RESEARCH ARTICLE

CORRELATION OF SERUM VITAMIN D AND BONE MINERAL DENSITY IN MALES MORE THAN 50 YRS WITH HIP FRACTURES

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ABSTRACT

Introduction: Bone strength is related to bone mineral density (BMD) as well as other properties of bone that are often termed bone quality .BMD correlates highly with fracture risk and allows the clinician to determine the need for pharmacological intervention. Vitamin D is vital for bone health because it assists in the absorption and utilization of calcium. There are various studies in elderly females with hip fractures correlating the Vitamin D levels with BMD. But very few studies have evaluated the serum Vitamin D levels and BMD in males more than 50 years.

Materials and Methods: Present study was conducted in the JIPMER Pondicherry between August 2012 and August 2013. A total of 41 male patients aged more than 50 years with fracture neck of femur and Intertrochanteric fracture were included. Blood samples were taken stored, processed and evaluated for vitamin D total (D2 and D3).BMD measurements were done in B/L hip region. The Values of Vitamin D obtained are shown into three groups as 0-20ng/ml-deficiency, 20-30ng/ml as insufficiency and>30ng/ml as normal Reference. BMD reference values were taken as T score <2.5 is osteoporosis.

Results: On analysis of Vitamin D we found that out of total 41 patients 9(22%) had vitamin D level <20ng /ml, 15(36%) had levels between20ng-30ng/ml and 17 (41%) had >30ng/ml. Of the 21 patients with Neck of femur fractures Vitamin D<20ng/ml are6(28.6%),20-30ng/ml are 9 (42.9) and >30ng/ml are 6(35.3).Vitamin D levels in 20 intertrochanteric fractures are<20ng/ml in 3(15%), 20-30ng/ml in 6(35%) and >30ng/ml in 11(55%).

Conclusion: We found 24 patients (58%) have vitamin D level <30ng/ml, more in patients with neck femur fracture than intertrochanteric fractures. Present study found that there is no statistically significant correlation between the vitamin D level and BMD in Hip fractures in elderly males (p=0.489).

INTRODUCTION

As elderly population continues to rise globally, osteoporosis became a major risk factor for fractures among elderly population in the modern world. Osteoporosis represents an increasingly important clinical and public health problem among older men and women (Cauley, 2002). Global demographic changes are expected to dramatically increase the burden of osteoporosis. By 2050, it is estimated that the number of individuals aged 65 and older will be nearly 1.55 billion worldwide. The increase among the aged population would result in an almost four fold increase in the number of hip fractures worldwide (Dhanwal *et al*., 2011). Osteoporosis can affect any bone in the body. The most typical sites of fractures related to osteoporosis are hip, spine and wrist.

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Fracture sites have been shown to be related to age. Wrist fractures are common in 50yrs, spine in 60yrs and hip fractures in the 70yrs. The rates of all three types of fracture increases with age, but the increased risk with age is most pronounced with hip fractures (Sadat-Ali *et al*., 2011). For men the life time risk of osteoporotic fracture is about 13% after age 50 and the site specific fracture risks are 6% for the hip, 5% for the spine, and 3% for the wrist. Of the three most common sites for osteoporotic fractures hip fractures pose the most significant insult to health status of individual .Increased mortality with hip fractures is related to the coexistence of medical comorbidities (Cooper *et al*., 1992). The most significant increase in hip fracture rates is expected to occur in third world countries particularly in Asia (Sadat-Ali *et al*., 2011). An estimated 1.3–1.7 million hip fractures occurred worldwide in 1990. By 2025, this number is expected to increase to almost 3million (Kanis *et al*., 2004). Vitamin D is vital for bone health because it assists in the absorption and utilization of calcium.

The major source of vitamin D is sunlight which the human body absorbs by exposure to sunlight through the conversion of precursors in the skin to active vitamin D. While considering the geographic distribution of osteoporosis and related fractures, deficiencies in vitamin D cannot be ignored. Vitamin D deficiency may predispose individuals to developing osteoporosis and subsequently osteoporotic fracture (Bischoff-Ferrari *et al.*, 2004). Bone strength is related to bone mineral density (BMD) as well as other properties of bone that are often termed bone quality .BMD correlates highly with fracture risk and allows the clinician to determine the need for pharmacological intervention. In clinical practice various determinants of bone quality are not generally measurable and therefore cannot help the clinician predict fracture risk, therefore the corner stone for evaluation is the measurement of BMD.

For hip fractures the use of BMD at each age outperformed the use of clinical risk factors alone as a predictive value .So that BMD improved sensitivity without loss of specificity for the prediction of osteoporotic fractures. BMD which measures the quantify of calcified bone is at present the gold standard for diagnosing of osteoporosis and osteopenia. There are various studies in elderly females with hip fractures correlating the low BMD with Vitamin D levels. But very few studies have evaluated the serum Vitamin Dlevels in elderly males. Keeping in mind the enormity of the problem and sparse Indian studies the present study was carried out to assess how vitamin D levels will correlate with the BMD in more than 50yr old men who sustain Hip fractures.

Aims and objectives

Aim

To assess the relationship of serum Vitamin D and BMD in males older than 50yrs with hip fractures.

Objectives

1. To assess the Serum Vitamin D level in males older than 50yrs with hip fractures.
2. To assess the BMD in male patients older than 50yrswith hip fractures.
3. To assess the correlation between BMD and Vitamin D in males older than 50yrs with hip fractures.

Review of literature

Demographic profile and risk factors of osteoporosis and fractures

A meta-analysis evaluating RCTS on assessment of BMD and treatment for osteoporosis in older individuals with fractures showed that less than 20 % of patients hospitalized with hip fractures are evaluated for bone density. Additionally less than 10% are ever treated with anti resorptive agents to prevent future fractures (Feldstein Feldstein *et al.*, 2003). A study by Peggy *et al.* (Cawthon *et al.*, 2009) estimated the loss of Femoral neck BMD and found that loss of BMD in men 85yrs of age (0.021 g/cm2) was 2.5 times greater than the loss

expected for men 65yr of age (0.008 g/cm2). Such bone loss in 85-yr-old men may be sufficient to increase the risk of hip fracture by 25%. Hypovitaminosis D affects both men and women in all age groups but mainly depends on external factors. However, there is evidence that women have lower levels than men in elderly Europeans (Van Dam *et al.*, 2007).Varying prevalence of Vitamin D deficiency in the elderly has been reported globally. One of the highest prevalence among postmenopausal women reported, in recent times has been from Croatia and France, where 92.5% and 89.9% of women had Vitamin D insufficiency (Marwaha *et al.*, 2011).

Hip fractures

Hip fractures are the most serious osteoporotic fractures and most of them follow a fall from the standing position, although they may also occur spontaneously. They are painful and nearly always necessitate hospitalization. There are, broadly speaking, two types of hip fracture, intracapsular (cervical or femoral neck fractures) and extracapsular (trochanteric) fractures, which differ somewhat in both natural history and treatment. Trochanteric fractures are more characteristically osteoporotic, and the increase in age- and sex specific risk of hip fracture is greater for trochanteric than for cervical fractures, and is more commonly associated with prior fragility fractures. In many countries they occur with equal frequency, though the average age of patients with trochanteric fractures is approximately 5 years older than that for patients with cervical fractures (Maggi *et al.*, 1991; Adebajo *et al.*, 1991).

Diagnosis of vitamin D deficiency

Vitamin D deficiency often presents with common, non-specific symptoms, such as muscular weakness predominantly of the proximal limb muscles, a feeling of heaviness in the legs, chronic musculoskeletal pain, and fatigue or easy tiring. However, the vast majority of cases are asymptomatic. The classic presentation of severe vitamin D deficiency is metabolic bone disease, osteomalacia in adults and rickets in children. Stress fractures in otherwise healthy adolescents and adults may indicate vitamin D deficiency. Radiograph of the wrist, alkaline phosphatase, and 25 (OH) vitamin D level are used for the diagnosis of vitamin D deficiency.Vitamin D deficiency is endemic in India despite the abundance of sunshine.

The interpretation of vitamin D levels was done with the solar zenith angle, minimal erythema dose, skin type, UV Index and geographical location. All studies have uniformly documented low dietary calcium intake compared to its Recommended Daily/Dietary Allowances (RDA) by Indian Council of Medical Research (ICMR). The vitamin D status of children is very low in both urban and rural population studied. Pregnant women and their new born had low vitamin D status. The effect of short course of loading doses of vitamin D does not have a lasting effect and a maintenance dose is needed. Low 25(OH) D levels have its implications of lower peak bone mass and lower BMD compared to west. There may be a public health need to fortify Indian foods with vitamin D (Harinarayan *et al.*, 2009). Severe and prolonged deficiency of vitamin D results in rickets in children and osteomalacia in adults, conditions

characterized by defective mineralization of boneosteomalacia will aggravate osteoporosis. Since both of them increases the risk of fracture. Vitamin D deficiency is rare in Europe and the USA, but is still common in the Middle East and the Asian subcontinent (Compston, 2004). A positive association between serum,25-dihydroxycholecalciferol concentration and BMD was found in middle-aged and elderly women, whereas an inverse relationship between serum PTH levels and BMD has been reported. Vitamin D supplementation prevents the reduction in BMD that occurs during the winter months in normal subjects. Trials of the administration of calcium and vitamin D to institutionalized elderly people have shown that relatively small amounts of vitamin D reduce non-vertebral fracture rates [18.] Maintaining an adequate vitamin D status in the elderly may also improve muscle strength and reduce both the risk and consequences of falling (Pfeifer and Minne, 1999).

