where the prevalence of glucose intolerance may be close to 40%. Several investigators have proposed that insulin has an effect on ovarian steroidogenesis. However there is no evidence about the influence of insulin resistance on oocyte depletion.[41]

In contrast the study by **Lopez et al**[54] on type 2 diabetic women, the age of menopause was similar in diabetics and non diabetics (49.68 and 49.76 respectively). Five-eight years since the diagnosis of type 2 diabetes did not have an impact on the instillation of menopause in their study. However they had excluded patients with previous history of irregular mensus which is an important determinant of age at menopause. This criteria must have selected DM patients with milder or better controlled disease.

This study found significantly higher BMD levels in Diabetic postmenopausal women with osteoporosis than non diabetics but it was not statistically significant while the T-scores were significantly higher in the diabetic group. Patients with type 2 diabetes mellitus display an increased fracture risk despite a higher BMD, which is mainly attributable to the increased risk of falling and decreased bone quality.[20] BMD showed a significant positive correlation with Zinc ($p<0.001$) and Calcium ($p<0.05$) in diabetic women while significant positive correlation with zinc and calcium and a significant negative correlation with phosphorous and Alkaline phosphates ($p<0.05$) in non- diabetic women.

Significantly higher BMI was observed in diabetic women than the non-diabetics ($p <0.01$) in this study. This is in accordance with Zargar et al.[85] The high

BMI in this study was attributable to significantly higher weight of the diabetic women ($p<0.001$).

BMI showed a significant positive correlation with BMD in the diabetic women($p<0.05$). The obesity commonly present in type 2 DM may have a cumulative protective effect on bone density. This is in accordance with **Brown et al.**[63]

Low BMI as a risk factor for osteoporosis has been shown by several studies.[47,48] Overweight and hyperinsulinemia have been postulated as two important features of T2DM which are positively correlated with BMD.[20] In obesity peripheral conversion of androgens to oestrogens by adipose tissue aromatase is increased and these hormones exert protective effects on bone.[86] Adipose tissue also releases a wide variety of adipokines that have been implicated either directly or indirectly in the regulation of bone remodelling. Insulin has an anabolic effect on bone due to its structural homology to IGF-1 by interacting with the IGF-1 receptor which is present on osteoblasts. The IGF-1 signaling pathway is crucial for bone acquisition. Individuals with T2DM usually have an excess of insulin and insulin levels could mediate in part a positive association between T2DM and elevated BMD. Moreover Body fatness may have an impact on the accuracy of DEXA based BMD measures. However such a measurement error is negligible.[20]

In this study BMI of diabetics showed a significant positive correlation with zinc ($P<0.05$). High BMI in T2DM may also have a protective influence in maintaining serum zinc levels and to compensate for the decrease in zinc attributed to zincuria seen in DM. BMI correlated positively with calcium as well but was not significant. There was no correlation between BMI and Phosphorous. It correlated negatively with ALP but was not significant. In the non diabetic women BMI

correlated positively with BMD, zinc, calcium and ALP and negatively with phosphorous but none of them were significant. **Ahlström T**[89] found a significant positive correlation between plasma calcium with BMI and insulin resistance. **Akter N**[90] in their study showed that serum calcium levels increase with increase in BMI. The increased intracellular calcium is an important second messenger that triggers various pathways that promote the accumulation of fat in adipose tissue including activation of lipogenesis by augmenting fatty acid synthase activity. Hence high BMI in Diabetic women in our study might act as a protective factor against hyperzincuria and hypercalciurea that may be associated with diabetes.

In our study out of the 30 diabetic women six were having FSG levels>200mg/dL. Out of which three diabetic participants were recently diagnosed with diabetes with duration of one month. Severe Hyperglycemia in these women can be a confounding factor. The rest three diabetic women may not have been under strict glycemic control.

CONCLUSION

Our study suggests that serum zinc, calcium, phosphorous and alkaline phosphatase are not significantly altered in type 2 diabetic postmenopausal women with osteoporosis as compared to non diabetic women with postmenopausal osteoporosis when overt nephropathy is excluded. Type 2 DM mellitus women may be at risk of early menopause. High BMI in type 2 DM may contribute to high BMD and may be a protective factor against zincuria and hypercalciurea as well.

