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CLINICAL STUDY

Serum vitamin D status and bone mineral density in fibromyalgia

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Abstract: *Objectives:* To compare serum vitamin D levels and bone mineral density (BMD) values in patients with fibromyalgia and healthy controls.
Background: The so far available reports of low levels of vitamin D and low BMD values in patients with fibromyalgia are inconsistent.
Methods: Serum 25-hydroxyvitamin D (25-OHD) levels and BMD values were measured in thirty women with fibromyalgia and compared with thirty age-matched healthy women. Serum calcium, phosphorus, alkaline phosphatase and parathyroid hormone (PTH) levels were also measured. All participants completed the Fibromyalgia Impact Questionnaire (FIQ) and Hospital Anxiety and Depression Score (HADS). Pain severity was assessed with visual analog scale (VAS).
Results: Mean serum 25-OHD levels did not differ between the groups (fibromyalgia 10.57±10.46, controls 10.87±5.52 ng/l; p=0.89); nor did the frequency of vitamin D deficiency (25-OHD≤20 ng/l) in each group (fibromyalgia 86.7 %, controls 96.7 %; p=0.353). Although, mean serum PTH level was found significantly higher in fibromyalgic patients than in controls (p=0.014), only one patient and two of controls had barely elevated PTH levels. There was no relationship between vitamin D level and FIQ score (p=0.707) or HADS (p=0.824) or pain VAS (p=0.414). BMD values in the patients with fibromyalgia were comparable to those in controls at both, the lumbar spine (p=0.866) and femur neck (p=0.61).
Conclusion: Neither vitamin D levels nor BMD values are different between women with and without fibromyalgia. In this cross-sectional study, mean serum PTH level was found higher in the fibromyalgic patients than in controls. Nevertheless, in order to confirm the findings of this preliminary study it is still necessary to perform a controlled longitudinal study *(Tab. 2, Fig. 2, Ref. 35).* Full Text in free PDF *www.bmj.sk.*
Key words: fibromyalgia, musculoskeletal pain, vitamin D, parathyroid hormone.

Fibromyalgia (FM) is a chronic musculoskeletal disorder characterized by widespread pain, decreased pain threshold, stiffness, sleep disturbances, depression and fatique (1). It occurs more commonly in women and has a prevalence of 2 % in general population (2). As the physical activitiy in patients with FM is impaired and their sunlight exposure is lower, they may be at increased risk of developing hypovitaminosis D, osteoporosis and osteomalacia (3, 4). Formerly, the decreased bone mineral density (BMD) values used to be measured at various sites in patients with FM (3, 5), which had an effect on inconsistency of findings (6–8).

As it is well known, vitamin D deficiency has also been linked to widespread musculoskeletal pain, fatique and depression as well as low BMD (9–12). In some earlier studies, low levels of vitamin D were reported in patients with persistent widespread musculoskeletal pain including FM (13, 14). However, other studies have not confirmed this finding (15, 16). The findings of lower vitamin D concentrations in FM patients, if confirmed, may be important in the future investigation and management of FM patients. In previous studies, low levels of vitamin D have been associated with increased risk of hip fracture in postmenopausal women, and up to 97% of patients hospitalized with hip fracture were found to have inadequate levels of vitamin D (12). To our knowledge, there are only two published studies analyzing the levels of vitamin D in patients with FM or persistent nonspecific musculoskeletal pain as compared with controls (5, 16). Other published studies did not include the control subjects (9, 13, 15).

In this study, we measured vitamin D levels and BMD values in primary FM patients and compared them with age-matched healthy controls. The possibility that socioeconomic, racial, and cultural factors might be important in vitamin D deficiency deserves further evaluation. This is the first study in which Turkish FM patients were compared with age-matched healthy controls for vitamin D levels and BMD values. We further investigated the relationship between vitamin D levels and various clinical parameters in FM patients.