BMD

Peak bone mass

The "peak bone mass" is the amount of bone tissue present at the end of skeletal maturation[20]. It is a major determinant of the risk of fracture due to osteoporosis since the mass of bone tissue at any time during adult life is the difference between the amounts accumulated at maturity and that lost with ageing. There is, therefore, considerable Interest in exploring ways to increase peak bone mass. Epidemiological studies indicate a 10% increase in peak bone mass in the Caucasian Female population would decrease the risk of hip fracture by about 30%. Such an increase would roughly correspond to the difference between peak bone mass in male and females as measured at the radial or femoral diaphyseal site (WHO, 1994).

Measurement of bone mass

Most information in the characteristics of skeletal growth during childhood and adolescence has been obtained by non-invasive techniques that enable bone mass to be measured at various sites in the skeleton with great precision and accuracy. The bone mass of a particular part of the skeleton is directly dependent on both the volume or size of the part concerned and the density of the mineralized tissue contained within its periosteal envelope.

The mean volumetric mineral density of bony tissue (in grams of hydroxyapatite per cm^3) can be determined non-invasively by quantitative computed tomography (QCT). The so-called "areal" or "surface" bone mineral density (BMD in g of hydroxyapatite per cm^2) can be determined by single- or dual-energy X-ray absorptiometry (SXA and DXA). The values generated by these techniques are directly dependent on both the size and integrated mineral density of the scanned skeletal tissue. The integrated mineral density is determined by cortical thickness, the number and thickness of the trabeculae, and the "true" mineral density corresponding to the amount of hydroxyapatite per unit volume of the bone organic matrix. Although the term BMD, without the additional "areal" qualification, is widely used, SXA and DXA do not measure the volumetric density (Gilsanz, 1998). The BMD is the summation of several structural components which may evolve

differently in response to genetic and environmental factors (Seeman and Hopper, 1997). Nevertheless, the term remains of clinical relevance in the assessment of gain or loss of bone mass, since BMD is directly proportional to bone strength, i.e. to the resistance of the skeleton to mechanical stress, both in vivo and in vitro.

Defining Osteoporosis by BMD

The World Health Organization (WHO, 1994) has established the following definitions based on BMD measurement at the spine, hip or forearm by DEXA devices.

Normal

BMD is within 1 Standard deviation of a "young normal" adult (T-score at -1.0 and above).

Low bone mass ("osteopenia")

BMD is between 1.0 and 2.5 SD below that of a "young normal" adult (T-score between -1.0 and -2.5).

Osteoporosis

BMD is 2.5 SD or more below that of a "young normal" adult (T-score at or below -2.5). Patients in this group who have already experienced one or more fractures are deemed to have severe or "established" osteoporosis.

Sex

Ross *et al* in their study found that osteoporosis in men based on BMD showing a strong relationship between bone density and fracture risk in men like that seen for women (Ross *et al*., 1993).

Among 1355 Japanese– American men, the incidence of vertebral fractures was increased significantly by 1.8-fold and 1.5-fold for every 1 SD decrease in BMD at the proximal and distal radius, respectively; the age-adjusted for other nonviolent fractures was 1.3 at both sites and was not statistically significant (Stegman *et al*., 1995). The age-adjusted a low trauma fracture among 498 Nebraska men was 1.7 and 1.8 per 1 SD decrease in BMD of the distal radius and ulna, respectively, (Lenchik *et al*., 2002) There was a similar relationship between distal radius BMD and fragility fractures in 654 Swedish men (Close *et al*., 2005).

Osteoporosis in men

With the aging of the population, osteoporosis in men is becoming an increasingly important public health problem. Aging men lose bone mineral density (BMD) at a rate of approximately1%per year, and one in five men over the age of 50yrs will suffer an osteoporotic fracture during their lifetime. Of all osteoporotic fractures, hip fractures contribute to the greatest morbidityand mortality, and almost 30% of all hip fractures occur in men (Kanis *et al*., 2004). The gender ratio of hip fracture varies geographically and by ethnic group. The gender ratio of hip fracture is 1.5 among US blacks compared

to 2.9 among US whites. Hip fracture rates are similar in African American women and Caucasian US men. The prevalence of vertebral fracture is also similar in Both groups. Trabecular and cortical BMD are similar in white men and Afro American women, but there are no ethnic differences in vertebral cross-sectional areas [43].

Age

According to data from the National Center for Injury Prevention and Control, US seniors aged 85 years and older had a much higher crude non-fatal and fatal fall rate compared to senior's aged 65 to 69 years (nonfatal:10.7 vs 2.5 per 100 persons and fatal: 143.7 vs 9.2 per100,000 persons) (Vondracek and Linnebur, 2009).

Sheryl F Vondracek *et al* found that Bone mineral density testing with central dual energy x-ray absorptiometry is essential and cost-effective in older population. It is important to remember that falls play a very important role in the risk for osteoporotic fractures, especially in the older senior. All older seniors should be evaluated annually for falls and strategies should be implemented to reduce fall risk in this population. The risk for vitamin D insufficiency and deficiency is high in the older senior and can contribute to falls and fractures (Vondracek and Linnebur, 2009).

Vitamin D

1, 25-dihydroxyvitamin D3 is the major steroid hormone involved in the mineral ion homeostasis regulation. Vitamin D and its metabolites are hormones and hormone precursors rather than vitamins.

Factors affecting vitamin D levels

Latitude, season, time of day, air pollution, cloud cover, melanin content of the skin, use of sun-blocking agents, age and the extent of clothing covering the body. An increase in the zenith angle of the sun during winter, early morning and late afternoon results in a longer path length for the UVB (ultraviolet) photons to travel through the ozone layer which efficiently absorbs them. Due to this vitamin synthesis occurs only between 10 am and 3 pm. Excessive exposures to sunlight will not cause vitamin D intoxication because sunlight degrades any excess pre-vitamin D3 and vitamin D (Chen *et al*., 2007).

Functions of vitamin D

1, 25 hydroxyvitaminD is recognized by osteoblasts receptors causing an increase in the expression of receptor activator of NFκBligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL and induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. It induces the expression of bone matrix proteins, osteocalcin and osteopontin. Adequate calcium and phosphorus levels promote the mineralization of the skeleton and maintain neuromuscular function. In kidney it increases the absorption of calcium and phosphorus (Holick, 2007).

Vitamin D levels

The reference level of serum 25 hydroxy vitamin Dis 30-60 ng/mL.Vitamin D insufficiency is defined by the concentration of serum 25 hydroxyvitaminD below 30ng/mL and deficiency below 10ng/mL (Kennel *et al*., 2010)

- Levels needed to prevent rickets and osteomalacia ~15ng/ml.
- Level that suppresses parathyroid hormone levels ~20 – 30 ng/ml.
- Levels needed to optimize intestinal calcium absorption~ 34ng/ml.
- Better neuro-muscular performance ~ 38ng/ml.
- Reduction in incidence ofintestinal cancers~ 38ng/ml.
- Level to reduce the risk of incident and worsening joint pain is 40ng/ml (Cannell and Hollis, 2008).

Relation of Vitamin D with BMD

Study by Hannan MT *et al* confirms that substantial racial and ethnic group differences in BMD and serum 25(OH) D in men. Serum 25(OH) D and BMD are significantly related to one another in White men only. This may have implications for evaluation of bone health and supplementation in men with low levels of 25(OH) D (Hannan *et al*., 2008). Kota *et al.* studied correlation between BMD and Vitamin D in a patients with or without fragility fractures they found there is no significant relation between BMD and Vitamin D (Kota *et al*., 2013). Gutierrez *et al* done a cross-sectional analysis of relatively young adults showed that BMD significantly decreased with declining 25 (OH) D concentrations in whites and Mexican-Americans, but not in blacks (Gutiérrez *et al*., 2011).

Aloia*et al*analyzed the change in BMD in 208 postmenopausal African-American women randomly assigned to receive either vitamin D3 supplementation or placebo for 3 years. In contrast to analogous studies of white subjects, they found no significant differences in BMD loss between the intervention groups despite a significant increase in 25(OH) D concentrations in the active group as opposed to the control groups (Aloia *et al*., 2005).

