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Evaluation of vitamin D level in newly diagnosed patient with osteoporosis

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Abstract
Background: Inadequate serum vitamin D [25(OH) D] concentrations are associated with secondary hyperparathyroidism, increased bone turnover and bone loss, which increase fracture risk in osteoporotic patients. Vitamin D status is determined by measuring the level of 25(OH) D in the serum and can be described as deficient, insufficient, normal and high.
Objective: The aim of this research was to determine the status of vitamin D in patients who have been recently given the diagnosis of osteoporosis, the adequacy of any vitamin D replacement and to correlate the status of vitamin D with bone mineral density (BMD). The study also aimed to screen for symptoms and signs of overt osteomalacia in these patients.
Patients and Methods: The research included 88 subjects (82 females and 6 males) at the time of confirming the diagnosis of osteoporosis. All the participates were sent for level of serum 25(OH) D as well as the levels of other biochemical bone profile (serum calcium, serum phosphorus and serum alkaline phosphatase). Their bone mineral density on the lumbar spine was measured by DEXA densitometer.
Results: The mean age of the patients (±SD) was 64.42 years (± 8.670). The mean (±SD) of their T-score was - 2.922 (±0.493). The mean (±SD) of 25(OH) D level was 23.38 ngm/ml (±17.1). In the whole study subjects, the prevalence of 25(OH) D inadequacy was 68.2 % when considering cutoffs of 30 ngm/ml. Subjects who used vitamin D supplement had higher concentration of 25(OH) D than non-users. None of the patients had symptoms and signs of overt or even latent tetany and none of them had frank clinical or radiological features of osteomalacia.

Keywords: osteoporosis, osteomalacia, bone

Introduction

1. Osteoporosis

1.1 Definition

Osteoporosis was defined at a 1993 consensus conference as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue with a resultant increase in fragility and risk of fracture (Brown and Josse, 2002) [5]. Osteoporosis is the most common bone disease in humans (National Osteoporosis Foundation, 2008) and is a major public health problem because of its age-associated exponential increase in prevalence, and its serious consequences in terms of mortality, morbidity and economic costs (Nguyen, 2004) [45]. The World Health Organization (WHO) operationally defines osteoporosis as "bone density 2.5 SDs below the mean for the young white adult women". The WHO definition of osteoporosis was established for Caucasian women, because of paucity of data for other population groups (Sanborn and Simmonds, 2002) [54].

1.2 Pathogenesis of osteoporosis

The mineralization of bone leading to compressive strength and rigidity begins with the precipitation of calcium and phosphate ions that begin to induce crystallization. As crystal growth occurs, mineralization spreads, resulting in the formation of mature, fully calcified bone, known as hydroxyapatite. This process is also underpinned by magnesium, small amounts of sodium and traces of fluoride (Sutcliffe, 2006) [58]. Skeletal fragility can result from: (a) failure to produce a skeleton of optimal mass and strength during growth; (b) excessive bone resorption resulting in decreased bone mass and micro architectural deterioration of the skeleton; and (c) an inadequate formation response to increased resorption during bone remodeling. In addition, the incidence of fragility fractures, particularly of the hip and wrist, is further determined by the frequency and direction of falls (Raisz, 2005) [52].

1.3 Pathophysiology of osteoporosis

Bone is a dynamic organ, with bone resorption by osteoclasts, bone formation by osteoblasts, bone mineralization, and quiescence occurring simultaneously. Bone mineralization requires calcium, phosphate, and magnesium, which provide strength and rigidity. Bone remodeling (i.e., resorption and formation) maintains skeletal strength by repairing microscopic damage. It also maintains the serum calcium concentration within the range needed for physiologic functions. Remodeling is regulated by estrogen, receptor activator of nuclear factor kB (RANK) ligand, parathyroid hormone (PTH), calcitonin, vitamin D, prostaglandins, interleukins, growth factors, tissue necrosis factor, bone morphogenic proteins, and various other hormones and cytokines (O'connell, 2006) [48].