Medications with oral hypolycemic drugs and/or insulin may reduce the zincuria and hypercalciurea that may accompany diabetes. Strict glycemic control is recommended. However to what extent it can prevent diabetes related fractures cannot be told as fractures in type 2 DM are attributable to poor bone quality rather than BMD. More studies are required in this arena on the role of zinc in postmenopausal women with Diabetic Osteoporosis.

Limitations of the study

1. Both newly diagnosed and old cases of diabetes were included.
2. We excluded patients with overt diabetic nephropathy but did not test for microalbuminuria in diabetics which is an early predictor of nephropathy.

SUMMARY

This study was undertaken with the aim of estimating serum zinc, calcium, phosphorous and alkaline phosphatase in diabetic postmenopausal women with osteoporosis and comparing their levels with non- diabetic postmenopausal women with osteoporosis. All the 30 diabetics participants belonged to type 2 diabetes category. The diagnostic modality for osteoporosis was DEXA scan based on the WHO definition of osteoporosis.

The present cross sectional study was conducted at Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between Jan 2011 to March 2012 on 60 postmenopausal women with osteoporosis including 30 diabetics and 30 non- diabetics in the age group of 45-75 years. The age of diabetics and non diabetics were comparable.

Serum zinc, calcium, phosphorous and alkaline phosphatase levels were estimated in both the groups. Following were the findings

- There was no significant difference in the four parameters between the two groups though zinc and calcium were lower while phosphorous and alkaline phosphatase were higher in the diabetic group.
- Diabetics attained menopause at a significantly earlier age than non- diabetics ($P<0.01$).
- Diabetic postmenopausal women with osteoporosis had higher BMD than non diabetics but it was not statistically significant while the T-scores were significantly higher in the diabetic group ($P<0.05$).
- BMI was significantly higher in diabetics than the non- diabetics ($P<0.01$).
- BMI of diabetics showed a significant positive correlation with BMD and zinc ($P<0.05$).

- BMI correlated positively with calcium as well but was not significant. There was no correlation between BMI and Phosphorous. It correlated negatively with ALP but was not significant. In non- diabetics BMI correlated positively with BMD, zinc, calcium and ALP but were not significant while it showed a non-significant negative correlation with phosphorous.
- Zinc did not correlate significantly with calcium, phosphorous and ALP in both the groups. It showed positive correlation with calcium and phosphorous and negatively with ALP in diabetics and positively with calcium and negatively with phosphorous and ALP in non diabetics.
- BMD showed a significant positive correlation with zinc and calcium in diabetic women and it correlated significantly in a positive manner with zinc and calcium and negatively with phosphorous and alkaline phosphatase in non- diabetics.

The diabetic women were on medication with oral hypoglycaemic drugs with four of them taking insulin as well.

Our study suggests that serum zinc, calcium, phosphorous and alkaline phosphatase are not significantly altered in type 2 diabetic postmenopausal women with osteoporosis as compared to non diabetic women with postmenopausal osteoporosis when overt nephropathy is excluded. High BMI in type 2 DM may be a protective factor against zincuria.

Medications with oral hypolycemic drugs and/or insulin may reduce the zincuria and hypercalciurea that may accompany diabetes. Strict glycemic control is recommended. However to what extent it can prevent diabetes related fractures cannot be told as fractures in type 2 DM are attributable to poor bone quality rather than BMD. More studies are required in this arena on the role of zinc in postmenopausal women with Diabetic Osteoporosis.

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ANNEXURE I - CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Title- A comparative study to correlate the levels of Serum Zinc, Calcium, Phosphorous and Alkaline Phosphatase in Post Menopausal women with Osteoporosis: Diabetics versus Non-Diabetics

Principal investigator- Dr _____

Guide- Dr _____

Mrs/Ms. …… you are invited to participate in our research study that is a study to compare the levels of serum Zinc, Calcium, Phosphorous and Alkaline Phosphatase in Diabetic and Non-Diabetic Postmenopausal women with Osteoporosis.

Participation in this study is completely voluntary. About 30 Diabetics and 30 Non-Diabetics with Osteoporosis will be enrolled in this study at J. N. Medical College, Belgaum under the supervision of Dr. _____, Department of Biochemistry, Jawaharlal Nehru Medical College, Belgaum. The study will be carried out by Dr. _____, Post Graduate Student, Department of Biochemistry, KLE University, Belgaum for her M.D. dissertation is to be submitted to KLE University, Belgaum.