Methods

Thirty women with primary FM who fulfilled the ACR criteria for FM (1) were consecutively enrolled for the study. Thirty

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age-matched (±2 years) healthy women, namely friends and relatives of the patients as well as medical school employees were recruited as controls. To eliminate the bias due to alterations of bone metabolism in menopause, premenopausal women aged 20 to 40 years were included in both, patient and control groups. All participants were sequentially picked during three months, between May and July, which corresponds to the warm months of the year in our city (latitude 40° 19.8' N). Therefore, seasonal variations did not confound the results. All participants were selected from physically active unveiled women who were used to walking outdoors at least ten minutes every day.

Women were excluded if they had an inflammatory rheumatic disease, known osteoporosis treated with antiresorptive drugs, renal disease, hepatic disease, malabsorption disorder, anticonvulsant therapy, malignancy or pregnancy. Women using calcium or vitamin D supplement in the past three months were excluded. In addition, women previously or currently treated with systemic corticosteroids as well as women with present or previous primary hyperparathyroidism, hyperthyroidism or diabetes mellitus were excluded. Smoking and alcohol consumption were also exclusion criteria.

The same researcher (EBO) assessed all participants. Pain severity was measured with 100 mm visual analog scale (VAS). Fibromyalgia Impact Questionnaire (FIQ) (17) and Hospital Anxiety and Depression Score (HADS) (18) were optained from all participants. The FIQ is a well-validated tool for the evaluation of outcome in fibromyalgia, while the total score is measured out of 100. The HADS is a validated tool designed for the assessment of mood disorders specifically in hospitalized patients and it is scored out of 42.

Serum parathyroid hormone (PTH), alkaline phosphatase, thyroid hormones, calcium and phosphorus levels were measured with routine laboratory methods. Serum 25-hydroxyvitamin D3 (25-OHD) was measured using high performance liquid chromatography method (with a commercial kit, Chromsystems). In accordance with that used in international studies, low levels of vitamin D were grouped as deficiency (25-OHD ≤20 ng/l) and severe deficiency (25-OHD ≤8 ng/l) (9, 15, 16, 19). Based on physiologic parameters such as optimal calcium absorption and PTH normalization, there is a controversy as to the appropriate definitions of vitamin D deficiency (20). Physiological deficiency is conservatively identified at <20 ng/ml, but optimal serum 25-OHD seems to be ≥32 ng/l (20–22). Severe vitamin D deficiency (25-OHD<8 ng/ml) leads to osteomalacia. The majority of patients with ostemalacia present with widespread musculoskeletal pain, especially in the shoulder and pelvic girdle, rib cage, and leg (23).

The BMD was measured at the lumbar spine (L1–4) and femur neck by dual-energy X-ray absorptiometry (DEXA) using a Hologic scanner (Discovery W, S/N 80215) in the same week when blood sample was collected.

This study was approved by the local Ethics Committee of the Gaziosmanpasa University. All study participants gave a written informed consent.

Normality was tested using the Kolmogorov-Smirnov test. A comparison of characteristics between FM patients and controls included a Chi-squared test of association for categorical variables and an independent t-test analysis for continuous variables. The non-normally distributed data were analysed using the Mann-Whitney U test. Fisher's exact test was used instead of the Chi-squared test when any of the cells in a cross-tabulation contained fewer than five subjects. Correlation analysis was performed by calculating Pearson's correlation coefficients. For all analyses, results were considered statistically significant for p values <0.05.