1.4 Types of osteoporosis

1.4.1 Primary osteoporosis

In the early 1980s, Riggs and Melton proposed the existence of two discrete types of involutional osteoporosis (Sipos *et al*., 2009) [56]: Type I (postmenopausal osteoporosis) occurs only in women due to decreased estrogen and increased osteoclast activity, typically in the years following

menopause, from age 50 to 70. Type II (senil osteoporosis) most commonly affects men and women over the age of 75 due to decreased osteoblast activity and decreased bone formation attributed to aging processes (Gruver, 2004).

1.5.2 Secondary osteoporosis

This is osteoporosis occur as a result of some underlying disease state or medications and may be corrected by treatment of the primary disease (Krawiecki *et al*., 2006).

1.6 Clinical features of osteoporosis

Osteoporosis is known as a silent disease because the deterioration of skeletal tissue proceeds with no outward symptom until a symptomatic fracture occurs, and thus the condition is under recognized and affected individuals are undertreated (NHMRC, 2010). Osteoporosis often presents as a clinically evident fracture. A low trauma fracture (following a fall from standing height or less) in someone aged over 45 should trigger the suspicion of osteoporosis.

In other cases, osteoporosis may present as backache, height loss, spinal deformity, or radiological osteopenia (Poole and Compston, 2006) [50].

1.7 Diagnosis of osteoporosis

Osteoporosis diagnosis is based on the measurement of bone mineral content, or BMD, which is a surrogate marker of bone strength and fracture risk (NOF of South Africa, 2010). BMD of the lumbar spine and the proximal femur are the best current predictors of future fracture risk and both sites should be measured (NOF, 2008). The most widely used are based on X-ray absorptiometry in bone, particularly dual-energy X-ray absorptiometry (DEXA), since the absorption of X-rays is very sensitive to the calcium content of the tissue of which bone is the most important source. (Kanis *et al*., 2008) [29]. The WHO classification originally has been proposed in 1994 for the hip BMD of postmenopausal Caucasian women, but in clinical practice is also used for DEXA measurements at the lumbar spine as well as for men (Sipos *et al*, 2009) [56].

Table 1: WHO Definition of Osteoporosis Based on BMD Measurements by DEXA

Definition	Bone Mass Density Measurement	T-Score
Normal	BMD within 1 SD of the mean bone density for young adult women	≥ –1
Low bone mass (osteopenia)	BMD 1–2.5 SD below the mean for young-adult women	between –1 and –2.5
Osteoporosis	BMD ≥2.5 SD below the normal mean for young-adult women	≤ –2.5
Severe or "established" osteoporosis	BMD ≥2.5 SD below the normal mean for young-adult women in a patient who has already experienced ≥1 fractures	≤ –2.5 (with fragility fracture[s])

2. Vitamin D

2.1 Over view

Vitamin D and its metabolites are important molecules that aid several processes in the human body. It is a fat-soluble pro vitamin that is synthesized in the skin with the aid of ultraviolet light B (UVB) or obtained from dietary sources (DeLuca and Zierold, 1998) [11].

2.5 Causes of vitamin D deficiency

The major cause of vitamin D deficiency is inadequate exposure to sunlight (Looker *et al*., 2008) [35]. The limited natural supply of vitamin D in food is other cause of vitamin D deficiency (Compston, 1998) [9]. Wearing a sunscreen with a sun protection factor of 30 reduces vitamin D synthesis in the skin by more than 95% (Matsuoka *et al*., 1987) [37]. People with a naturally dark skin have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin D as a person with a white skin (Hintzpeter *et al*., 2008) [21]. Patients with one of the fat malabsorption syndromes and bariatric patients are often unable to absorb the fat-soluble vitamin D, and patients with nephrotic syndrome lose 25(OH) D bound to the vitamin D-binding protein in the urine (Holick, 2007) [31]. Patients on a wide variety of medications, including anticonvulsants and medications to treat AIDS/HIV, are at risk because these drugs enhance the catabolism of 25(OH) D and 1, 25(OH) 2D (Zhou *et al*., 2006) [61]. Aging is associated with decreased concentrations of 7-dehydrocholesterol, the precursor of vitamin D3 in the skin. A 70-years-old has ~25% of the 7-dehydrocholesterol that a young adult does and thus has a 75% reduced capacity to make vitamin D3 in the skin (Holick and Chen, 2008) [22].