PURPOSE OF THE STUDY

Postmenopausal women are at increased risk of osteoporosis. Moreover the incidence of Diabetes is increasing in India. Trace element levels like zinc along with routine biochemical bone markers like Ca, P and ALP are altered in osteoporosis. Diabetics are at increased risk of fractures. So the study is being done to compare the levels of these elements in Diabetic and Non-Diabetic postmenopausal women with osteoporosis.

PROCEDURE

If you agree to participate in this research you will be asked the relevant history and will be subjected to clinical examination. You will be requested to come in the fasting state the next day. 5ml of blood will be collected by intravenous route by pricking a small blood vessel which may give rise to small amount of pain. It will be subjected to glucose, zinc, calcium, phosphorous and alkaline phosphatase estimation.

RISKS AND BENEFITS

There are no risks involved in this procedure. If any complications arise during the procedure you will be treated in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum with the best of our knowledge and the availability of resources in the free hospital. There will be no compensation or payment for such medical treatment.

During the course of the study you will be informed of any significant new findings such as changes in the risks and benefits resulting from participation in the research.

OPTIONS

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

PRIVACY AND CONFIDENTIALITY

The only people who will know that you are a research participant are members of the research team. No information provided by you or about you during the research will be disclosed to others without your written consent.

INSTITUTIONAL POLICY

Your participation in this study is voluntary, whether or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum

COST FOR PARTICIPATION

You will not be charged for the test to be carried out on your blood sample.

FINANCIAL INCENTIVE FOR PARTICIPATION

You will not receive any remuneration for participating in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Dr. _____, Principal, Jawaharlal Nehru Medical College, KLE University, Belgaum and Chairman of Jawaharlal Nehru Medical College, Institutional Ethics Committee of Human Subjects Research, Telephone No. _____.

If you have questions as a participant in our study, you can contact the study investigator Dr._____, Mobile No. _____ or the research guide Dr. _____ , Department of Biochemistry, Phone No. 0831-2473777 (Extension) _____.

CONSENT STATEMENT

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

Signature or left thumb print of participant or legally authorized representative.

Participant's Name:	Participant's Signature or thumb print:
Experimenter's Name:	Experimenter's Signature:
Witness' Name:	Witness' Signature:
Guardian's Name:	Guardian's Signature or thumb print:
Date:	Place:

ANNEXURE II: PROFORMA

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

TITLE: A Comparative study to correlate the levels of serum Zinc, Calcium, Phosphorous and Alkaline Phosphatase in Post Menopausal women with Osteoporosis: Diabetics versus Non Diabetics

I. Patient Identification

Name :	OPD No. :
Age :	Sex :
Occupation:	Religion :

II. Presenting Complaints:

Past History

H/o similar episodes in the past

H/o fracture or trauma

H/o treatment for the above complaints

H/o Diabetes Mellitus

H/o treatment for Diabetes

H/o polycystic ovarian syndrome

Family History

H/o Diabetes in the family

Personal History

Sleep

Appetite

Diet

Bowel and Bladder Habits

H/o smoking

H/o alcohol consumption

Menstrual History- Age at menarche, parity, age at menopause

III. General Physical Examination

Weight (Kg)	:	Pulse	:
Height (m)	:	Blood pressure:	
Pallor	:	Respiratory rate:	
Icterus	:	Temperature	:
Clubbing	:	Oedema	:
Cyanosis	:		

Lymphadenopathy:

Systemic examination

CVS :

Respiratory :

Abdominal :