Results

Table 1 summarizes the demographic, clinical and laboratory variables in the two groups. The mean age was 32.23±6.76 years (ranging from 20 to 40) in the patient group and 30.13±4.19 years (ranging from 22 to 38) in the age-matched healthy control group. As expected, no significant difference was found for age between the two groups (p=0.154). Height, weight and body mass index were not significantly different between the patients and controls (p>0.05 for each). There was no significant differ-

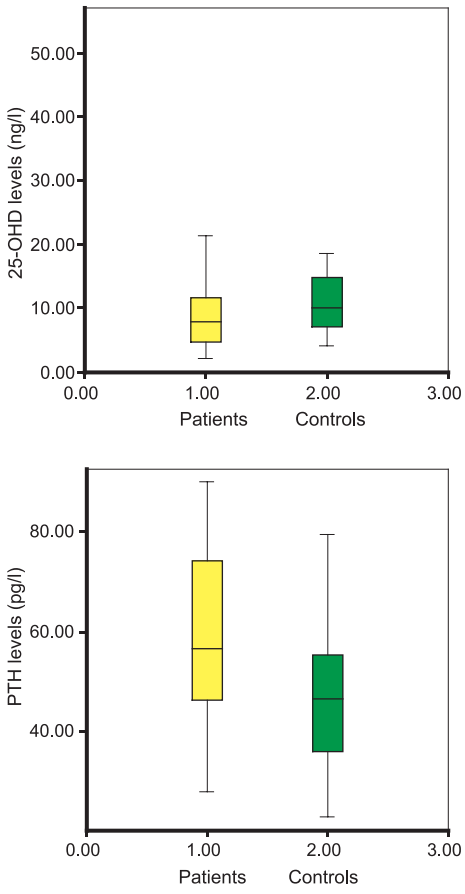

Fig. 1. Showing the shape of the distribution, central value, and variability of 25-hydroxyvitamin D (25-OHD) and parathyroid hormone (PTH) levels in patients with fibromyalgia and age-matched healthy controls.

Tab. 1. Demographic, clinical and laboratory results in the patients with fibromyalgia and age-matched healthy controls.

Variables	Patients with fibromyalgia (n=30)	Age-matched healthy controls (n=30)	p
Age (years)	32.23±6.76	30.13±4.19	0.154
Weight (kg)	68.70±13.63	64.56±12.19	0.221
Height (cm)	159.27±6.86	158.67±4.95	0.699
Bady mass index (kg/m^2)	27.29±6.20	25.66±4.81	0.259
Disease duration (month)	32.66±19.66	-	-
Number of tender points (0-18)	15.33±1.89	1.50±1.35	**<0.001***
Pain VAS (0-100mm)	66.43±12.38	-	-
FIQ score (0-100)	64.70±14.33	26.86±15.17	**<0.001***
HADS (0-42)	20.86±6.03	11.56±5.32	**<0.001***
Serum 25-OHD level (ng/l)	10.57±10.46	10.87±5.52	0.890
Serum 25-OHD≤20 ng/l (deficiency)	26 (86.7%)	29 (96.7%)	0.353
Serum 25-OHD≤8 ng/l (severe deficiency)	17 (56.7%)	10 (33.3%)	0.069
Serum calcium (mg/dl)	9.22±0.49	9.11±0.51	0.386
Serum phosphorous (mg/dl)	3.54±0.56	3.57±0.46	0.786
Alkaline phosphatase (U/l)	68.36±20.93	63.86±23.41	0.436
Parathyroid hormone (pg/l)	59.95±17.64	48.46±17.36	**0.014***
Lomber (L1-4) BMD (mg/cm^2)	1.01±0.11	1.01±0.11	0.866
Femur neck BMD (mg/cm^2)	0.79±0.09	0.78±0.10	0.610

VAS: visual ttanalog scale, FIQ: fibromyalgia impact questionnaire, HADS: hospital anxiety and depression score, 25-OHD: 25-hydroxyvitamin D, BMD: bone mineral density. Data are number (percentage) or mean±standard deviation. * Statistically significant result (p<0.05)

ence in mean serum calcium, phosphorus or alkaline phosphates levels between the two groups (p>0.05 for each). In addition, all study participants had normal serum calcium, phosphorus and alkaline phosphates levels. Although mean serum PTH level was significantly higher in the FM patients than in controls (p=0.014), only 1 of 30 patients and 2 of 30 controls had barely elevated PTH levels (up to 90 pg/l). Reference values of our laboratory for serum PTH level were 12–88 pg/l.