2.6 Consequences for the Skeleton of Vitamin D Deficiency

Vitamin D deficiency during the first 2 years of life results in rickets. In adults, vitamin D deficiency can predispose or exacerbate osteoporosis and induce osteomalacia (Holick, 2005b) [23]. Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed. The interaction of $1,25(OH)_2D$ with the vitamin D receptor increases the efficiency of intestinal calcium absorption to 30 to 40% and phosphorus absorption to approximately 80% (Holick, 2007a) [23]. Vitamin D deficiency results in abnormalities in calcium, phosphorus, and bone metabolism. Specifically, vitamin D deficiency causes a decrease in the efficiency of intestinal calcium and phosphorus absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels. Secondary hyperparathyroidism maintains serum calcium in the normal range at the expense of mobilizing calcium from the skeleton and increasing phosphorus wasting in the kidneys (The Endocrine Society, 2011). PTH stimulates the formation of osteoclasts, which in turn dissolves the bone matrix and mineral to release the calcium into the extracellular space. Secondary hyperparathyroidism can precipitate and exacerbate both osteopenia and osteoporosis, increasing risk of fracture. PTH also causes phosphorus loss in the urine resulting in a low-normal serum phosphorus level. The cumulative effect of higher PTH levels, secondary to poor calcium and vitamin D nutrition (secondary hyperparathyroidism), is an inadequate calcium-phosphate product necessary for the mineralization of the collagen matrix, an increase in bone remodeling leading to significant loss of bone and an increased fracture risk (Holick, 2007b; Nieves, 2005) [24, 46]. Vitamin D deficiency and secondary hyperparathyroidism can contribute not only to accelerated bone loss and increasing fragility, but also to neuromuscular impairment that can increase the risk of falls (Raisz, 2005) [52].

2.7 Evaluation of Vitamin D Deficiency

Measurement of 25(OH) D is the only means to determine

whether a patient is vitamin D deficient or sufficient. Levels of 25-hydroxyvitamin D <20 ng/ml represent deficiency and between 20 ng/ml (50 nmol/l) and 30 ng/ml (75 nmol/l) represent insufficiency (Elliott, 2010) [14].

5. The aim of the study

The aim of this research was to determine the status of vitamin D level in patients that have been recently given the diagnosis of osteoporosis.
The study also aimed to screen for symptoms and signs of overt osteomalacia in these patients

Subject and Methods

The study was conducted between the periods from December 2018-July 2019. Participates were patients sent for assessment of their BMD from Rheumatology Outpatient Clinic in Ibn Sina Teaching Hospital and the Orthopaedic Outpatient Clinic in Al-Jamhori Teaching Hospital in Mosul to DEXA unit in Al-Jamhori Teaching Hospital in Mosul. Patients were included in the study if they fulfilled the criteria of diagnosis of osteoporosis (having T-score ≤ - 2.5 measured by DEXA scan) and agreed to participate in the study.
Patients were excluded from the study if
Patients were questioned regarding vitamin D replacement and the dose and duration of the supplement. All patients were screened for symptoms of osteomalacia including bone pain, difficulty in climbing stair, difficulty in getting out of a chair and for symptoms of tetany (parasthesia and carpal spasm). All patients were examined for waddling gait, bone tenderness and proximal muscle weakness. They were tested for the presence of positive Trousseau's and Chvostek's signs as follows:
Trousseau's sign: a blood pressure cuff is placed around the arm and inflated to a pressure greater than the systolic blood pressure and held in place for 3 minutes. This will occlude the brachial artery. In the absence of blood flow, the patient's hypocalcaemia and subsequent neuromuscular irritability will induce spasm of the muscles of the hand and forearm. The wrist and metacarpophalangeal joints flex, the Distal Inter Phalangeal (DIP) and Proximal Inter Phalangeal (PIP) joints extend, and the fingers adduct.
Chvostek's sign: abnormal reaction to the stimulation of the facial nerve. By tapping facial nerve at the angle of the jaw, the facial muscles on the same side of the face will contract momentarily (typically a twitch of the nose or lips). All participate were sent for the following laboratory investigations:

- Serum 25(OH) D: measured by ELISA using kits purchased from Euro Immune –Deutschland.
- Serum calcium: measured by colorimetry using kits Biolabo; made in France.
- Serum phosphorus: measured by colorimetry using kits Biolabo; made in France.
- Serum alkaline phosphatase measured by colorimetry using kits Biolabo; made in France.

The patients were considered to have vitamin D deficiency if serum 25(OH) D was less than 30 ng/ml. Severe deficiency was defined as serum 25(OH) D less than 10 ng/ml (Elliott, 2010; Kennel *et al*, 2010) [14] X-Ray of the pelvis was obtained for patients with elevated serum alkaline phosphatase to search for the radiological sign of osteomalacia. Bone mineral density was measured by DEXA using Lunar DDX, USA machine.

The study was approved by the Scientific Research Committee of the Department of Medicine, College of Medicine, University of Mosul and from the Research Committee of Nineveh Directorate of Health.
Data were analyzed using:
Independence t-test for two means.
Z-test for two proportions.
Fisher's exact test for small proportions.
Chi square test.
P value was significant if it is ≤ 0.05.

Results

The results of the data analysis of the studied group or patients. The mean age of the patients (±SD) was 64.42 years (± 8.670), ranging from47to92 years. Their mean of T-score (±SD) was - 2.922 (±0.493) ranged between (-2.5) to (-5.0). The mean (±SD) of serum 25(OH) D level was 23.38 ngm/ml (±17.1) ranged from 2.120 to 89.23ngm/ml. (Table 3-1).

Table 2: The mean age, T-score, serum25 (OH) D, calcium, phosphorus and alkaline phosphatase of the studied group.

Parameters	Mean±SD	Range
Age(Years)	64.420±8.670	(47.000)-(92.000)
T-score	-2.9227±0.4931	(-5.000)-(-2.500)
25(OHD(ngm/ml)	23.38±17.10	(2.120)-(89.23)
s. calcium(mmol/L)	2.1938±0.2737	(1.140)-(2.750)
S. Phosphorus(mmol/L)	1.0874±0.2626	(0.510)-(1.830)
S.ALP(U/L)	73.01±26.05	(22.00)-(135.00)

Twenty patients (22.7 %) were receiving vitamin D supplements as a mean dose of 560 I.U /day. The mean duration of therapy was 2.7 months.Low serum level of vitamin D, defined as aserum 25(OH)D level (< 30 ngm/ml), was found in 60 patients (68.2%), while the remaining 28 patients (31.8%) had normal level (≥30 ngm/ml).Of those patients with vitamin D deficiency, 40 patients (45.5 % of the total number of patients) were having mild deficiency (serum 25(OH)D level 10-30 ngm/ml), while 20 patients (22.7% of the total number of patients) had severe deficiency (serum 25(OH)D level less than 10ngm/ml). There was a weak positive correlation between serum 25(OH) D level and T-score. (Figure 1).There was no statistical difference between patients with normal and low serum 25(OH) D regarding their mean age or T-score. Similarly, their mean serum calcium, phosphorus and alkaline phosphatase were not significantly different. (Table 3-2).

