Vitamin D Levels in Women with Systemic Lupus Erythematosus and Fibromyalgia

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ABSTRACT. ***Objective.*** Many patients with systemic lupus erythematosus (SLE) and fibromyalgia (FM) may spend less time exposed to the sun than healthy individuals and thus might have low vitamin D levels. It is known that hydroxychloroquine (HCQ) inhibits conversion of 25(OH)- to $1,25(OH)_2$-vitamin D both *in vitro* and in patients with sarcoidosis. We assessed winter serum 25(OH)- and $1,25(OH)_2$-vitamin D levels in patients with SLE and FM.

Methods. We recruited 25 consecutive female SLE and 25 female FM patients in London, Ontario, between January and March 2000. Subjects completed a brief questionnaire. Serum levels of 25(OH)-, $1,25(OH)_2$-vitamin D, and parathyroid hormone (PTH) were measured.

Results. In SLE patients mean 25(OH)-vitamin D was 46.5 nmol/l and mean $1,25(OH)_2$-vitamin D was 74.4 pmol/l. In FM patients these means were 51.5 nmol/l and 90.1 pmol/l, respectively. Serum 25(OH)-vitamin D levels did not significantly differ between SLE and FM patients, nor after adjusting for age and vitamin D, milk consumption, and sun block use. In 14 of the SLE patients and 12 of the FM patients 25(OH)-vitamin D levels < 50 nmol/l were found. SLE patients not using vitamin D supplements had lower 25(OH)-vitamin D levels than those who did. $1,25(OH)_2$-vitamin D tended to be lower in the SLE compared to the FM patients. This difference could be attributed to HCQ use: HCQ users (n = 17) had lower $1,25(OH)_2$-vitamin D levels than nonusers (n = 33); the mean adjusted difference was 24.4 pmol/l (95% CI 2.8–49.9).

Conclusion. Half the SLE and FM patients had 25(OH)-vitamin D levels < 50 nmol/l, a level at which PTH stimulation occurs. Our data suggest that in SLE patients HCQ might inhibit conversion of 25(OH)-vitamin D to $1,25(OH)_2$-vitamin D. (J Rheumatol 2001;28:2535–9)

Key Indexing Terms:
25(OH)-VITAMIN D $1,25(OH)_2$-VITAMIN D FIBROMYALGIA
SYSTEMIC LUPUS ERYTHEMATOSUS HYDROXYCHLOROQUINE

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Vitamin D levels in young adults and elderly vary widely across Europe and North America[1-4]. Important factors are latitude, physical health, food fortification, vitamin supply, and sun exposure[2]. Yet low vitamin D levels are frequently reported in parts of the world where sunlight is plentiful[1,2,5].

There is discussion in the literature on the cutoff point for vitamin D deficiency. Thomas, *et al* studied a hospital population of 290 patients, among whom 57% had 25(OH)-vitamin D levels < 37.5 nmol/l[6]. Even among 77 patients without risk factors for vitamin D deficiency and younger than 65 years of age, 42% had 25(OH)-vitamin D levels < 37.5 nmol/l. Such levels are considered deficient because parathyroid hormone (PTH) levels increase[6,7]. Moreover, at these levels postmenopausal women have a reduced vertebral bone mineral density[8]. In 1998 the threshold for vitamin D deficiency was redefined in a study on the relationship between 25(OH)-vitamin D and PTH before and after administration of vitamin D[9]. At levels of 50 nmol/l and lower, stimulation of PTH was seen[9]. The conclusion was that levels of 25(OH)-vitamin D < 50 nmol/l have to be

considered deficient[9]. PTH levels approach their lowest range when serum 25(OH)-vitamin D level is > 70 nmol/l[7,10-12]. Heaney[13] and Vieth[14] argued that individuals exposed to the sun in lower latitudes always have serum 25(OH)-vitamin D > 100 nmol/l, reflecting a healthy threshold to prevent pathophysiological effects on bone.

Patients with lupus often seek to prevent sun exposure by wearing a hat or scarf and by using sun screen. Recently, Redlich, *et al* reported a high frequency of osteoporosis in premenopausal women with SLE[15]. Hence vitamin D deficiency should be prevented in patients with SLE, whose bone likely is already made more vulnerable by disease activity and prednisone use. Some patients with SLE appear to be aware of the risk of vitamin D insufficiency and use vitamin D supplements.