Mean 25-OHD level was 10.57±10.46 ng/l in the patient group and 10.87±5.52 in the control group (p=0.89) (Tab. 1). The percentages of women with vitamin D deficiency (25-OHD≤20 ng/l) were not found different between the patients and controls (86.7% and 96.7% respectively, p=0.353). Although the prevalence of severe vitamin D deficiency was higher in FM patients (25-OHD≤8 ng/l) than in controls, this difference did not reach the statistically significant level (56.7 % and 33.3 % respectively, p=0.069). The shape of the distribution, central value, and variability of serum 25-OHD and PTH levels in the two groups are shown in Figure 1.

When comparing the FM patients with age-matched healthy controls, BMD results were not different at both, the lumbar spine (p=0.866) and femur neck (p=0.61) (Tab. 1). Figure 2 shows the shape of the distribution, central value, and variability of BMD results at the lumbar spine and femur neck in the patients and controls.

As expected, the functional impairment assessed by FIQ score was significantly greater in FM patients than in age-matched healthy controls as (p<0.001) (Tab. 1). In addition, the FM patients were more anxious and depressive as assessed by HADS (p<0.001).

Correlations between 25-OHD levels and various parameters in FM group are shown in Table 2. Serum 25-OHD level was not correlated with BMD values at both the lomber spine (p=0.761) and femur neck (p=0.560). There was no relationship between vitamin D level and FIQ score (p=0.707) or HADS (p=0.824). Additionally, neither pain intensity measured with VAS (p=0.414) nor number of tender points (p=0.91) was correlated with 25-OHD level. Except alkaline phosphates (r=0.678, p<0.001), 25-OHD level was not related to serum calcium, phosphorous or PTH levels (p>0.05 for each).

Tab. 2. Pearson correlation coefficients for 25-hydroxyvitamin D level versus various parameters in the patients with fibromyalgia (n=30).

Variables	25-hydroxyvitamin D	
	r	p
Pain VAS	r=-0.155	p=0.414
Number of tender points	r=0.022	p=0.910
FIQ score	r=0.071	p=0.707
HADS	r=0.042	p=0.824
Serum calcium	r=0.141	p=0.456
Serum phosphorous	r=-0.027	p=0.887
Parathyroid hormone	r=0.115	p=0.546
Alkaline phosphates	**r=0.678**	**p=0.001***
Lomber (L1-4) BMD	r=0.058	p=0.761
Femur neck BMD	r=0.111	p=0.560

VAS – visual analog scale, FIQ – fibromyalgia impact questionnaire, HADS – hospital anxiety and depression score, BMD – bone mineral density. * Statistically significant correlation (p<0.05).

Discussion

In this study, we compared vitamin D levels and BMD values between FM patients and age-matched healthy controls. According to the results of this work, neither serum 25-OHD levels nor BMD values were significantly different between the patients and controls. The prevalence of vitamin D deficiency (25-

