

Vitamin D metabolites as clinical markers in autoimmune and chronic disease

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Recent research has implicated vitamin D deficiency (serum levels of 25-hydroxyvitamin D <50nmol/L) with a number of chronic conditions including autoimmune conditions such as multiple sclerosis, lupus, psoriasis and chronic conditions such as osteoporosis, osteoarthritis, metabolic syndrome, fibromyalgia and chronic fatigue syndrome. It has been assumed that low levels of 25-hydroxyvitamin D (25-D) accurately indicate vitamin D storage and vitamin D receptor (VDR) mediated control of calcium metabolism and innate immunity. To evaluate this assumption, 25-D and 1,25-dihydroxyvitamin D₃ (1,25-D) levels were measured in 100 Canadian patients with these conditions. Additionally, other inflammatory markers (CK, CRP) were measured. Results showed a strong positive association between these autoimmune conditions and levels of 1,25-D >110 pmol/L. However, there was little association with vitamin D deficiency or the other inflammatory markers, meaning the results challenge the assumption that serum levels of 25-D are a sensitive measure of the autoimmune disease state. Rather, these findings support the use of 1,25-D as a clinical marker in autoimmune conditions. High levels of 1,25-D may result when dysregulation of the VDR by bacterial ligands prevents the receptor from expressing enzymes necessary to keep 1,25-D in a normal range.

Key words: autoimmune disease, 1,25-dihydroxyvitamin D₃, 25-hydroxyvitamin D, C-reactive protein, creatinine kinase

Introduction

There is increasing interest in the role of vitamin D deficiency in a number of chronic health problems including autoimmune diseases.¹⁻⁸ However, other studies have shown a deleterious or no beneficial effect of vitamin D supplementation in certain diseases.⁹⁻¹⁸ The effects of vitamin D are the result of genomic and non-

genomic actions mediated by the active form of vitamin D, calcitriol, also known as 1,25-dihydroxyvitamin D₃ (1,25-D). Yet most of the studies evaluating vitamin D and its association with disease are based on 25-hydroxyvitamin D (25-D) serum levels and not 1,25-D.

The definition of deficiency of 25-D is variable. One author recently surveyed the literature and determined that 25-D deficiency begins at or below 80 nmol/L.¹⁹ Still, a number of studies have found levels below that to be common in healthy subjects.²⁰⁻²⁴

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Because of these ambiguities, an evaluation of vitamin D metabolites was conducted on 100 Canadian patients residing in the Pacific Northwest who suffer from diseases that have been associated with vitamin D deficiency.²⁵ Two markers of inflammation, creatinine kinase²⁶ and C-reactive protein were also measured.^{27, 28}

Materials and Methods

Blood samples from 100 randomly selected patients presenting with clinical criteria indicating the presence of autoimmune and associated diseases were drawn and analyzed by Lifelabs, located in Burnaby, British Columbia, Canada.

Of the 100 patients, 26 were male and 74 female and ranged in age from 20 to 67 years. Patients with classical autoimmune disease totaled 30: nine with metabolic syndrome, 43 with chronic fatigue syndrome/fibromyalgia, 12 with post-Lyme disease syndrome,²⁹ and six with osteoarthritis (see Figure 1).

Patients were measured for the presence of four blood markers - elevated levels of C-reactive protein, elevated levels of creatinine kinase, deficient levels of 25-D and elevated levels of 1,25-D. Elevated levels of C-reactive protein were determined by a finding of 5 mg/L or greater. Elevated levels of creatinine kinase were determined by a finding of above 300 U/L (males), 200 U/L (females). 25-D deficiency was determined by the finding of levels at or below 50 nmol/L.

Samples to be tested for 1,25-D were refrigerated and then frozen within twelve hours after withdrawal. 25-D was measured using the Diasorin LIAISON chemiluminescence immunoassay. 1,25-D was measured using the Diasorin radioimmunoassay.