We undertook this pilot study to measure 25(OH)-vitamin D and $1,25(OH)_2$-vitamin D levels and to relate these to milk intake and vitamin D supply. We also compared the vitamin D levels of lupus patients with those of patients with fibromyalgia (FM). In addition we studied if hydroxychloroquine (HCQ), used by many SLE patients as an immunosuppressant, lowered the conversion of 25(OH)-vitamin D to $1,25(OH)_2$-vitamin D. Because of this effect HCQ is used in patients with sarcoidosis to treat hypercalcemia[16-18].

MATERIALS AND METHODS

Subjects. All subjects were recruited from the outpatient rheumatology clinic of the university campus of the London Health Sciences Centre in London, Ontario. In January, February, and March 2000, consecutive patients with lupus and primary FM were given an information letter and asked to participate. Inclusion criteria were female sex, age 18–65 years, Caucasian race, and either SLE or FM in accord with the American College of Rheumatology classification criteria[19,20]. Any potential subject who met both sets of classification criteria (for SLE and FM) was excluded, as were patients with liver or kidney dysfunction defined as any abnormality in serum levels of alkaline phosphatase, aspartate or alanine aminotransferase, blood urea nitrogen or creatinine. All subjects completed a health questionnaire asking about medication, vitamin D supplementation, diet, life habits, previous operations, and weight loss.

Laboratory assays. Liver and kidney function were measured as well as serum ionized calcium, phosphate, and alkaline phosphatase. Intact serum PTH was measured by the immunoradiometric method (IRMA) using kits provided by Nichols Institute Diagnostics (San Juan Capistrano, CA, USA; normal values 10–60 ng/l). Serum 25(OH)-vitamin D was measured by the Dia Sorin radioimmunoassay (Dia Sorin, Stillwater, MN, USA; normal values 25–250 nmol/l). The method for $1,25(OH)_2$-vitamin D measurement was the calf thymus radio-receptor assay with cartridge extraction of serum that was prelabelled with a tracer amount of $1,25(OH)_2$-vitamin D to monitor for recovery. Normal values for $1,25(OH)_2$-vitamin D are 40 to 140 pmol/l. The vitamin D measurements were done at Mount Sinai Hospital in Toronto.

Data analysis. Using Student's t test for unpaired groups, group mean vitamin D levels (both 25(OH)- and $1,25(OH)_2$-vitamin D) were compared between patients with FM and SLE. Data are presented in Table 2 as mean difference and corresponding 95% confidence intervals (CI). In multivariate linear regression analysis, differences were adjusted for other known determinants of vitamin D levels including age, vitamin D supplement and milk consumption, and sun block use. Similar analyses were performed comparing patients taking and not taking HCQ.

RESULTS

Table 1 displays the characteristics of the 25 women with FM and 25 with SLE. The patients with FM were, on average, 5 years older than the patients with SLE. SLE patients used more drugs that potentially could interfere with vitamin D metabolism, but their intake of vitamin D also tended to be higher than in women with FM. SLE patients more frequently used sun block than FM patients, even during the winter months in which the study was conducted. Liver function, kidney function, serum ionized calcium and alkaline phosphatase all were within normal limits. Serum phosphate was low in 2 patients.

Table 2 summarizes the vitamin D levels for 25(OH)- and $1,25(OH)_2$-vitamin D by disease category and use of HCQ. There was no significant difference between FM and SLE patients with respect to 25(OH)-vitamin D levels, even after adjusting for age and vitamin D supplement, milk, and sun block use. Twelve FM patients (48%) and 14 SLE patients (58%) had 25(OH)-vitamin D levels < 50 nmol/l. SLE patients not taking vitamin D supplements had lower 25(OH)-vitamin D levels than those who did; the mean difference was 21.8 nmol/l (95% CI 5.8–38.0). Seventy-seven percent of the SLE patients not taking vitamin D supplements had a 25(OH)-vitamin D level < 50 nmol/l (n = 14).

Figure 1 illustrates $1,25(OH)_2$-vitamin D levels in SLE patients taking and not taking HCQ, and in patients with FM, none of whom used HCQ. Because the $1,25(OH)_2$-vitamin D levels were virtually the same in the SLE and FM patients not taking HCQ, these 2 subject groups were combined. The mean difference in $1,25(OH)_2$-vitamin D levels between HCQ users and all nonusers (SLE and FM) was statistically significant: 29.1 pmol/l (95% CI 11.2–46.9). This difference decreased slightly after adjusting for age and vitamin D supplement, milk, and sun block use to 24.4 pmol/l (95% CI 2.8–45.9). Three SLE patients using HCQ had $1,25(OH)_2$-vitamin D levels < 40 pmol/l. However, their 25(OH)-vitamin D levels were 49, 50, and 66 nmol/l.