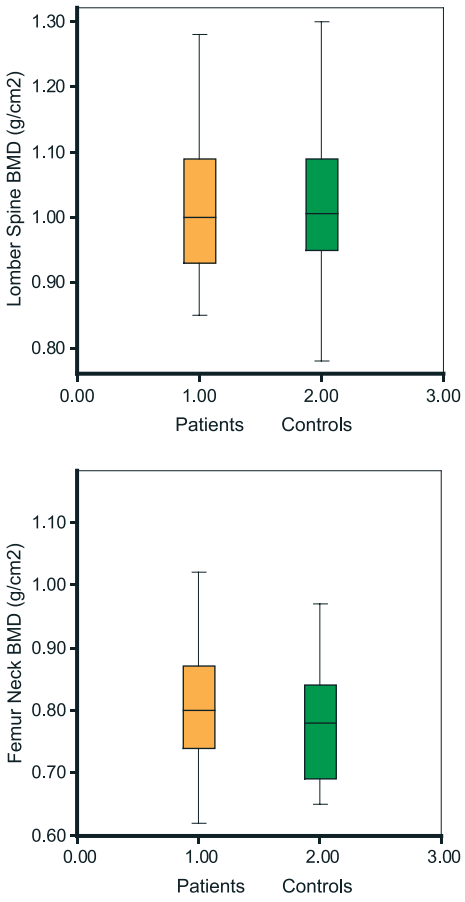

Fig. 2. Showing the shape of the distribution, central value, and variability of the bone mineral density (BMD) results at both lomber spine and femur neck in patients with fibromyalgia and age-matched healthy controls.

OHD=20 ng/l) was consistent in these two groups. The only difference between them was in mean serum PTH level, which was higher in the patient group. Additionally, there was no correlation between vitamin D level and functional status (FIQ score), or pain (VAS), or anxiety and depression (HADS) in the FM group.

Previously, low levels of vitamin D used to be associated with widespread unexplained musculoskeletal pain, and it has been suggested that all patients with persistent nonspecific musculoskeletal pain including FM be screened for hypovitaminosis D (9, 24–28). It is not known whether hypovitaminosis D contributes to FM symptoms as a cause or whether it is a result of the disease. There are evidences for both instances. In the first instance, vitamin D receptors are found in neurons and glial cells, in the brain, and although their exact function remains unclear, vitamin D may function in a fashion, which is similar to that of other neurosteroids (29). Today, central mechanisms are also held responsible in FM pathophysiology (30). In the second instance, patients with FM are less exposed to sunlight due to reduced functional ability as compared with healthy controls (3, 4).

Up to the present, low levels of vitamin D have been reported in a very variable frequency ranging from 38 to 93 % in persistent widespread musculoskeletal pain including FM (9, 15). However, most of previous studies describing an association between hypovitaminosis D and persistent widespread musculoskeletal pain did not include a control population (9, 13, 15, 25, 31). Because vitamin D insufficiency is present in a significant percentage of normal population living in temperate latitudes, the observation that a high percentage of patients with persistent widespread musculoskeletal pain have vitamin D insufficiency may be doubtful (24, 32). Therefore, larger controlled studies are needed to resolve whether vitamin D levels are lower in patients with FM or persistent widespread musculoskeletal pain than in healthy individuals living in the same area. However, to our knowledge, there are only two controlled studies published on this subject (5, 16). In the first controlled study, Al-Allaf et al (5) found out that 25-OHD deficiency (<20 ng/l) was significantly more frequent in FM patients than in controls (45% and 18.9 %, respectively). Nevertheless, in their study, patients were found to have higher BMI compared with controls. According to previous reports, high BMI is related to lower vitamin D values, possibly by reducing its serum levels as a result of storing lipid-soluble vitamin D in fat tissue (33, 34). In the second controlled study, Warner and Arnspiger (16) found out that vitamin D insufficiency (9–20 ng/l) is not more common in patients with persistent widespread musculoskeletal pain than it is in controls. Nevertheless, they chose a population of patients with osteoarthritis as controls. Patients with osteoarthritis are likely to spend more time indoors and are older than FM patients. These factors might be associated with lower vitamin D levels as well as an increasing chance of vitamin D insufficiency being associated with persistent widespread musculoskeletal pain . In our study, mean serum 25-OHD levels as well as the frequency of itamin D deficiency (25-OHD ≤20 ng/l) were consistent in both, FM patients and age-matched healthy controls. Although, the vitamin D deficiency was more severe in a greater proportion of FM patients (25-OHD ≤8 ngl/l) than in controls (56.7 % and 33.3 %, respectively), this difference was not statistically significant.