MATERIALS AND METHODS

Present was conducted in the Department of Orthopaedics in collaboration with the Department of Biochemistry, JIPMER Pondicherry, India. Approval was obtained prior to the study from JIPMER Scientific advisory committee and ethical clearances were obtainedInstitute Ethical committee (Human Studies) [Reference Notice .IEC /SC/2012/4/162.].This is a cross sectional study of single group. Subjects were enrolled in the study based on inclusion and exclusion criteria.

Inclusion criteria

All male patients aged more than 50 years presenting to orthopedic department with

1. fracture neck of femur

2. Intertro chanteric fracture

Exclusion criteria

1. Patients who are taking calcium and vitamin D3 supplementation.
2. Patients with Secondary osteoporosis
3. Patients with Pathological fractures/Malignancy
4. Patients Bilateral hip fractures
5. Patients with Associated Acetabular and Pelvic fractures
6. Patients with Open hip fractures
7. Patients undergone small and large intestine surgeons & renal problems.

This study was conducted from August 2012 to August 2013. All the patients presenting to the department of orthopedics in emergency and OPD with proximal femoral fractures satisfying inclusion criteria were included into the study. The demographic details of the patient were collected and recorded asperproforma in Appendix 1. A valid informed consent in English and Vernacular language was taken in the format approved by the institute ethical committee.

The patients were then subjected to x ray pelvis with Bilateral Hips and lateral view of affected hip. Five ml of Blood was collected from the ante cubital vein, the samples were centrifuged and serum was separated aliquoted and stored in -70^0centigrade. The stored samples were processed by using immunodiagnostic 25hydroxy vitamin D total (D2 and D3) Enzyme linked Immunosorbentassay (ELISA) kit [KAP1971DIA source Immuno Assays S.A Rue du Bosquet,2 B-1348 Louvain-la Neuve, Belgium] and the values obtained were expressed in ng/ml. Values of Vitamin D obtained are shown into three groups as 0-20ng/ml-deficiency, 20ng/ml-30ng/ml as insufficiency and>30ng/ml as normal Reference.

Vitamin D Assay

Immunoenzymetricassay for the in vitro quantitative measurement of 25-hydroxyvitaminD2and D3 (25OH-D2and 25OH-D3)in serum. Vitamin D is the generic term used to designate Vitamin D2 or ergoclalciferol and vitamin D3or Cholecalciferol.vitaminD2 is metabolised in a similar way to vitamin D3 both contribute to the overall vitamin D status of an individual it is the reason why it is very important to measure both forms of 25hydroxy vitamin D equally for a correct diagnosis of vitamin D deficiency, insufficiency or intoxication.

Principle

The 25OH Vitamin D total ELISA is a solid phase enzyme linked immunosorbent Assay performed on microtiter plates. During first 2hrs incubation step ,at room temperature, total 25 OH vitamin D(D2and D3)present in calibrators, control and sample is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody .After one washing step a fixed amount of 25OH Vitamin D labelled with biotin in presence of horsedish peroxide(HRP),compete with unlabelled 25OH vitamin D2and25OH vitaminD3present on the binding sites of the specific monoclonal antibody.

After 30 minutes incubation at room temperature the microtiter plate is washed to stop the competition reaction The chromogenic solution (TMB) is added and incubated for 15 minutes .The reaction is stopped with the addition of stop solution and the microtiterplate is then read at the appropriate wave length .The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH vitamin D (D2 and D3) concentration. Calibration curve was plotted and the total 25OH vitamin D(D2and D3)concentrations of the sample are determined by dose interpolation from the calibration curve.

Procedure

Vitamin D

Number of strips for the run was selected.
↓
50ul of each calibrator, control and sample was pipetted into the wells
↓
150ul of incubation bufferwas pipetted into all the wells
↓
All wells were Incubatedfor 2 hours at room temperature on a plate shaker (300 to 700rpm)
↓
Working HRP conjugate solution was prepared.
↓
Aspiration of liquid was done from each well.
↓
Plate washed 3 times by dispensing 0.4ml of wash solution into each well and aspirate content of each well
↓
200 ul of working HRP solutionwas pipetted into each well and incubated on the micro titre plate for 30 minutes at room temperature on a plate shaker
↓
Plates were washed 3 times by using 0.4 ml wash solution into each well and aspirating content into each well
↓
100ul of chromogenic solution was pipetted into each well within fifteen minutes following washing step
↓
Micro titre plate was incubated for 15 minutes at room temperature on a plate shaker
↓
100ul of stop solution was pippeted into each well
↓
Absorbance were read at 450 nm (reference filter 630 nm or 650 nm) with in 1 hour

$$\text{Calculation of B/B0} = \frac{\text{OD (calibrator, control, sample) x100}}{\text{OD (zero calibrator)}}$$

Reference range:30-120ng/ml.

Bone mineral density

Patients who satisfied the inclusion criteria were subjected to BMD of bilateral hips.

In this study, all DEXA measurements were performed and analysed by technician trained by NORLAND and certified by the International Society for Clinical Densitometry Germany. Estimation of BMD was done on both the hips on fracture side and non fractureside. It was estimated at femoral neck, Greater trochanter and Total Hip .T scores and Z scores were obtained and analyzed.

Table 1. Definition of T score and Z score[21]

T-SCORE	T score is the number of standard deviations a patient BMD value is above or below a young reference value for individuals of same ethnic group
Z-SCORE	The Z score is the number of standard deviations that the patients BMD value is above or below reference value for individual of same age ethnic back ground and gender

Table 2. WHO Classification of T-score[21]

0 to -0.99	Normal
-1 to -2.499	osteopenia
≤ -2.5	osteoporosis
≤-2.5 with fracture	severe osteoporosis

Statistical analysis

All the data were tabulated and recorded in Microsoft excel 2007. Analysis was done by IBMs statistical package for social sciences (SPSS) version 20.0 (2011). Mean and standard deviation for all obtained values were done correlation analysis were performed between BMD and vitamin D and scatter plot was also given. All statistical analysis was carried out for 95% confidence interval and p value <0.05 was considered as significant.

RESULTS

The present study was a cross sectional study conducted during study period.

Fracture types

Of the total 41 patients evaluated 21 had fracture neck of the femur and 20 patients had intertrochanteric fractures as shown in Figure 8.

Age

Total 41 patients included in the study. The youngest is 50yrs and oldest is 79yrs with a mean age of 62.20yrs. Eighteen patients were in age group of 50-59yrs,15 patients in age group of 60-69 ,8 patients are in age group >70yrs. The same has been represented in Figure 9.

**Distribution of fractures types in different age groups**

Of the total 18 patients in the age group 50-59, 7(32.8%) patients had intertrochanteric and 11(68.1%) had fracture neck of the femur. In the age group 60-69, out of 15 patients 6(40%) had intertrochanteric fractures and 9(60%) are neck of femur fractures. In the age group 70-79 out of 8 patients 7(90%) had intertrochanteric and 1 (10%) had fracture neck of the femur same shown in Table 3.

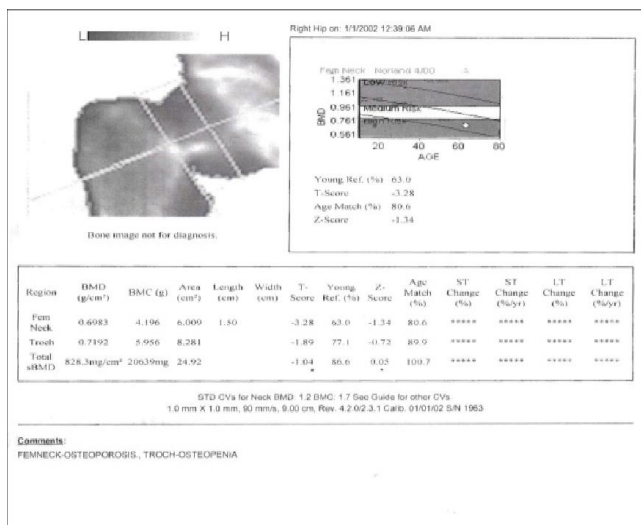**(A)**
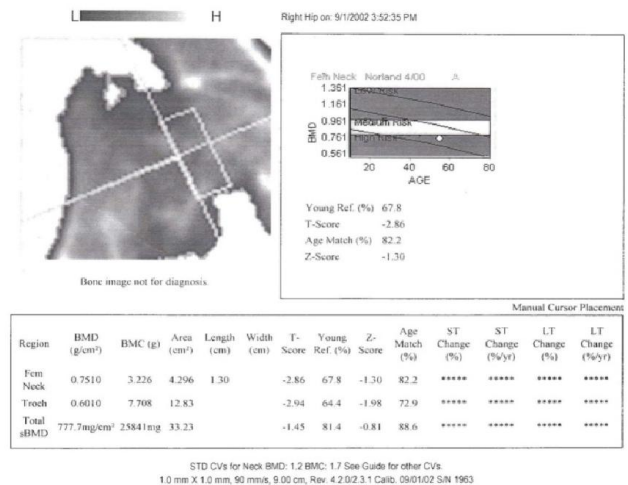
(B)
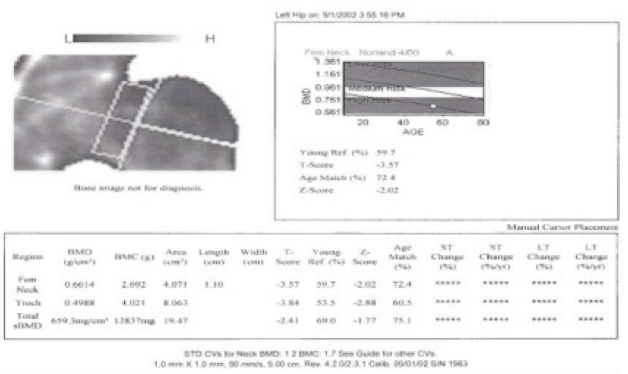
(C)