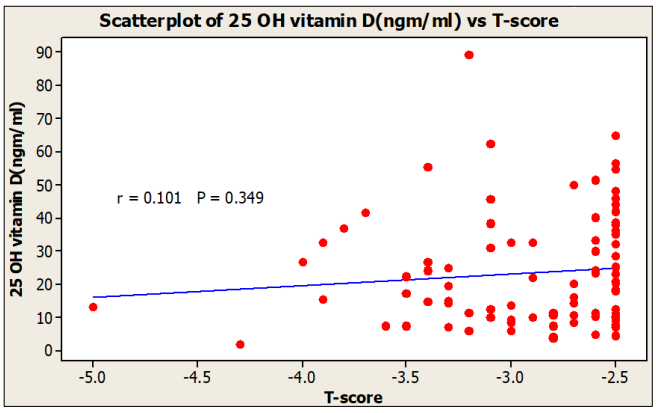

Fig 1: Correlation between serum level of 25(OH) D in all patients and T-score

Table 3: Comparison between serum 25(OH) D level and the age, T-score, s. calcium, s. phosphorus and s. alkaline phosphatase.

Parameters	Low 25(OH) D (<30 ngm/ml) n=60 Mean ± SD	Normal 25 (OH)D (≥30 ngm/ml) n=28 Mean ± SD	P-value [a]
Age (years)	63.6±8.97	66.1±7.87	0.214
T-score	- 3.0±0.52	- 2.8±0.44	0.261
S. calcium (mmol/L)	2.2±0.30	2.3±0.19	0.242
S. phosphorus(mmol/L)	1.1±0.24	1.2±0.30	0.096
S. alkaline phosphatase (U/L)	72.3±27.50	74.6±23.10	0.704

[a]Independent t-test for tow means was used.
P- Value significant if it is ≤ 0.05.

There is no statistically significant differences between age, BMD, 25(OH) D, serum calcium, serum phosphate and serum alkaline phosphatase in all studied subjects or even within subgroups of low and normal serum 25(OH) D level. None of our patients had symptoms and signs of overt or even latent tetany and none of them had frank osteomalacia with the typical waddling gait. Bone pain and tenderness were prevalent in vitamin D deficient patients, but it was also present in those having normal serum level of vitamin D. No symptom or sign was found as sensitive indicator of vitamin D deficiency. No clinical signs of overt or latent tetany (Trousseau's and Chvostek's signs) were noticed in any patients including those with hypocalcaemia (Table 3-3). None of the patients with high serum alkaline phosphatase showed radiological signs of osteomalacia.

Table 4: The relation between clinical findings of osteomalacia and 25(OH) D serum level in the studied patients.

Symptoms and signs	Low vitamin D "< 30 ngm/ml" n = 60 No. (%)	Normal vitamin D "≥ 30 ngm/ml" n = 28 No. (%)	P-value[a]
Symptoms			
Bone pain	55 (91.7)	26 (92.9)	0.844
Difficulty in climbing stairs	18 (30.0)	7 (25.0)	0.620
Difficulty in getting out	4 (6.7)	2 (7.1)	0.935 [b]
Symptoms of tetany	0 (00.0)	0 (00.0)	---
Waddling gait	0 (00.0)	0 (00.0)	---
Signs			
Bone tenderness	31 (51.7)	14 (50.0)	0.884
Proximal muscles weakness	5 (8.3)	0 (00.0)	0.173 [b]
Trousseau's sign	0 (00.0)	0 (00.0)	---
Chvostek's sign	0 (00.0)	0 (00.0)	---

[a] Z-test for two proportions was used.
[b] Fisher's exact test was used because of small proportions.

Only one patient had high serum 25(OH) D level (he was receiving vitamin D800IU/day for 6 months).
Vitamin D deficiency was present in 45% of patients already receiving vitamin D supplement, while 75% of patient without vitamin D replacement had low serum vitamin D level (p=0.011).
Mean serum 25(OH)D was 34.2 ngm/ml in those receiving vitamin D supplement and 20.0 ngm/ml in those not receiving supplement (p=0.001).(Table 3-4)

Table 5: The comparison between patients receiving vitamin D and patients not receiving vitamin D.