Table 1. Clinical characteristics of the patients at enrollment.

	Fibromyalgia, n = 25	SLE, n = 25
Age, yrs (SD)	44.5 (6.2)	39.4 (10.0)
Drug use		
Hydroxychloroquine	0 (0)†	17 (68)
Prednisone (5–14 mg/day)	0 (0)	9 (36)
Azathioprine	0 (0)	2 (8)
Vitamin D supplement ≥ 400 IU/day	4 (16)	7 (28)
Calcium supplement ≥ 500 mg/day	4 (16)	10 (40)
Dietary vitamin D intake (≥ 250 cc milk/day*)	13 (52)	19 (76)
Exposure to daylight > 30 min/day	17 (68)	17 (68)
Sun block use (factor ≥ 20)	12 (48)	22 (88)

† Data in parentheses are percentages. * In Canada 250 cc standard milk is enriched with 90 IU vitamin D.

Table 2. Vitamin D and parathyroid hormone levels according to patient characteristics.

	Fibromyalgia*, n = 25	SLE No HCQ, n = 8	SLE HCQ, n = 17
Serum 25(OH)-vitamin D, nmol/l (SD)	51.5 (17.1)	43.5 (20.0)	48.0 (20.0)
< 25 nmol/l (%)	1 (4)	1 (13)	3 (18)
< 37.5 nmol/l (%)	6 (24)	4 (50)	5 (31)
< 50 nmol/l (%)	12 (48)	6 (75)	8 (50)
Crude difference, FM – SLE (95% CI)	5.0 (–5.6 to 15.6)		
Adjusted** difference, FM – SLE (95% CI)	7.8 (–4.7 to 20.3)		
Crude difference HCQ, no – yes (95% CI)	1.5 (–9.8 to 12.9)		
Adjusted** difference HCQ, no – yes (95% CI)	0.2 (–12.9 to 12.5)		
Serum 1,25$(OH)_2$-vitamin D, pmol/l (SD)	90.1 (29.5)	98.4 (38.0)	63.1 (26.4)
< 40 pmol/l (%)	0 (0)	0 (0)	3 (18)
Crude difference, FM – SLE (95% CI)	15.8 (–2.4 to 33.9)		
Adjusted** difference, FM – SLE (95% CI)	6.8 (–16.5 to 29.7)		
Crude difference HCQ, no – yes† (95% CI)	29.1 (11.2 to 46.9)		
Adjusted** difference HCQ, no – yes† (95% CI)	24.4 (2.8 to 45.9)		
Serum parathyroid hormone, ng/l (SD)	34.5 (12.8)	32.1 (16.0)	33.6 (25.1)

* No patient with FM used HCQ. ** Adjusted for age and vitamin D supplement, sun block, and milk use. † Analysis combined patients with FM and SLE.

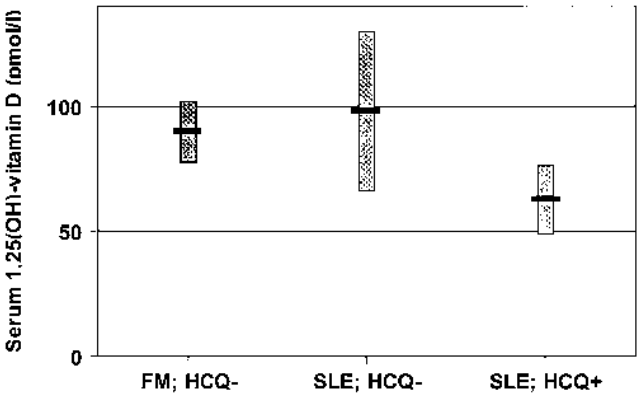

Figure 1. Mean 1,25$(OH)_2$-vitamin D levels and corresponding 95% CI according to patient group and HCQ use. HCQ–: no use of HCQ, HCQ+: HCQ used.

The mean PTH level in patients with 25(OH)-vitamin D levels < 50 nmol/l (n = 23) was 41.0 (± 21.2) ng/l and in those with levels > 50 nmol/l (n = 23) it was 26.7 (± 9.8) ng/l; the mean difference was 14.3 ng/l (95% CI 4.4–24.1).