Figure 1. A X ray pelvis with bilateral Hip in a 50yr old showing Intertrochanteric fracture on right side B and C: DEXA scan report of the same patient on fracture side and non fracture side
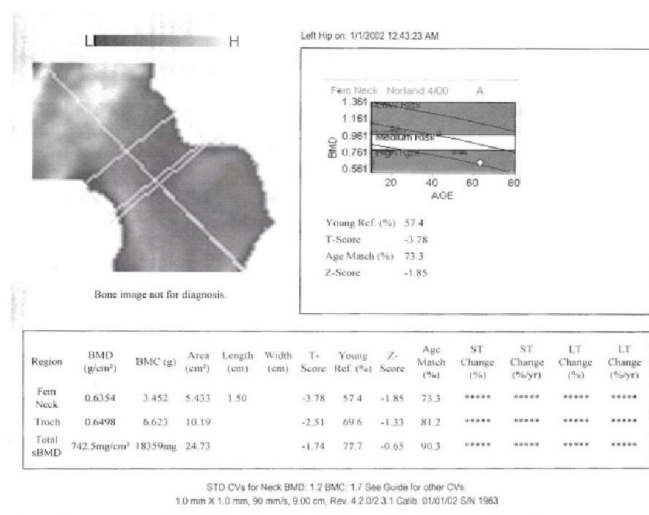
(A)

(B)

(C)

Figure 2. A: X ray pelvis with bilateral hip in a 60yr old showing fracture Neck of femur on right side. B and C: DEXA scan report of the same patient on fracture side and non fracture side
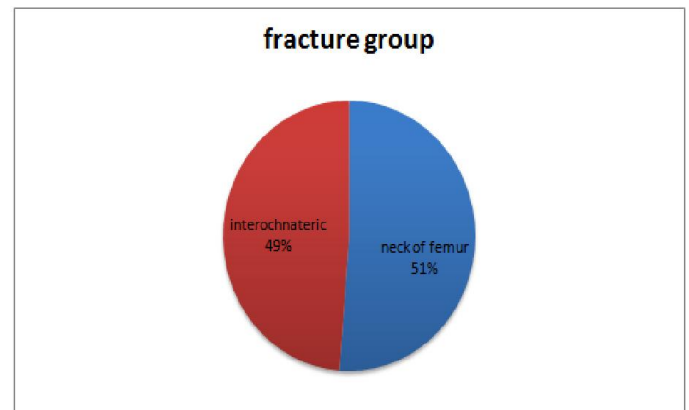

Figure 3. Showing the Bone Densitometer (NORLAND) used in the study

Figure 4. Piechart showing Distribution of fracture types in the study population
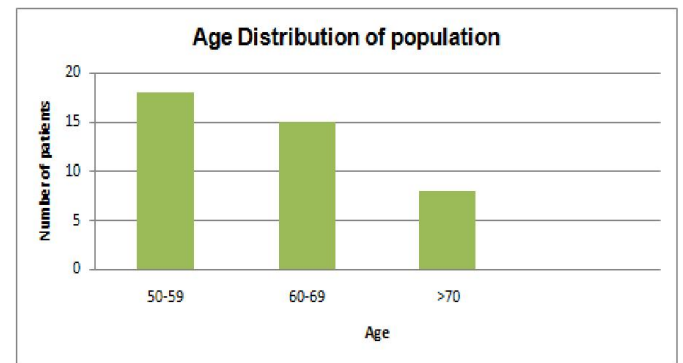

Figure 5. Bar chart showing Age distribution of study population

Table 3. Distribution of fractures types in different age groups

Age(years)	Intertrochanteric(%)	Neck of femur(%)	Total(%)
50-59	7(32.8)	11(68.1)	18(100)
60-69	6(40)	9(60)	15(100)
70-79	7(90)	1(10)	08(100)

Time duration between injury to presentation

Patients included in the study presented to our department at variable time since injury. Nearly 82.92% (34) patients

presented with 10 days of injury and remaining presented after 10 days.As most patients were presented with in ten days after injury in our study population there was not much variation in assessment as only 7 patients presented after 10 days of trauma same is shown in Table 4.

Table 4. Time duration betweeninjury to presentation

Duration(days)	Number of patients	Percentage (%)
<10 days	34	82.92
>10 days	7	17.08

BMD distribution among different age groups on fracture side

Among the total T score on fracture side we found that 11(26.8%) patients had osteoporosis, 17(41.5%) patients had osteopenia, and 13 6(31.7%) had normal values. It shows that 63.3 %(28) patients had low BMD on fracture side. On analysing we found that there was no significant association between age group and total T score on fracture side as p value is 0.868 .The distribution of T scores in relation to age group has been shown in table 5 and Figure 6.
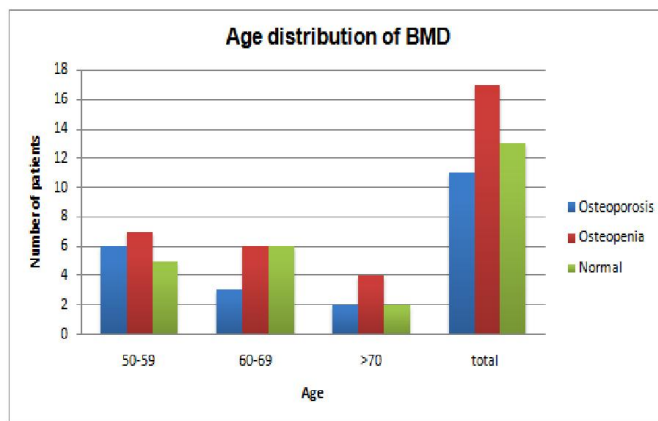

Figure 6. Bar chart showing BMD distribution among different age groups on fracture side

Distribution of vitamin D among different age groups:

On analysis of Vitamin D in relation to different age groups we found that in the age group of 50-59yrs 4(22%)showing <20ng/ml ,8 (44%) showing 20-30ng/ml and6(33%) showing

Table 5. BMD distribution among different age groups on fracture side

Age group	Osteoporosis (%)	Osteopenia (%)	Normal (%)	Total	P value
50-59	6(33.3)	7(38.9)	5(27.8)	18 (100)	0.868
60-69	3(20)	6(40.0)	6(40)	15(100)	
>70	2(25)	4(50)	2(25)	8(100)	
Total	11(26.8)	17(41.5)	13(31.7)	41(100)	

Table 6. Correlation between fracture type and BMD

Fracture	Normal (%)	Osteopenia (%)	Osteoporosis (%)	Total (%)	P value
Neck of femur	6(28.5)	8(38)	7(33.3)	21(100)	0.986
Intertrochanteric	7(35)	9(45)	4(20)	20(100)	
Total	13	17	11	41	

Table 7. Distribution of vitamin D among different age groups

Age	<20ng/ml (%)	20-30ng/ml (%)	>30ng/ml (%)	Total (%)	P value
50-59	4(22)	8(44)	6(33)	18(43.9)	0.593
60-69	3(20)	5(33.3)	7(46.6)	15(36.6)	
70-79	2(25)	2(25)	4(50)	8(19.5)	
Total	9(22)	15(36)	17(41)	41(100)	

Table 8. Correlation of BMD between fracture side and non fracture side

BMD	Group	Mean	SD	r value	P value
Total T score	Fracture	-1.55	1.29	0.622	<0.001
	Non Fracture	-1.88	1.00		
Total Z score	Fracture	-0.49	1.46	0.729	<0.001
	Non Fracture	-0.91	0.96		

Correlation between fracture type and BMD

Of the total 41 patients 21 had fracture of the neck femur, in these 7(33.3%) showing osteoporosis, 8 (38%) showing osteopenia, 6 (28.5%) were normal. Among 20 intertrochanteric fractures, 4(20%) had osteoporosis, 9(45%) had osteopenia, 7(35%) were normal, same shown in table 6 figure7.We found that there was no association between fracture types and BMD.