25 (OH)D (ngm/ml)	Patients receiving vitamin D No. (%)	Patients not receiving vitamin D No. (%)	P- value
Vitamin D deficiency (<30ngm/ml)	9 (45 %)	51 (75.0)	0.011 [a]
No vitamin D deficiency (≥30ngm/ml)	11 (55%)	17 (25.0)	
Total	20 (22.7)	68 (77.3)	------
Mean ± SD (ngm/ml)	34.2 ± 21.5	20.0 ±14.2	0.001 [b]

A Chi squar test was used
B Independent t-test for two mean was used

Discussion

Vitamin D deficiency was so common (68.2 %) in the osteoporotic patients who participated in this study. About one third of them (33.3%) had severe deficiency, despite of the widely recognized importance of vitamin D in the management of this disease. There are numerous studies in which a large prevalence of deficiency of vitamin D in patient with osteoporosis was proven. Kuchuk *et al*, 2009 [31] showed in their study - which included 7441 postmenopausal women with osteoporosis from 29 countries on six continents (North America, South America, Europe, Asia, Africa, Australia) - a high prevalence of low serum 25(OH) D < 10 and 10-20 ngm/ml in many countries; especially South and South eastern Europe. In another large study which included 2589 women with postmenopausal osteoporosis from 18 countries, grouped into five regions: Europe (Sweden, United Kingdom, Germany, The Netherlands, France, Switzerland, Hungary and Spain), the Middle East (Turkey and Lebanon), Asia (South Korea, Japan, Thailand and Malaysia), Latin America (Mexico, Brazil and Chile) and Australia, the prevalence of dificiency of vitamin D to those regions ranged from 53.4% to 81.8%. Overall, 63.9% of women had serum 25(OH) D levels <30 ng/ml. High prevalence of vitamin D dificiency, defined as serum 25(OH) D levels <30 ng/ml), was seen in South Korea (92.1%), Japan (90.4%), Lebanon (84.9%), Turkey (76.7%), United Kingdom (74.5%), Germany (68.0%), Mexico (67.1%) and Spain (64.7%) (Lips *et al.*, 2006). In United Kingdom, in 330 patients with fragility

fractures from Glasgow, Belfast and Medway, mean concentrations of 25(OH) D ranged from 16 to 20.9 ng/ml and 83.7 to 96.4% of patients had 25(OH) D concentrations < 32 ng/ml and 55.8 to 73.2% had 25(OH) D concentration < 20 ng/ml (Dixon *et al*., 2006). In a systematic review of 30 publications reporting prevalence estimates for vitamin D inadequacy in populations with osteoporosis associated with other disorders, the prevalence of 25(OH) D concentrations < 12 ng/mL ranged from 12.5 to 76%, while prevalence rates reached 50 to 70 % of patients with a history of fracture(s) (Gaugris *et al*., 2005) [16]. An evaluation of vitamin D status in patients with osteoporosis is essential for two main reasons: First, vitamin D deficiency causes defective bone mineralization and leads to low bone mass.