Although, the mean serum vitamin D level and the prevalence of vitamin D deficiency were consistent in the two groups, the mean serum PTH level was found significantly higher in the patient group. However, only one patient and two control subjects had barely elevated PTH levels. The rest of FM patients and controls had normal serum PTH levels. The small sample size used in this study may have prevented us from analyzing properly this result, or theoretically, patients with FM may be more sensitive to low levels of vitamin D, which could have led to higher serum PTH levels in our study. Almost no previous studies on patients with FM or persistent widespread musculoskeletal pain were focused on serum PTH level (3, 6, 8, 9, 13, 15, 16). Only Al-Allaf et al. reported data about serum PTH level in FM patients and as a result they found no significant difference in PTH level between patients and controls (5).

Additionally, the results from this study revealed that there was no significant correlation between 25-OHD level and clinical

symptoms including pain severity (VAS), anxiety and depression (HADS) as well as functional disability (FIQ score). Reed et al. also reported that musculoskeletal complaints and depressive symptoms did not correlate with the severity of 25-OHD insufficiency (35). Similarly, Armstrong et al. found no significant relationship between 25-OHD level and FIQ score as well as pain severity. But in their study, lower levels of 25-OHD were related to higher HADS (13).

In consistency with previous studies, BMD values at both, lumbar spine and femur neck were not found different in the two groups in this study (6–8). Al-Allaf et al (5) reported comparable BMD results at the lumbar spine in FM patients to that in controls, and lower BMD at the mid-distal forearm in FM patients when compared to controls. They did not measure BMD at the femur neck. On the other hand, Swezey et al. found out that FM patients as compared to controls had decreased BMD in both, lumbar spine and femur neck (3). In the present study, our patients were younger than those in Swezey's study. Therefore the duration of the disease in our patients was relatively shorter which may have prevented us from finding different BMD values between the patients and controls.

In conclusion, the frequency of vitamin D deficiency in patients with FM is not different and does not distinguish them from the rest of the population. Additionally, BMD values at both the lumbar spine and femur neck are consistent in women with and without FM. In this study, mean serum PTH level was found significantly higher in FM patients when compared to healthy controls. However, with the exception of one patient and two of controls, all paticipants had normal serum PTH levels. We do not know whether these different PTH levels within normal limits, were physiologically important. Because our study was cross-sectional and included only a limited number of participants, a controlled longitudinal study including more participants may be beneficial to confirm this preliminary finding.

References

1. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL et al. The American College of Rheumatology 1990 criteria for he classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990; 33: 160–172.

2. Wolfe F, Ross K, Anderson J, Russell IJ, Herbert L. The prevalence and characteristics of fibromyalgia in the general population. Arthritis Rheum 1995; 38: 19–28.

3. Swezey RL, Adams J. Fibromyalgia: a risk factor for osteoporosis. J Rheumatol 1999; 26: 2642–4.

4. Dobkin PL, Abrahamowicz M, Fitzcharles MA, Drista M, da Costa D. Maintenance of exercise in women with fibromyalgia. Arthritis Rheum 2005; 53: 724–731.

5. Al-Allaf AW, Mole PA, Paterson CR, Pullar T. Bone health in patients with fibromyalgia. Rheumatology (Oxford) 2003; 42: 1202–1206.

6. Appelboom T, Schoutens A. High bone turnover in fibromyalgia. Calcif Tissue Int 1990; 46: 314–317.

7. Jacobsen S, Gam A, Egsmose C, Olsen M, Danneskiold SB, Jensen GF. Bone mass and turnover in fibromyalgia. J Rheumatol 1993; 20: 856–859.

8. Jensen B, Wittrup IH, Bliddal H, Danneskiold-Samsoe B, Faber J. Bone mineral density in fibromyalgia patients-correlation to disease activity. Scand J Rheumatol 2003; 32: 146–150.

9. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clin Proc 2003; 78: 1463–1470.

10. Jorde R, Waterloo K, Saleh F, Haug E, Svartberg J. Neuropsychological function in relation to serum parathyroid hormone and serum 25-hydroxyvitamin D levels The Tromso study. J Neurol 2006; 253: 464–470.

11. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004; 79: 362–371.

12. Simonelli C, Weiss TW, Morancey J, Swanson L, Chen YT. Prevalence of vitamin D inadequacy in a minimal trauma fracture population. Curr Med Res Opin 2005; 21: 1069–1075.

13. Armstrong DJ, Meenagh GK, Bickle I, Lee ASH, Curan ES, Finch MB. Vitamin D deficiency is associated with anxiety and depression in fibromyalgia. Clin Rheumatol 2007; 26: 551–554.

14. Macfarlane GJ, Palmer B, Roy D, Afzal C, Silman AJ, O'Neill T. An excess of widespread pain among South Asians: are low levels of vitamin D implicated? Ann Rheum Dis 2005; 64: 1217–1219.

15. Block SR. Vitamin D deficiency is not associated with nonspecific musculoskeletal pain syndromes including fibromyalgia. Mayo Clinic Proc. 2004; 79: 1585–1586.

16. Warner AE and Arnspiger SA. Diffuse musculoskeletal pain is not associated with low vitamin D levels or improved by treatment with vitamin D. J Clin Rheumatol 2008; 14: 12–16.

17. Burckhardt CS, Clark SR, Bennett RM. The fibromyalgia impact questionnaire: development and validation. J Rheumatol 1991; 18: 728–33.

18. Aylard PR, Gooding JH, McKenna PJ, Snaith RP. A validation study of three anxiety and depression self-assessment scales. J Psychosom Res 1987; 31: 261–268.

19. Grootjans-Geerts I, Wielders JP. A pilot study of hypovitaminosis D in apparently healthy, veiled, Turkish women: severe vitamin D deficiency in 82% [in Dutch]. Ned Tijdschr Geneeskd 2002; 146: 1100–1101.

20. Hollis BW, Wagner CL. Letter to the editor. N Engl J Med 2005; 352: 515.

21. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998; 351: 805–806.

22. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 2003; 22: 142–146.

23. Reginato AJ, Coquia JA. Musculoskeletal manifestations of osteomalacia and rickets. Best Pract Res Clin Rhematol 2003; 17: 1063–1080.

24. Serhan E, Newton P, Ali HA, Walford S, Singh BM. Prevalence of hypovitaminosis D in Indo-Asian patients attending a rheumatology clinic. Bone 1999; 25: 609–611.

25. Al Faraj S, Al Mutairi K. Vitamin D deficiency and chronic low back pain in Saudi Arabia. Spine 2003; 28: 177–179.

26. Mascarenhas CV, Mobarhan S. Hypovitaminosis D-induced pain. Nutr Rev 2004; 62: 354–359.

27. Richardson JP. Vitamin D deficiency-the once and present epidemic. Am Fam Physician 2005; 71: 241–242.

28. Meyer C. Scientists probe role of vitamin D: deficiency a significant problem, experts say. J Amer Med Ass 2004; 292: 1416–1417.

29. Perez-Fernandez R, Alonso M, Segura C, Munoz I, Garcia-Caballero T, Diguez C. Vitamin D receptor gene expression in human pituitary gland. Life Sci 1997; 60: 35–42.

30. Bennett RM. Emerging concepts in the neurobiology of chrome pain: evidence of abnormal sensory processing in fibromyalgia. Mayo Clin Proc 1999; 74: 385–398.

31. Nellen JFJB, Smulders YM, Jos Frissen PH, Slaats EH, Silberbusch J. Hypovitaminosis D in immigrant women: slow to be diagnosed. Br Med J. 1996; 12: 570–572.

32. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med 2002; 112: 659–662.

33. Worstman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000; 72: 690–693.

34. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003; 88: 157–161.

35. Reed SD, Laya MB, Melville J, Ismail SY, Mitchell CM, Ackerman DR. Prevalence of vitamin D insufficiency and clinical associations among veiled east african women in Washington state. J Womens Health 2007; 16: 206–213.

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