>30ng/ml .In age group of 60-69, 3(20%)had <20ng/ml,5(33.3%)had values between 20-30ng/ml ,7(46.6%) had >30ng/ml. In age group of 70-79 2 (25%) had <20ng/ml, 2(25%) had values between 20-30ng/ml, 8(19.5%) had >30ng/ml. Vitamin D less than 30ng/ml same shown in table 7 and figure We found there was no association between age and Vitamin D as P value is 0.593.
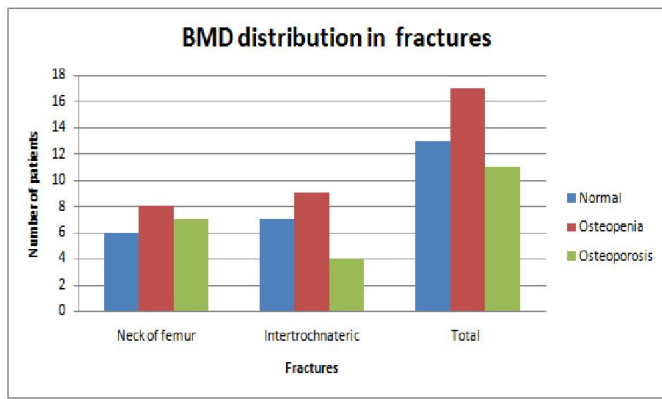
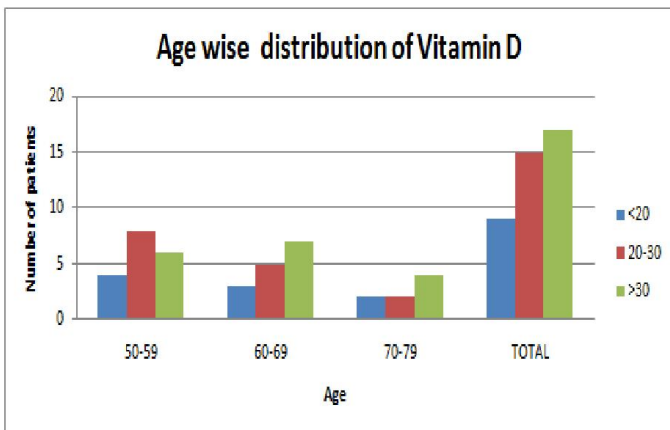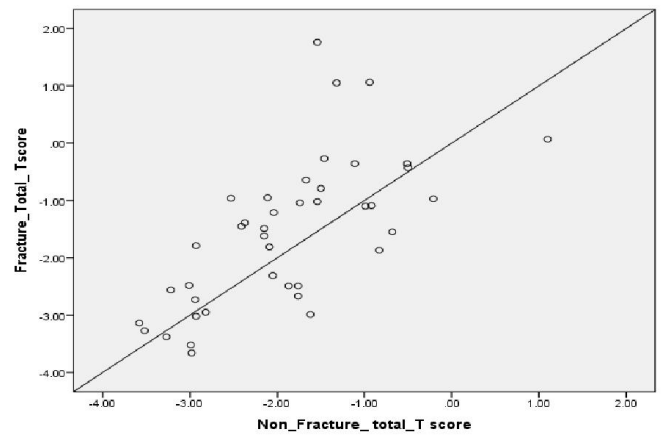**Figure 7. Bar chart showing BMD distribution among fractures**

(A)

Figure 8. Bar chart showing Distribution of Vitamin D among different age groups

(B)

Figure 10. (A &B) Scatter diagram showing correlation between the fracture sidesZ score with non fracture side Z score

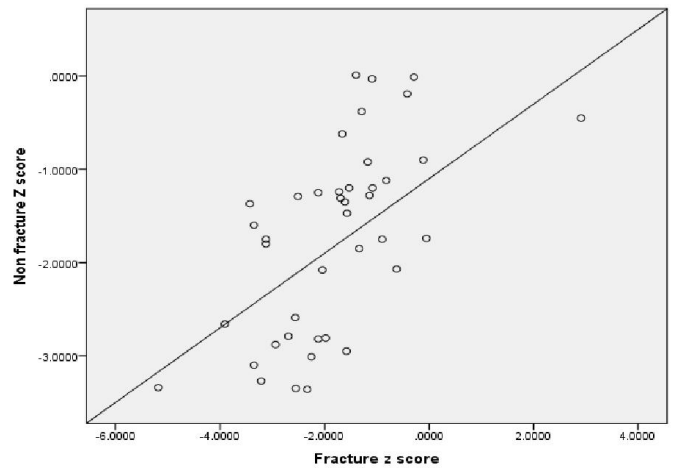## Correlation of BMD between fracture side and non fracture side

The mean value of total T scores on fracture side is -1.55 and on non fracture side is -1.88.Total T score on fracture side and non fracture side was showing significant correlation as both sides showing similar mean BMD and P value is <0.001.same shown in table 8 .Mean total Z scores on fracture side is -0.49 and on non fracture side is -0.91.Total Z score on Fracture side and non fracture side was showing significant correlation as both sides showing similar mean BMD and P value is <0.001 same was shown as scatter diagrams in table 8 and Figure9 and 10.

Correlation between Neck and Trochanter BMD values between fracture and non fracture sides

The mean value of neck T score and Z score on fracture side is -3.44 and -1.57 and on non fracture side is -3.28 and 1.37 respectively. On analysis we found that there is correlation between T scores and Z scores as mean values of T scores and Z scores are within same range p value is<0.001 same shown in Table 9. Mean values of Trochanteric T score and Z score on fracture side is -2.7and -1.6 and on non fracture side is -3.08 and -1.9 respectively.We found that there is significant correlation between T scores and Z scores as mean values are within same range p value is <0.001same shown in Table 9.

Table 9. Correlation between Neck and Trochanter BMD values between fracture and non fracture sides

BMD	Group	Mean	SD	r value	P value
Neck T score	Fracture	-3.44	1.18	0.580	<0.001
	Non fracture	-3.28	0.95		
Neck Z score	Fracture	-1.57	1.24	0.636	<0.001
	Non fracture	-1.37	1.02		
Troch T score	Fracture	-2.79	1.19	0.595	<0.001
	Non fracture	-3.08	1.06		
Troch Z score	Fracture	-1.68	1.16	0.672	<0.001
	Non fracture	-1.93	1.04		

Distribution of Vitamin D among fracture groups

Of the total 41 patients 9 (22%) had vitamin D level <20 ng /ml, 15 (36%) had levels between 20ng-30ng/ml and 17 (41%) had >30ng/ml. Of the 21 patients with Neck of femur fractures Vitamin D<20 are 6(28.6%), 20-30 are 9 (42.9) and >30 are 6(35.3).

Total 20 with intertrochanteric fractures Vitamin D<20 are 3(15%), 20-30ng/ml are 6(35%) and >30 are 11(55%). We didn't found association between fracture types and Vitamin D as P value was 0.489 same shown in Table 10 and Figure 11.

Table 10. Distribution of Vitamin D among fracture groups

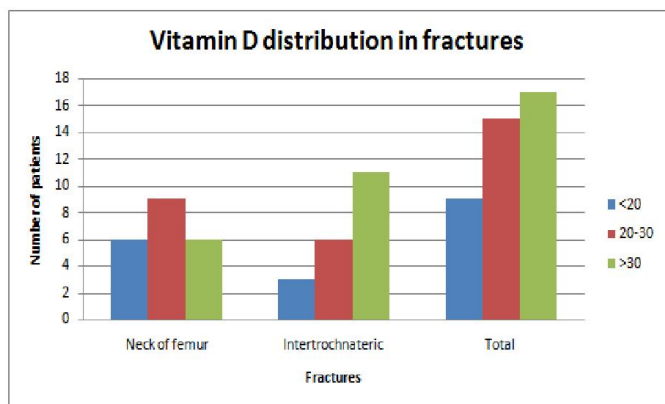| Fracture group	VITD 0-20 ng/ml(%)	VITD20-30ng /ml(%)	VITD>30ng/ ml (%)	P value
Neck of femur(21)	6(28.6)	9(42.9)	6(35.3)	0.489
Intertrochanteric(20)	3(15)	6(30)	11(55)	
Total	9(22)	15(36)	17(41)	

Figure 11. Bar chart showing Distribution of Vitamin D among fracture group

Correlation between Vitamin D with Total T and z scores

Correlation of BMD with vitamin D was not found significant in both fracture and non fracture side. Total T score on fracture side and non fracture side shows no correlation with Vitamin D. Total Z score on fracture and non fracture side shows no correlation with Vitamin D as P value is >0.05 in all. Same has been shown in table 11 and scatter diagrams in figures 12 (A and B),13 (A and B).