Investigations

Serum Zinc

Serum Calcium

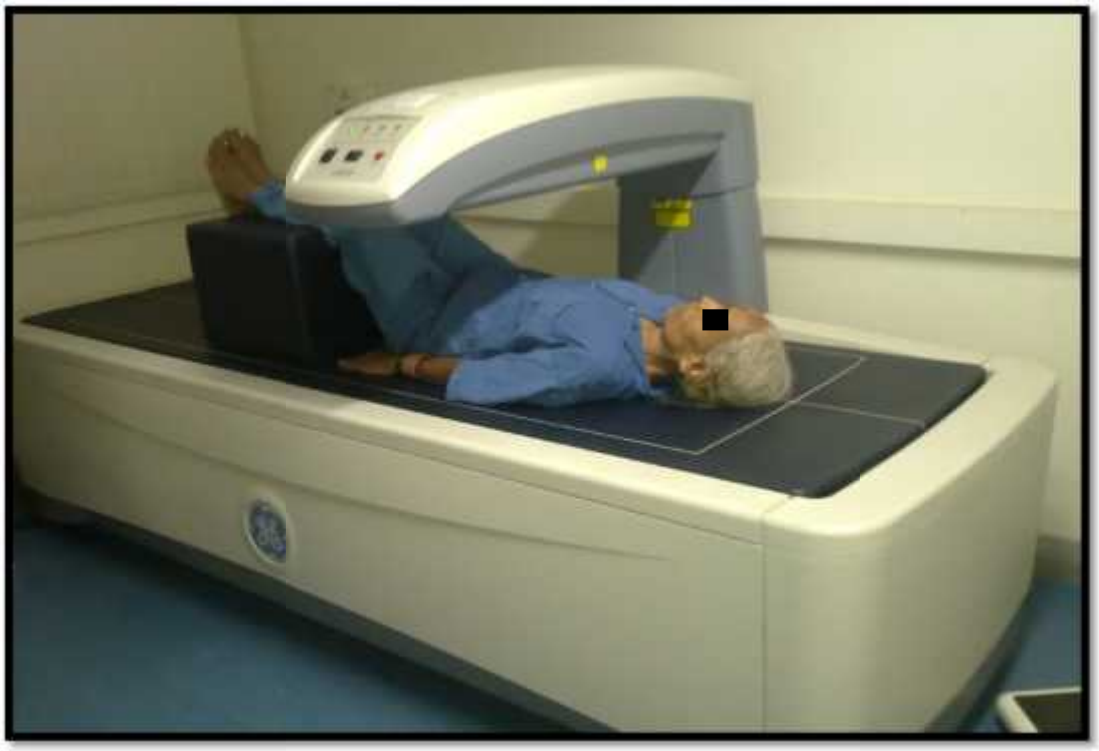
Serum Phosphorous

Serum Alkaline Phosphatase

Final Diagnosis

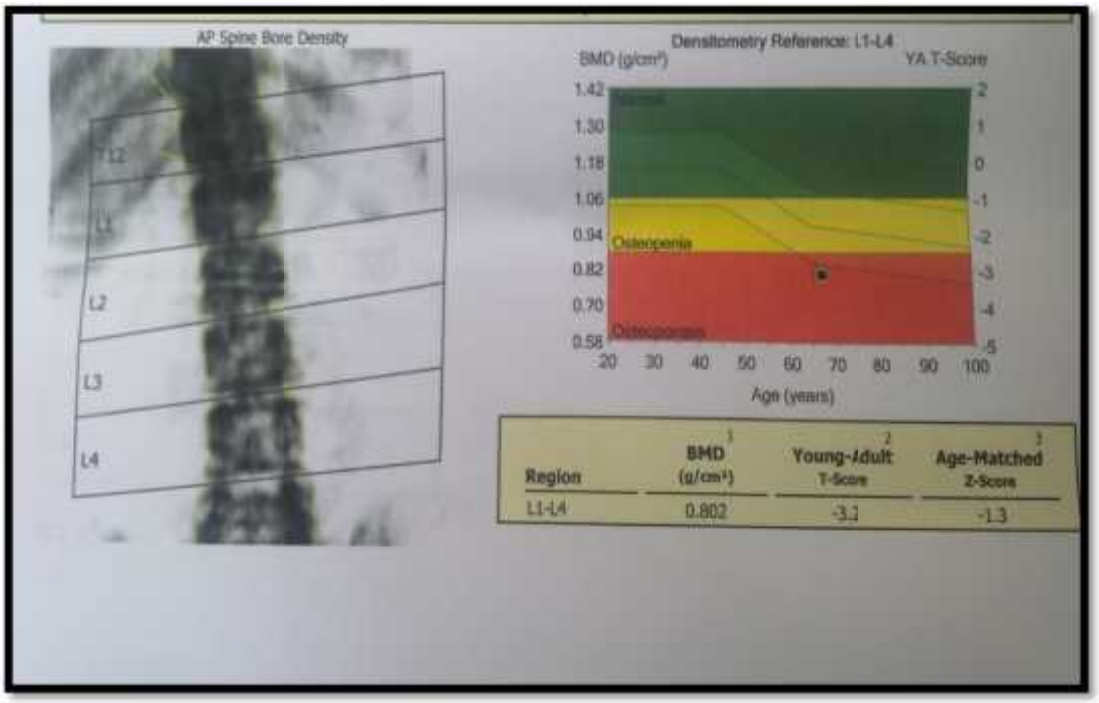
ANNEXURE III: PHOTOGRAPHS

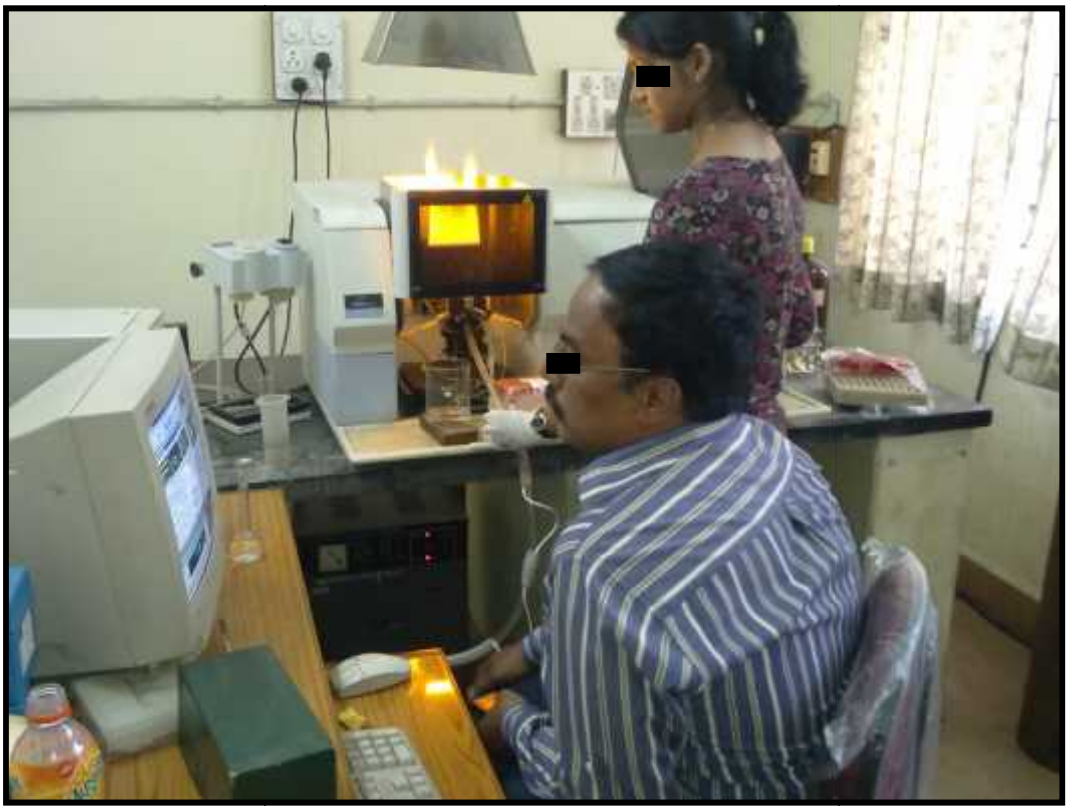
Photograph 1: DEXA Scan of Lumbar Spine



Photograph 2: DEXA Evaluation Apparatus

Photograph 3: DEXA Evaluation chart of the lumbar spine



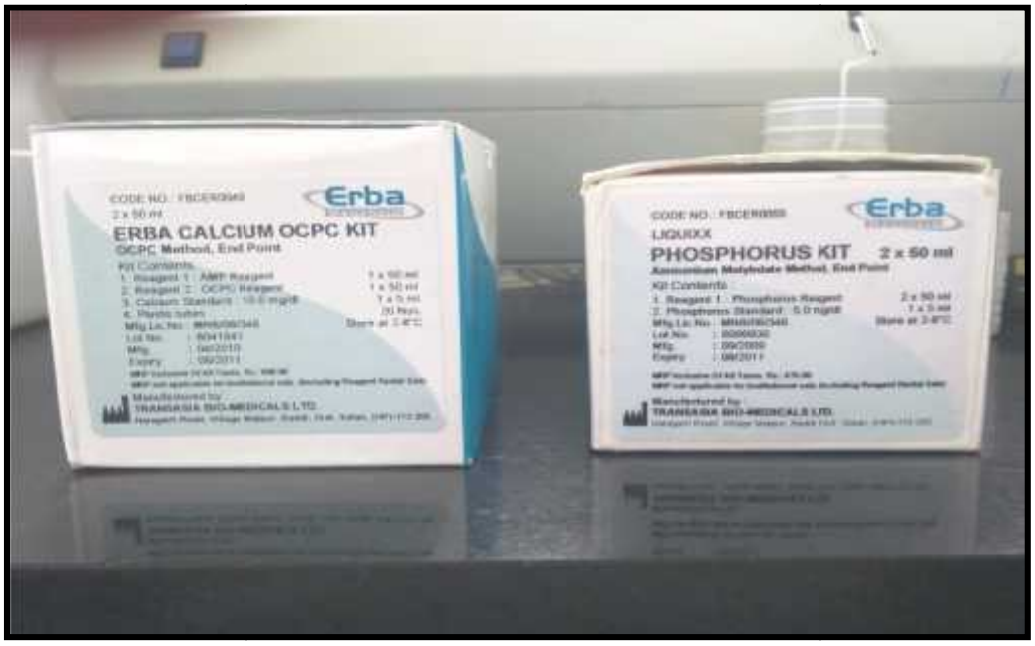


Photograph 4: Investigator performing analysis of serum zinc on Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

Estimation of Calcium, phosphorous and alkaline phosphatase

Photograph 5: ERBA Chem 5 Semi-auto analyser

Photograph 6:Automated pipettes used for various tests



Photograph 7: calcium and phosphorous kits

Photograph 8: Alkaline phosphatase and glucose kits

ANNEXURE-IV: MASTER CHART

MASTERCHART- DIABETICS