Second, optimal vitamin D repletion in patients with osteoporosis is important to maximize the response to anti-resorptive therapy in terms of both BMD changes and anti-fracture efficacy. (Ali *et al*., 2011) [1]. BMD continues to decline in 5 to 15% of patients with osteoporosis despite treatment with etidronate or alendronate. Heckman *et al*., 2002 [20] demonstrated that the concurrent use of vitamin D (1000 IU/d) was associated with an increased lumbar spine (1.45%) and femoral neck BMD (1.15%) in those who had previously experienced a decline in BMD while on bisphosphonate therapy alone. Vitamin D insufficiency was the most frequently identified cause of bone loss in patients with declining BMD during bisphosphonate therapy. Correction of vitamin D insufficiency in these patients led to a significant rebound in BMD (Geller *et al*., 2008). In cases where adequate supplementation is not ensured or, alternatively, good vitamin D levels have not been reached, the assessment of the therapeutic response is probably less certain (Diez-perez and Gonzalez-macias, 2008) [12]. In this study there was no difference in T- score between the two groups of normal and low serum 25(OH) D level although a weak positive correlation was found between T-score and vitamin D serum level. Our findings are in accordance with previous reports demonstrating the absence of any association between 25(OH) D and BMD (Tsai *et al*., 1997, Chapuy *et al*., 1997, Malavolta *et al*., 2005 and Hosseinpanah *et al*., 2008) [60, 6, 27]. In the study of Chapuy *et al*., 1997 [39] of 440 French women aged 75–90 years, BMD of the proximal femur had no association with 25(OH) D and PTH. Likewise, in the Tsai *et al*., 1997 [60] study of 262 Chinese urban women, aged 40–72 years and with a mean 25(OH) D of 30.6 ngm/ml, there was no association between 25(OH) D either with BMD or with bone markers. Moreover, Hosseinpanah *et al*., 2008 [27] demonstrate the absence of any association between 25(OH) D and BMD in their study which included 200 subjects, 89 of them were osteoporotic. On the other hand, a number of studies revealed a positive association between serum 25 (OH) D levels and BMD (Collins *et al*., 1998; Lips *et al*., 2001 and Mezquita –Raya *et al*., 2001) [40]. The probable association between 25(OH) D and BMD may vary at various sites of densitometry measurement owing to different composition of trabecular and cortical bone tissue, in distinct level of vitamin D, in institutionalized subjects and in elderly. Ooms *et al*., 1995 [7] studied the association between 25(OH)D and BMD in 330 women aged ≥70 years and found an association between 25(OH) D and BMD of femoral neck and trochanter only in a subgroup of subjects with 25(OH) D <12 ng/ml. Some studies found an association between 25(OH) D and BMD in institutionalized subjects (Meunier *et al*., 1994) [39]. Some other studies found a positive association between 25(OH) D and BMD in the elderly (Ooms *et al*., 1995; Sahota *et al*., 1999; Melin *et al*., 1999) [7, 53, 38].

Our results showed that there is no difference in the mean of serum calcium, phosphate and alkaline phosphatase in the two groups. Various relationships of calcium, phosphate, and alkaline phosphatase with serum vitamin D levels have been reported. Some studies have reported a significant correlation between 25(OH) D and calcium and between 25(OH) D and alkaline phosphatase levels (Preece *et al*.,; Nisbet *et al*., 1990) [47], although other studies have not shown any correlation between these biochemical parameters and serum 25(OH) D level (Singh *et al*., 2004; Smith *et al*., 2005; Batra *et al*., 2006; Hashemipour *et al*., 2006) [55, 57, 2, 19]. Routine measurements of calcium, phosphate, and alkaline phosphatase are not reliable predictors of hypovitaminosis D, even when vitamin D insufficiency has been sufficient to produce a PTH response and the only reliable way to confirm this is to do vitamin D levels (Smith *et al*., 2005; Batra *et al*., 2006) [57, 2]. Therefore the finding of normal ALP, calcium or phosphate should not be interpreted as implying normal vitamin D status. Frank features of osteomalacia were not present even in severely vitamin D deficient patients. Although patients with low vitamin D levels present many nonspecific symptoms like backache, body ache, leg pain, and thigh pain, not all are osteomalacic. How does one explain the absence of clinical osteomalacia in individuals with very low vitamin D levels? Is it likely that even at low vitamin D levels, there is enough 1, 25(OH) $_{2D}$ to maintain homeostasis, primarily patients do not typically present with overt clinical signs and symptoms until the deficiency is severe and prolonged. A parallel can be drawn with iron deficiency anemia; until body iron stores are exhausted, iron deficiency does not manifest as anemia (Kanekar *et al*., 2010) [28]. In our study radiological signs of osteomalacia were not presents in patients with elevated serum alkaline phosphatase. These signs only occur in severe and advanced cases of vitamin D deficiency (Eriksen and Glerup, 2002) [15]. Twenty subjects (22.7%) out of 88 participated in the study were receiving vitamin D supplement with a mean dose of 560 IU/day; nine subjects (45%) of them were found to had vitamin D deficiency (25(OH) D less than 30 ngm/ml) despite of this replacement, which indicates that this replacement was inadequate. The question remains which dose is recommended?. A recent review on this matter suggests that the dose of vitamin D in the management of osteoporosis should be no less than 700–800 IU per day (Bischoff-Ferrari, 2007) [3]. By supplementation of 400 IU per day, a mean level of only 24 ngm/ml was achieved (Lips P *et al*., 1996). In a study on supplementation of vitamin D in institutionalized elderly with a very good compliance, 90% of participants achieved a serum 25(OH) D above 20 ngm/ml after 4 months of a daily supplementation with 600 IU of vitamin D (Chel *et al*., 2008) [7]. As was shown in a meta-analysis on vitamin D and fracture prevention, a dose of 700–800 IU per day appears to reduce the risk of hip and any nonvertebral fractures in older persons (Bischoff-Ferrari *et al*., 2005) [4]. Supplemental use of vitamin D less than 800 IU/d and lack of physician counseling regarding the importance of vitamin D were two risk factors independently and significantly associated with vitamin D inadequacy that could easily be addressed through physician and patient education. These results underscore the need for better education of the public and physicians regarding the optimization of vitamin D status in the care of