DISCUSSION

During winter about half our patients with SLE and FM had 25(OH)-vitamin D levels less than 50 nmol/l. There was no significant difference in mean 25(OH)-vitamin D levels in SLE and FM patients. The mean levels of 25(OH)-vitamin D in the SLE and FM patients are typical winter averages for healthy adults[14]. We observed PTH stimulation in subjects with 25(OH)-vitamin D levels < 50 nmol/l, as reported[6,7].

The literature about vitamin D levels in SLE and FM is sparse. In 21 patients with SLE 25(OH)-vitamin D levels were lower than in healthy volunteers[21]. In another study of 12 adolescent patients with SLE, 7 were found to have subnormal 1,25$(OH)_2$-vitamin D levels[22]. The complaints of patients with FM resemble those of patients with vitamin D deficiency. Reginato, *et al* described 2 patients, initially diagnosed with FM, who eventually appeared to be suffering from osteomalacia due to vitamin D deficiency with secondary FM[23]. Thus it may be useful to measure vitamin D levels in patients with FM.

Three-quarters of the patients with SLE in our study who did not take vitamin D supplements had 25(OH)-vitamin D levels < 50 nmol/l. In these patients, who also have other risk factors for osteomalacia such as disease activity and the use of prednisone, 25(OH)-vitamin D should be measured, and if lower than 50 nmol/l these patients should be advised to take vitamin D on a daily basis, especially during winter months in colder climates. Over-the-counter multivitamins in general contain up to 400 IU of vitamin D per tablet. We asked patients about their use of multivitamins. Many did take multivitamins, but not on a daily basis. If they do use multivitamins without further vitamin D supplementation, they may not be protected from having low vitamin D levels in winter (Vieth R., personal communication).

Hydroxychloroquine, used by many patients with SLE as an immunosuppressant, is also prescribed in patients with sarcoidosis to treat hypercalcemia[16-18]. This effect is probably mediated by inhibition of the conversion of 25(OH)-vitamin D to 1,25$(OH)_2$-vitamin D[16]. Theoretically this effect might also be achieved by influence of HCQ on the disease process itself. Our study shows lower 1,25$(OH)_2$-vitamin D levels in HCQ users (63.1 pmol/l) compared to nonusers (92.1 pmol/l), whereas 25(OH)-vitamin D levels

were the same in both groups. This difference remained statistically significant after adjusting for age and vitamin D supplement, milk, and sun block use. These data suggest that, in SLE patients as well, HCQ inhibits the conversion of 25(OH)-vitamin D into 1,25$(OH)_2$-vitamin D. Theoretically, this effect could also be due to accelerated breakdown of 1,25$(OH)_2$-vitamin D. HCQ is known to lower lipid levels[24]. If HCQ were to lower 1,25$(OH)_2$-vitamin D by lowering lipid levels, one also would expect lower 25(OH)-vitamin D levels; however, that was not the case in our study. Low 1,25$(OH)_2$-vitamin D levels might impair the efficiency of intestinal calcium absorption and contribute to a negative calcium balance by the mobilization of calcium from the bone[25]. Three SLE patients who did use HCQ had 1,25$(OH)_2$-vitamin D levels < 40 pmol/l. However, their 25(OH)-vitamin D levels were normal (between 49 and 66 nmol/l).

The question arises whether patients with rheumatoid arthritis (RA) who use HCQ also have lower 1,25$(OH)_2$-vitamin D levels, and if this ultimately might affect bone. If this is found to be true, SLE and RA patients undergoing longterm HCQ therapy should be checked for both their 25(OH)-vitamin D and 1,25$(OH)_2$-vitamin D levels. If these are low, vitamin D supplementation would be advisable.

Patients using HCQ also might experience muscle weakness due to the possible direct toxic effect of HCQ on muscle[26-28]. However, cases have also been described in which muscle weakness occurred in the absence of electromyographic or muscle biopsy abnormalities[26-29]. In these cases HCQ might have been the cause of the muscle weakness by limiting the conversion of 25(OH)-vitamin D to 1,25$(OH)_2$-vitamin D.

Vitamin D plays a role not only in bone metabolism, but also in immunomodulation, as recently illustrated by the higher incidence of active tuberculosis in vitamin D deficient Gujarati Asian immigrants in London, UK[30].