SL. NO	Name	Age(years)	Age at Menopause (years)	Diabetes Duration (years)	Height(cm)	Weight(kg)	BMI(kg/m^2)	BMD(g/cm^2)	T- score	Zn(µg/dL)	Ca(mg/dL)	P(mg/dL)	ALP(IU/L)	FSG(mg/dL)
1	PPV	55	46	10	157	70	28.6	0.815	-2.8	42	10	5.4	100	80
2	LDS	63	47	2	147	55	25.5	0.785	-3.3	69	8.3	5.1	56	88
3	SLK	57	45	6	150	55	24.4	0.731	-3.7	47	7	5.2	92	180
4	BGB	55	48	1	155	64	26.7	0.831	-2.5	62	10.9	3.2	78	148
5	GRP	60	47	3	154	48	20.24	0.801	-3.2	44	8	5.3	104	252
6	YSL	53	46	2	158	70	28	0.873	-2.7	68	8.6	4.5	93	175
7	SNR	50	44	2	148	70	32	0.865	-2.5	66	8.4	3.3	88	154
8	VJS	58	46	0.08	150	48	21.3	0.856	-2.7	45	6.8	5.5	100	272
9	SSG	47	45	0.08	158	64	25.6	0.81	-3.1	79	6.7	5.8	86	185
10	CYK	49	48	0.08	154	58	24.46	0.837	-2.9	78	9.6	5.8	132	256
11	CNH	60	48	1	151	66	28.94	0.841	-2.8	60	8.7	4.8	56	174
12	JYP	65	44	5	157.5	52	20.83	0.734	-3.7	45	8.3	4.5	104	130
13	HBJ	63	44	0.67	150	59	26.22	0.809	-3.1	54	6.7	4.8	122	100
14	RTK	52	45	3	146	70	32.84	0.903	-2.5	96	6.8	3.6	84	124