Table 11. Correlation between VITDwith Total T and z scores

| BMD	Correlation with VITD	r value	P value
Total T score	Fracture	0.023	0.88
	Non Fracture	0.064	0.31
Total Z score	Fracture	0.040	0.80
	Non fracture	0.008	0.96

Correlation between vitamin D and T score, Z scores in Neck and Trochanter region

Correlation of BMD of neck and trochanteric region with vitamin D was not found significant in both fracture and non fracture side. Neck T score on fracture side and non fracture side shows no correlation with Vitamin D. Neck Z score on fracture and non fracture side shows no correlation with Vitamin D3 as P value is >0.05. Trochanteric T score on fracture side and non fracture side shows no correlation with Vitamin D. Trochanteric Z score on fracture and non fracture side shows no correlation as P value is >0.05 same shown in Table12.

DISCUSSION

This study investigated the association between Bone mineral density and Vitamin D3 in males more than 50yrs, with proximal femur fractures. The purpose of taking only male population in this study was that until now only limited studies

on osteoporosis in elderly males with hip fracture are available in literature. As osteoporosis is well documented in elderly females than elderly males, we studied male population only. Only the elderly male patients more than 50yrs taken into consideration as this group of people are prone to have hip fractures due to trivial fall.
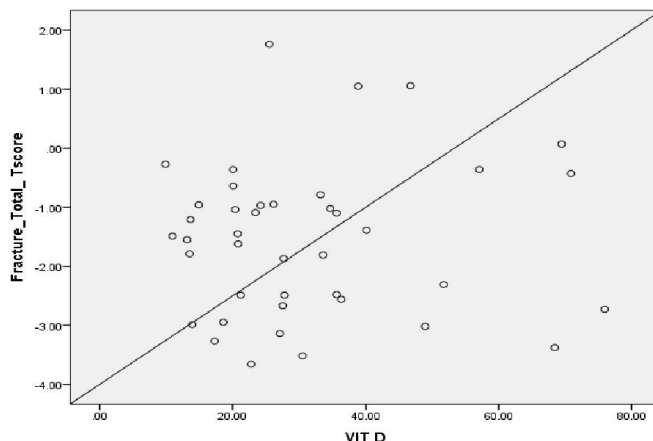
(A)
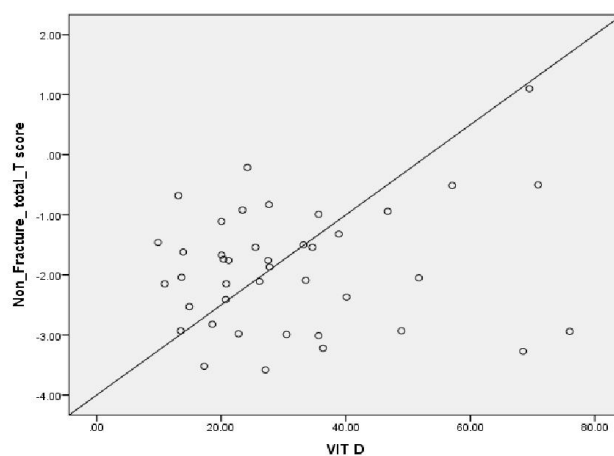
(B)

Figure 17. (A&B) Scatter diagram showingcorrelation between the non fracture T score and Vitamin D

Age: The mean age group of patients in this study was 62.2(50-79) and all were below 80yrs males this is in par with other studies[38,39,40.] Who have included either males or females.

None of the studies reviewed patients below a lower limit of 50yrs. Whereas in this study group the patients were aged between 50-80yrs. In our study the patients included have a wide range of distribution in age. So the bias of age related osteoporosis might have avoided by this.
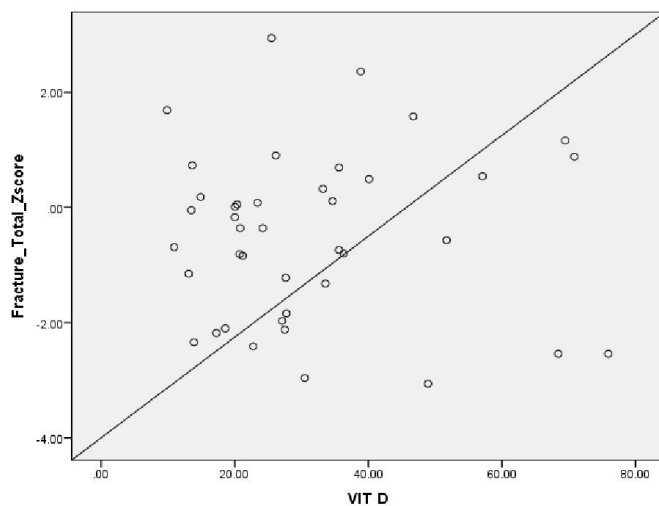
(A)
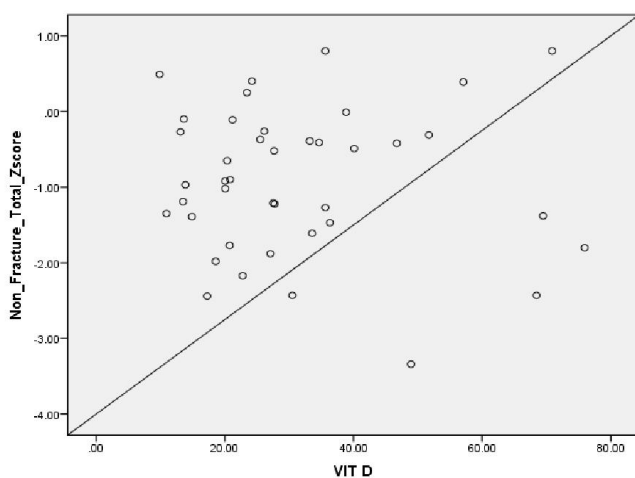
(B)

Figure 19. (A&B) Scatter diagram showing correlation between the non fracture Total Z score and Vitamin D

Table 12. Correlation between vitamin D and T score, Z scores in Neck and Trochanter region

BMD	Correlation with VIT D	r value	P value
Neck T score	Fracture	0.108	0.50
	Non fracture	0.012	0.94
Neck Z score	Fracture	0.084	0.60
	Non fracture	0.007	0.96
Trochanteric T score	Fracture	0.105	0.46
	Non fracture	0.116	0.95
Trochanter Z score	Fracture	0.088	0.49
	Non fracture	0.143	0.11

Age distribution of fractures

The fractures in this study group showed an age predilection as fracture neck of femur occurred mostly in younger age group (50-59)than older age group as compared to intertrochanteric which occurred mostly in age group 70 to 79. This is in confirmation with existing literature. (Table14). In our study we have included almost equal number of patients with fracture

neck of femur and Intertrochanteric femur. As the age of the patients increases they are more prone to have Intertrochanteric fracture than neck of femur fracture following trivial fall. This may be due to the fact that the T score and Z scores have shown more osteoporotic in trochanteric region than in neck region in this age group. Although other factors like nature of injury, velocity of injury, muscle and fat cover around hip might also have contributed to this distribution of fracture pattern.

Duration

In this study of 41 patients, 34(82.91%) presented within 10 days of injury and only 7(17.08%) presented after 10 days. So the effect of duration on BMD in the fractured hip could not be evaluated due to lack of pre fracture baseline BMD in affected hip. In our study 7 patients presented more than 10 days after injury and have been non ambulatory .So disuse osteopenia might have occurred in these patients in view of bed rest. This factor might have contributed to the low BMD but we found no significant correlation between duration and BMD.

BMD

Most of the studies that have done analysis on BMD have taken total T-score as a standard to correlate with hip fractures or Vitamin D. In the analysis of BMD the T score are more significant as it represents the same ethnic group. In the hip region also mostly the total T score will be taken for the study purpose. In our study we have analyzed BMD as Total T score and Z score as well as taken individual Neck and Trochanteric region T scores and Z scores separately for the correlation with Vitamin D. To the best of our knowledge no similar study has been done in English literature evaluating the relationship between neck and trochanteric region BMD values with serum vitamin D level in male patients.

Table 13. Comparison between other studies in BMD at different sides

Study	Hannan et al[32]	Sunil et al[33]	Our study
Year	2007	2013	2013
populations	331(blacks)	108	41
Age	48±12.8yrs	62.5	62.3
Total T score	1.09±0.15	0.72±0.15	-1.55±1.2
T score in femoral neck	0.94±0.15	Not done	-3.44±1.18
T score in tochanter	0.81±0.14	Not done	-2.79±1.19

We also found there was a significant correlation of T score and Z scores of neck and trochanter region on comparing between fracture and non fracture sides. When compared to other studies [32] we found that our patients have more osteoporosis in the neck region than in the trochanter region on both sides .in our study only male patients have been evaluated whereas in other studymales and females are included. When compared the total T score we found only osteopenia on both the fracture side and non fracture side this is comparable with other studies[32, 33]. we have found significant osteoporosis in both neck and trochanter regions on both fracture and non fracture sides yet we have some patients with trochanteric fracture and some have neck fracture on only one side.in view of this other factors like mode of injury, velocity of injury, muscle wasting might have contributed significantly to the type of fracture and side involved.