In summary, almost half our SLE and fibromyalgia patients had 25(OH)-vitamin D levels less than 50 nmol/l, at which level parathyroid hormone stimulation occurs. Our data further suggest that in patients with SLE HCQ inhibits the conversion of 25(OH)-vitamin D to 1,25$(OH)_2$-vitamin D, which may affect bone metabolism and immune modulation.

REFERENCES

1. McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. Am J Med 1992;93:69-77.
2. van der Wielen RP, Lowik MR, van den Berg H, et al. Serum vitamin D concentrations among elderly people in Europe. Lancet 1995;346:207-10.
3. Jacques PF, Felson DT, Tucker KL, et al. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr 1997;66:929-36.
4. Delvin EE, Imbach A, Copti M. Vitamin D nutritional status and related biochemical indices in an autonomous elderly population. Am J Clin Nutr 1988;48:373-8.
5. Romagnoli E, Caravella P, Scarnecchia L, Martinez P, Minisola S. Hypovitaminosis D in an Italian population of healthy subjects and hospitalized patients. Br J Nutr 1999;81:133-7.
6. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. N Engl J Med 1998;338:777-83.
7. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis Int 1997;7:439-43.
8. Villareal DT, Civitelli R, Chines A, Avioli LV. Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. J Clin Endocrinol Metab 1991;72:628-34.
9. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency [letter]. Lancet 1998;351:805-6.
10. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337:670-6.
11. Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Ann Intern Med 1991;115:505-12.
12. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE. Dietary calcium and vitamin D intake in elderly women: effect on serum parathyroid hormone and vitamin D metabolites. Am J Clin Nutr 1998;67:342-8.
13. Heaney RP. Lessons for nutritional science from vitamin D [editorial]. Am J Clin Nutr 1999;69:825-6.
14. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 1999;69:842-56.
15. Redlich K, Ziegler S, Kiener HP, et al. Bone mineral density and biochemical parameters of bone metabolism in female patients with systemic lupus erythematosus. Ann Rheum Dis 2000;59:308-10.
16. Adams JS, Diz MM, Sharma OP. Effective reduction in the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia with short-course chloroquine therapy. Ann Intern Med 1989;111:437-8.
17. Peris P, Font J, Grau JM, Martinez dOM, Filella X, Munoz-Gomez J. Calcitriol-mediated hypercalcaemia and increased interleukins in a patient with sarcoid myopathy. Clin Rheumatol 1999;18:488-91.
18. O'Leary TJ, Jones G, Yip A, Lohnes D, Cohanim M, Yendt ER. The effects of chloroquine on serum 1,25-dihydroxyvitamin D and calcium metabolism in sarcoidosis. N Engl J Med 1986;315:727-30.
19. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
20. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160-72.
21. Muller K, Kriegbaum NJ, Baslund B, Sorensen OH, Thymann M, Bentzen K. Vitamin D_3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D_3 in patients with systemic lupus erythematosus. Clin Rheumatol 1995;14:397-400.
22. O'Regan S, Chesney RW, Hamstra A, Eisman JA, O'Gorman AM, Deluca HF. Reduced serum 1,25-$(OH)_2$ vitamin D_3 levels in prednisone-treated adolescents with systemic lupus erythematosus. Acta Paediatr Scand 1979;68:109-11.
23. Reginato AJ, Falasca GF, Pappu R, McKnight B, Agha A. Musculoskeletal manifestations of osteomalacia: report of 26 cases and literature review. Semin Arthritis Rheum 1999;28:287-304.
24. Rahman P, Gladman DD, Urowitz MB, Yuen K, Hallett D, Bruce IN. The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs. J Rheumatol 1999;26:325-30.
25. Holick MF. Microgravity-induced bone loss — will it limit human space exploration? [comment]. Lancet 2000;355:1569-70.

26. Avina-Zubieta JA, Johnson ES, Suarez-Almazor ME, Russell AS. Incidence of myopathy in patients treated with antimalarials. A report of three cases and a review of the literature. Br J Rheumatol 1995;34:166-70.
27. Richards AJ. Hydroxychloroquine myopathy. J Rheumatol 1998;25:1642-3.
28. Estes ML, Ewing-Wilson D, Chou SM, et al. Chloroquine neuromyotoxicity. Clinical and pathologic perspective. Am J Med 1987;82:447-55.
29. Wang C, Fortin PR, Li Y, Panaritis T, Gans M, Esdaile JM. Discontinuation of antimalarial drugs in systemic lupus erythematosus. J Rheumatol 1999;26:808-15.
30. Wilkinson RJ, Llewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000;355:618-21.