15	MHB	72	48	5	148	56	25.6	0.864	-2.6	66	9.5	5	130	305
16	RHK	70.4	47	5	154	62	26.14	0.712	-3.9	47	6.9	5.6	92	140
17	GK	68	48	1.5	141	63	31.7	0.876	-2.5	64	8.9	4.8	121	160
18	ML	60	46	15	160	80	31.25	0.871	-2.5	70	9.2	4	88	138
19	RA	69	49	2	141	50	25.2	0.833	-2.5	72	8	4.3	58	156
20	SJ	64	43	10	156	56	23	0.776	-3.4	45	6.3	4.5	57	155
21	BI	48.9	45	0.08	153	71	30.3	0.887	-2.6	60	8.8	4.1	77	305
22	SMV	57	45	2	152	76	32.9	0.903	-2.5	75	8.5	3.9	70	142
23	RHS	55	46	1	161	76	29.32	0.832	-2.5	62	8.8	4.3	68	106
24	PDP	52.4	46	0.50	150	73	32.44	0.904	-2.5	79	9.4	3.8	74	143
25	SGM	68.5	46	1.5	151	59	25.9	0.66	-2.8	55	6.8	4.3	112	248
26	MHD	57	48	3	158	47	18.8	0.9	-2.5	75	7.9	3.3	88	153
27	SRG	65	49	8	160	70	27.3	0.844	-2.8	57	8.3	3.8	92	92
28	SK	74	42	6	157	61	24.7	0.807	-3.1	50	7.2	6.2	111	146
29	AA	52.3	45	4	159	60	23.7	0.895	-2.5	77	8.7	3.9	107	106
30	APH	62.7	44	3	152	69	29.9	0.896	-2.5	63	8.6	3.4	134	121