Table 14. Comparison of this study with others in relation to fractures and vitamin D

Study	Cooper *et al*. (2003)	Erem *et al*. (2002)	Bakthiaroviya *et al*. (2006)	Rajesh *et al*. (2010)	This study
Year	1998	2002	2006	2010	2013
Population	41hipfracture 40controls	21women with hip fracture& 20postmenopausal women	64fracturecases 97controls	43 Hip fractures	41 proximal femoral fractures
Age(yrs)	Cases77.4±8.6 Controls:73.3±10.5	Cases:76.7±6.5 y Controls:75.4 ±6.3	Cases:68.8±9.5 Controls: 70.2 ±8.3	62.2±12.3	62.2yrs
Sex	Only males	Only females	Cases: 69% female Controls: 55% female	9 men and 34females	Only males
Vitamin D(ng/ml) (mean±SD)	Fracture patients: 23.5±14.5, controls: 35.75±23.5	Cases26.9± 5.0 Controls:24.9± 20.5	Cases:22.4 ±11.4 Controls:28.1 ±10.1	9.9±4.8	31.4±17.3
Results	Men with hip fractures had significantly lowered25(OH) D levels vs. controls.	No significant difference in 25(OH)D levels in hip fracture patients vs. controls	25(OH)D levels significantly lower in hip fracture cases vs controls. .	96.7% of patients with fractures has vitamin deficiency	58% of patients showing < 30ng/ml we didn't found significant difference in Vitamin D levels in hip fractures.

Table 15. Showing correlation of Vitamin D and BMD with our study and others

Study	Cooper *et al*. (2003)	Aloia *et al*. (2005)	Marian *et al*. (2008)	Sunil *et al*. (2013)	Our study
year	2003	2005	2007	2013	2012-2013
Age	56.5±4.2	61.2 ± 6.3	48±12.8	62.5±6.4	62.3±
population	187	208	331 blacks	108	41
sex	Post-menopausal women	Post-menopausal women	Male and females	Males and females	males
VitaminD (ng/ml)	82.6 ± 27.0	17.2 ± 6.64	25.0± 14.7	21.3±0.5	31.4±17.3
Total T score	Not done	−0.59±0.84	1.09±0.15	0.72±0.15	-1.55±1.2
Neck T score	0.813 ± 0.128	Not done	0.94±0.15	Not done	-3.44±1.18
Trochanter T score	0.666±0.099	Not done	0.81±0.14	Not done	-2.79±1.19
VIT D and BMD	No association between serum 25(OH) D and BMD.	No association between serum 25(OH) D and BMD.	No association between serum 25(OH) D and BMD.in black males	No association between serum 25(OH)D and BMD Pvalue=0.473	No association between serum Vitamin D and BMD Pvalue= 0.88 on fracture side

Age with BMD

BMD tends to decline in older population because of age related bone loss. The exact age of attainment of peak bone density at various skeletal sites remains controversial as does the age at which bone loss begins at each site. But in our study all age groups shows no association with age and BMD as p value is 0.868.In our study population BMD on fracture side shows 11(26.8%) patients with osteoporosis 17(41.5%) with osteopenia and 13(31.7%) as normal .it shows that 28(63.3%) patients had lower BMD.In our study age group (50-59) were showing more osteoporosis than older age group this may not be correlating with existing literature as young patients showing more osteoporosis in our study this may be due to the small group of population taken for study.

Fracture correlation with Vitamin D

In this study out of the total 41 patients Vitamin D deficiency was found in 9 patients, insufficiency in 15 patients and 17 patients had normal values. On analyzing further we found that patient with neck of femur fracture had relatively low vitamin D (14 patients had Vit D <30ng/ml) when compared to the patients with intertrochanteric fracture (9 patients had Vit D<30ng/ml).Compared with other studies we found similar correlation except for one study[40] (Table No: 15).

Most of other studies have included females in their evaluation whereas only one study compared with male patients [42] (cooper *et al.*, 1998). We found similar correlation when compared with other studies. In our study we found that >50% (17 patients) have normal vitamin D levels more in patients with trochanter fracture than with neck of femur fracture. But we found almost equal incidence of fracture in both neck and trochanter region inspite of variable Vitamin D levels in our study population.

Correlation of Vitamin D and BMD

In our study population with proximal femur fractures vitamin D with mean value is of 31.4ng/ml and BMD has mean total T score of -1.55±1.2 and mean neck T score has -3.44±1.18 and mean trochanteric score has -2.79±1.19.

On evaluating the total T score we found only osteopenia on both the fracture side and non fracture side this is comparable with other studies[32].There was no correlation between Vitamin D and total T score and Z score in both fracture and non fracture side. The BMD was found to be lower level in patients with neck of femur fracture than intertrochanteric fracture .But no correlation was found between vitamin D and BMD scores at neck and trochanter region this is agreement with other studies who also have found no correlation (Table 15). Most of the studies have taken females for evaluation where Post-menopausal osteoporosis might have a definitive impact on the result but only one study has taken male patients for evaluation (Hannan *et al.*, 2008) we have included only male patients with hip fracture for the study.

In our study we have evaluated the results of Vitamin D and BMD on both fracture and non fracture side and also in neck and trochanteric region respectively. As our study was time bound we had few limitations like sample size was small and this was a single center study which will not reflective entire population. Duration of fracture at the time of presentation was not able to assess due to less sample size. The other factors like mechanism of injury velocity of injury soft tissue cover and muscle strength around hip region were not assessed. All these factors may have impact on the result of our study. Despite these limitations, this study has several unique strengths. This study sample represents one of the few patient - based studies patients presenting to tertiary institute centre like JIPMER which represent almost south Indian population Till now studies on fracture patients with measurement of BMD and vitamin D was done separately, no study has been done in correlating BMD with Vitamin D in Indian male population with hip fractures where more morbidity and mortality is seen. Direct serum measures of serum vitamin D were used instead of indirect measures such as questionnaire-based scales or food frequency intakes of vitamin D-rich foods. As a result, the data more accurately reflect the impact of vitamin D status on BMD

Conclusion

This was a cross sectional study aimed at studying the correlation of Serum vitamin D and BMD in males more than 50yrs with hip fractures who presented to the JIPMER. BMD of both hips were analysed by using DEXA scan and Vitamin D was estimated from blood.Mean and standard deviation for all obtained values were done and correlation analysis was performed between BMD and vitamin D. The results showed that most of the hip fractures were occurred in younger age group than older age group. Fracture neck of femur occurred in young patients compared to older patients as intertrochanteric fractures occurred mostly in older age. We found significant correlation of T-scores and z scores of neck and trochanter regions on comparing between fracture and non fracture sides .Vitamin D analysis found that 9 had deficiency (<20ng/ml),15 had (20-30ng/ml),17 had (>30ng/ml).We found 24 patients (58%) have vitamin D level <30ng/ml, more in patients with neck femur fracture than intertrochanteric fractures. We found no correlation between serum vitamin D and total T score and Z scores of both fracture side and non fracture side another was no correlation with neck and trochanteric T scores and Zscores.

Our preliminary study found that male patients with fracture neck of femur tend to be more osteoporotic (Low BMD) than those with intertrochanteric fractures. The vitamin D levels were lower in patients with neck of femur fracture than in intertrochanteric fractures. But there appears to be no direct relationship between the vitamin D level and BMD in elderly males with proximal femoral fractures. These may emphasize that in the male patients with hip fractures the vitamin D may not have critical role in development of osteoporosis .But the treatment of such patients with vitamin D supplements to prevent hip fractures is still debatable .However further studies in very large groups and controls may bring more light about this.

REFERENCES

Adebajo, A.O., Cooper, C. and Evans, J.G. 1991. Fractures of the hip and distal forearm inWest Africa and the United Kingdom. Age Ageing. Nov;20(6):435-8.

Aguado, P., del Campo, M.T., Garcés, M.V., González-Casaús M.L., Bernad, M., Gijón-Baños, J., Martín Mola, E., Torrijos, A. and Martínez, M.E. 2000. Low vitamin D levels in outpatientpostmenopausal women from a rheumatology clinic in Madrid, Spain: theirrelationship with bone mineral density. *Osteoporos Int.,* 11(9):739-44.

Aloia, J.F., Talwar, S.A., Pollack, S. and Yeh, J. 2005. A randomized controlled trial ofvitamin D3 supplementation in African American women. *Arch. Intern. Med,* Jul25;165(14):1618-23.

Ardawi, M.S., Maimany, A.A, Bahksh, T.M., Nasrat, H.A., Milaat, W.A., Al-Raddadi, R.M. 2005. Bone mineral density of the spine and femur in healthy Saudis. Osteoporos Int., Jan;16(1):43-55.