postmenopausal women with osteoporosis (Holick *et al*., 2005) [26].

Conclusion

This study points out a high prevalence of vitamin D inadequacy in patients with osteoporosis even among subjects receiving vitamin D supplements.

The minority of our patients were receiving vitamin D supplementation at the time of diagnosis of osteoporosis; The dose received was not sufficient to achieve normal serum 25(OH) D in significant proportion of patients. Clinical and radiological manifestation of osteomalacia is either quite common and nonspecific (like bone pain and tenderness) or very uncommon and late to develop (like waddling gait and radiological manifestation). Routine measurements of calcium, phosphate, and alkaline phosphatase are not reliable predictors of hypovitaminosis D, even when vitamin D insufficiency has been sufficient to produce a PTH response and the only reliable way to confirm this is to do vitamin D levels.

Recommendation

1. In patients with documented osteoporosis, calcium and vitamin D supplementation should be a first line component of the osteoporosis care, along with anti-resorptive or anabolic treatment.
2. Lack of physician counseling regarding the importance of vitamin D might be an important factor responsible for wide spread prevalence of vitamin D deficiency.
3. The available evidence suggests that the dose of vitamin D in the management of osteoporosis targeting fall and fracture prevention should be not less than 700–800 IU per day.
4. More studies are needed to address the amount of vitamin D intake necessary to maintain serum 25(OH) D to an adequate concentration which prevents secondary hyperparathyroidism and minimizes the possibility of further bone loss.
5. Future research should focus on comparative vitamin D supplementation trials testing higher doses of vitamin D. Another question to be addressed in future research is whether and in what dose calcium is adding value to the fracture efficacy of vitamin D.
6. Because replacement therapy is inexpensive, simple, and safe, a widespread screening for vitamin D insufficiency and the normalization of vitamin D status should alter our routine medical practice and have important public health implications.

Abbreviation

Table 6

Abbreviation	The original form
BMD	Bone mineral density
BMC	Bone mineral content
CKD	Chronic kidney disease
DEXA	Dual energy X-ray absorptiometry
IU	International Unit
$1,25(OH)_2D$	1,25 dihydroxy vitamin D
25(OH)D	25 hydroxy vitamin D
ng/ml	Nano gram/millileter
nmol/l	Nano mol/millileter
NHMRC	National Health and Medical Research Council
NIH	National Institute of Health
NOF	National Osteoporosis Foundation
OPG	Osteoproteregin
QCT	Quantitative computed tomography
PTH	Parathyroid hormone
RANK	Receptor Activator of Nuclear factor K-B
RANKL	Receptor Activator of Nuclear factor K-B Ligand
SD	Standard deviation
VDR	Vitamin D resistant
WHO	World Health Organization

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