MASTERCHART- NON-DIABETICS

SL. NO	Name	Age(years)	Age at menopause(years)	Height(cm)	Weight(kg)	BMI(kg/m^2)	BMD(g/cm^2)	T- score	Zn(µg/dL)	Ca(mg/dL)	P(mg/dL)	ALP(IU/L)	FSG(mg/dL)
1	SVP	55	47	156	55	22.6	0.823	-3	85	8.9	4.5	89	88
2	SNS	63	46	151	50	21.9	0.764	-3.5	79	7.8	5.2	102	94
3	MB	57	48	141	53	26.7	0.81	-3.1	82	7.9	4.9	99	92
4	AJ	54	47	150	39	17.33	0.704	-4	67	6.9	5	112	97
5	SG	60	46	154	54	22.8	0.723	-3.8	62	7.5	4.1	100	81
6	MLW	53	48	151	60	26.3	0.859	-2.6	67	10.2	4.2	88	79
7	MNG	50	47	153	51	21.8	0.891	-2.6	94	9.3	3.8	70	85
8	RKH	57.6	46	152	56	24.2	0.788	-2.9	76	7.8	4.6	71	98
9	JSH	46.8	45	157	62	25.2	0.836	-2.9	73	9.3	3.3	74	87
10	LXM	49.5	48	150	35	15.6	0.869	-2.5	63	8.9	3.5	55	90
11	GP	60	47	154	48	20.2	0.801	-3.2	57	8.4	3.9	88	84
12	KLW	65	45	162	50	19.1	0.675	-4.2	59	5.3	5.6	89	82
13	ANV	63	44	144.5	50	23.8	0.509	-5.6	47	5.9	5.2	111	97
14	PRM	52	48	156	66	27.1	0.876	-2.7	69	9.6	3.1	74	75
15	KLV	72	47	157.5	45	18.1	0.765	-3.5	53	7.8	3.2	80	88
16	SNT	70	48	140	44	22.5	0.767	-3.4	56	6.9	4	86	92
17	CCR	68	47	146	59	27.7	0.832	-2.9	99	7.8	3.8	91	91
18	AP	60.4	49	154	59	24.9	0.883	-2.5	92	9.8	2.8	95	86
19	SS	69	49	144	45	21.7	0.861	-2.7	65	10.4	5.8	87	81
20	SRD	64	48	162	59	22.5	0.764	-3.5	72	8.6	6.5	87	88
21	CNW	48.4	46	143.5	59	28.7	0.696	-4	52	7.5	4.8	104	93
22	MLT	57	46	154	41	17.3	0.866	-2.8	63	8.5	3.5	77	92
23	LXD	55.7	49	152	58	25.1	0.895	-2.5	94	9.4	4.8	61	90
24	SRK	52.7	48	140	41	20.9	0.795	-3.2	57	7.4	5	98	94
25	USH	68	46	138.5	50	26.1	0.602	-4.8	54	7.8	5.1	103	88
26	LXS	56.2	47	150	60	26.7	0.866	-2.5	62	8.6	5.2	59	73
27	RKG	65	49	155	70	29.13	0.832	-2.9	55	9	3.4	92	80
28	HKH	74.5	50	155	55	22.9	0.865	-2.5	67	10.8	3.5	83	97
29	BRM	52	46	160	76	29.7	0.778	-3.4	59	8.5	3.3	99	95
30	SVP	62	49	148	62	28.3	0.842	-2.8	66	9.6	4	93	71

KEY TO MASTER CHART

Sl. No	-	Serial number
Zn	-	Zinc
Ca	-	Calcium
P	-	Phosphorous
ALP	-	Alkaline Phosphatase
kg	-	Kilogram
m	-	metre
cm	-	centimetre
g	-	gram
mg	-	milligram
dL	-	decilitre
µg/dL	-	Microgram per decilitre
IU/L	-	International units per litre
FSG	-	Fasting Serum Glucose