Arya, V., Bhambri, R., Godbole, M.M. and Mithal, A. 2004. Vitamin D status and its relationshipwith bone mineral density in healthy Asian Indians. Osteoporos Int. 2004Jan;15(1):56-61.

Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994;843 :1-129.

Bakhtiyarova, S., Lesnyak, O., Kyznesova, N., Blankenstein, M.A., Lips, P. 2006. Vitamin Dstatus among patients with hip fracture and elderly control subjectsinYekaterinburg, Russia. *Osteoporos Int.,* 17(3):441-6.

Bischoff-Ferrari, H.A., Dietrich, T., Orav, E.J., Dawson-Hughes, B. 2004. Positiveassociation between 25-hydroxy vitamin D levels and bone mineral density: apopulation-based study of younger and older adults. *Am. J. Med,* May1;116(9):634-9.

Bonjour, J.P., Theintz, G., Law, F., Slosman, D. and Rizzoli, R. 1994. Peak bone mass. *Osteoporos. Int.,* 4 Suppl 1:7-13

Cannell, J.J. and Hollis, B.W. 2008. Use of vitamin D in clinical practice. *Altern. Med Rev.,* Mar;13(1):6-20.

Cauley, J.A. 2002. The determinants of fracture in men. J Musculoskeletal Neuron Interact, 2(3):220–1.

Cawthon, P.M., Ewing, S.K., McCulloch, C.E., Ensrud, K.E., Cauley, J.A., Cummings, S.R. and Orwoll, E.S. 2009. Osteoporotic Fractures in Men (MrOS) Research Group. Loss of hip BMD in oldermen: the osteoporotic fractures in men (MrOS) study. *J. Bone Miner Res.,* Oct;24(10):1728-35.

CChapuy, M.C., Arlot, M.E., Delmas, P.D. and Meunier, P.J. Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. *BMJ,* 1994 Apr 23;308 (6936):1081-2..

Chen, T.C. Chimeh, F., Lu, Z., Mathieu, J., Person, K.S., Zhang, A., Kohn, N. Martinello, S., Berkowitz, R., Holick, M.F. 2007. Factors that influence the cutaneous synthesis anddietary sources of vitamin D. *Arch Biochem. Biophys.* 2007 Apr 15;460(2):213-7.

Close, J.C. Lord, S.L., Menz, H.B. and Sherrington, C. 2005. What is the role of falls? *Best Pract Res Clin. Rheumatol.,* 2005 Dec;19(6):913-35.

Compston, J. US and UK guidelines for glucocorticoid-induced osteoporosis:similarities and differences. *Curr. Rheumatol Rep.,* 2004 Feb;6(1):66-9..

Cooper, C., Campion, G. and Melton, L.J. 1992. Hip fractures in the elderly: a worldwide projection. *Osteoporosis International,* 2:285–9.

Cooper, L, 2003. Clifton-Bligh PB, Nery ML, Figtree G, Twigg S, Hibbert E, Robinson BG. Vitamin D supplementation and bone mineral density in early postmenopausalwomen. Am J ClinNutr. 2003 May;77 (5):1324-9.

Dhanwal, D.K., Dennison, E.M., Harvey, N.C. and Cooper, C. Epidemiology of hip fracture: Worldwide geographic variation. *Indian J. Orthop.,* 2011 Jan;45(1):15-22.

Erem, C., Tanakol, R., Alagöl, F., Omer, B. and Cetin, O. 2002. Relationship of bone turnoverparameters, endogenous hormones and vit D deficiency to hip fracture in elderly postmenopausal women. *Int. J. Clin. Pract.,* Jun;56(5):333-7

Feldstein Feldstein, A., Elmer, P.J., Orwoll, E., Herson, M. and Hillier, T. 2003. Bone mineral density measurement and treatment for osteoporosis in older individuals with fractures: a gap in evidence-based practice guideline implementation. Arch Intern Med. Oct 13; 163(18):2165-72.

Gilsanz, V. 1998. Bone density in children: a review of the available techniques and indications. *Eur. J. Radiol.,* Jan;26(2):177-82.

Gutiérrez, O.M., Farwell, W.R., Kermah, D. and Taylor, E.N. 2011. Racial differences in therelationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. *Osteoporos. Int.,* Jun;22(6):1745-53.

Hannan, M.T., Litman, H.J., Araujo, A.B., McLennan, C.E., McLean, R.R., McKinlay, J.B., Chen, T.C. and Holick, M.F. 2008. Serum 25-hydroxyvitamin D and bone mineral density in a racially and ethnically diverse group of men. *J. Clin. Endocrinol. Metab.,* Jan;93(1):40-6

Harinarayan, C.V. and Joshi, S.R. Vitamin D status in India--its implications andremedial measures. J Assoc Physicians India. 2009 Jan;57:40-8.

Holick, M.F. 2007. Vitamin D deficiency. *N Engl J Med.,* Jul 19;357(3):266-81

Kanis, J.A., Johnell, O., De Laet, C. 2004. A meta-analysis of previous fracture and subsequent fracture risk. *Bone densitometry,* (35):375.

Kennel, K.A., Drake, M.T. and Hurley, D.L. 2010. Vitamin D deficiency in adults: when to testand how to treat. *Mayo Clin Proc.,* 2010 Aug; 85(8):752-7; quiz 757-8.

Khadgawat, R., Brar, K.S., Gahlo, M., Yadav, C.S., Malhotra, R., Guptat, N., Tandon, N. 2010. High prevalence of vitamin D deficiency in Asian-Indian patients with fragility hipfracture: a pilot study. *J Assoc Physicians India,* Sep;58:539-42. Erratumin: J Assoc Physicians India. 2010 Oct;58:630.

Kota, S., Jammula, S., Kota, S., Meher, L. and Modi, K. 2013. Correlation of vitamin D, bonemineral density and parathyroid hormone levels in adults with low bone density. *Indian J. Orthop.,* 2013 Jul;47(4):402-7.

Lenchik, L., Kiebzak, G.M. and Blunt, B.A. 2002. International Society for Clinical Densitometry Position Development Panel and Scientific Advisory Committee. What is the role of serial bone mineral density measurements in patient management? *J. Clin. Densitom.,* 5 Suppl:S29-38.

Maggi, S., Kelsey, J.L., Litvak, J. and Heyse, S.P. 1991. Incidence of hip fractures in the elderly: a cross-national analysis. Osteoporos Int. Sep;1(4):232-41.

Marwaha, R.K., Tandon, N., Garg, M.K., Kanwar, R., Narang, A., Sastry, A., Saberwal, A. and Bandra, K. 2011. Vitamin D status in healthy Indians aged 50 years and above. J AssocPhysicians India. Nov, 59:706-9.

Pfeifer, M., Minne, H.W. 1999. Vitamin D and Hip Fracture. *Trends Endocrinol. Metab.,* Dec;10(10):417-420.

Rola El-Rassi, Ghassan Baliki Ghada El-Hajj Ful heihan, 2002. Vitamin D status in Middle East and Africa. *AM. J. med,* 2:674–8.

Ross, P.D., Yhee, Y.K., Davis, J.W. and Wasnich, R.D. 1993. Bone density predicts fracture incidence among elderly men. Proceedings of the Fourth International Symposium on Osteoporosis and Consensus Development Conference, Hong Kong, 27 March–2 April 1993.

Sadat-Ali, M., Al Elq, A.H., Al-Turki, H.A., Al-Mulhim, F.A., Al-Ali, A.K. 2011. Influence of vitamin D levels on bone mineral density and osteoporosis. *Ann Saudi Med,* Nov-Dec;31(6):602-8.

Schaafsma, A., van Doormaal, J.J., Muskiet, F.A., Hofstede, G.J., Pakan, I., van der Veer, E. 2002. Positive effects of a chicken eggshell powder-enriched vitamin-mineralsupplement on femoral neck bone mineral density in healthy late post-menopausalDutch women. *Br J Nutr.* Mar;87(3):267-75.

Seeman, E. and Hopper, J.L. 1997. Genetic and environmental components of the populationvariance in bone density. *Osteoporos. Int.,* 7Suppl 3:S10-6.

Stegman, M.R., Heaney, R.P. and Recker, R.R. 1995. Comparison of speed of sound ultrasound with single photon absorptiometry for determining fracture odds ratios. *J Bone Miner Res.,* Mar;10 (3):346-52.

Van Dam, R.M., Snijder, M.B., Dekker, J.M., Stehouwer, C.D., Bouter, L.M, Heine, R.J., Lips, P. 2007. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: the Hoorn Study. *Am. J. Clin. Nutr.,* Mar;85(3):755-61.

Vondracek, S.F. and Linnebur, S.A. 2009. Diagnosis and management of osteoporosis in the older senior. *ClinInterv Aging.,* 2009;4:121-36. Epub 2009 May 14.

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