There appears to be a lack of consensus as to the normal serum levels of 1,25-D (see Table 1) with various authors citing ranges from 39 - 110 pmol/L,³⁰ 33 - 160 pmol/L,³¹ 60 - 156 pmol/L,³²

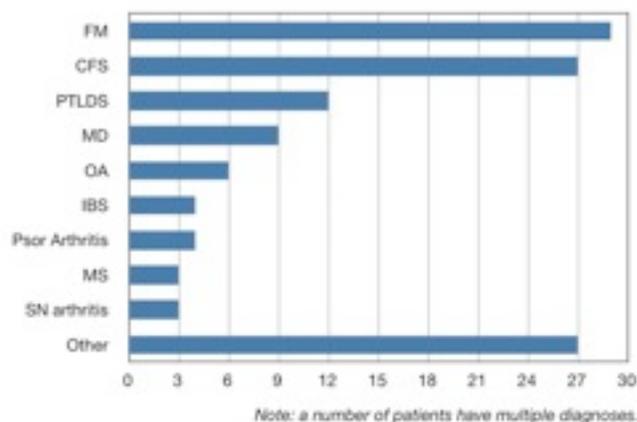


Figure 1. Patients with a given diagnosis.

47 - 162 pmol/L³³ and 36 - 108 pmol/L.³⁴ The threshold for elevated 1,25-D was selected as 110 pmol/L based on the observation that all healthy patients in a clinical care setting showed levels under this range.³⁵

Furthermore, levels of 1,25-D have been shown to drop below 110 pmol/L in patients participating in later stages of a therapy in which a VDR agonist and pulsed low-dose antibiotics are used to eliminate bacteria thought to cause the vitamin D dysregulation observed in autoimmune disease.³⁵

Results

Levels of 25-D ranging from a low of 20 nmol/L to 50 nmol/L were found in 26 patients (see Figure 2). None had below normal levels of 1,25-D (< 40 pmol/L).

Interestingly, 1,25-D rather than 25-D served as a more accurate measure of a chronic inflammatory disease state.

Elevated levels of 1,25-D ranging from 110 pmol/L to a high of 350 pmol/L were found in 85 patients. Of these patients, 19 had 25-D levels below 50 nmol/L. The mean level of 1,25-D observed in our sample (143.46 pmol/L +/- 45.56)

Table 1. Selected research of serum values for 1,25-D

Population	Age	Value of Serum 1,25-D
100 normotensive male industrial employees with no history of disease ³⁰	25 to 64 years (range)	39 - 110 pmol/L
173 subjects at high and moderate risk for coronary heart disease ³¹	Two groups analyzed: 63.3±7.1 years; 29.5±10.6	33 - 160 pmol/L
1,903 chronic kidney disease patients who were not prescribed vitamin D ³²	70.1 (mean)	60 - 156 pmol/L
10 healthy Inuit children ³⁴	5 to 17 years (range)	36-108 pmol/L
1,384 premenopausal and 1,084 postmenopausal women ⁴⁸	33.1±10.1 years (premenopausal); 65.4±8.1 years (postmenopausal)	35.5 pg/ml (mean for premenopausal women); 39.1 pg/ml (mean for postmenopausal women)

was significantly higher than the laboratory threshold value of 110 pmol/L ($p < 0.0001$ by one-sample t-test).

Levels of C-reactive protein of higher than 5.0 mg/L were observed in 17 patients, with the highest being 62.67 mg/L.

Creatinine kinase above the normal reference range were observed in 12 patients with the highest being 1109 U/L in a male and 562 in a female.

In diagnosed autoimmune patients, 10 out of 30 were found to have 25-D levels < 50 nmol/L while 27 out of 30 showed 1,25-D levels > 110 pmol/L. 5 out of 30 had C-reactive protein levels > 5.0 mg/L. 5 out of 30 had creatinine kinase levels above normal.

Levels of 25-D below the normal range were observed in two out of nine patients with metabolic syndrome while six had elevated levels of 1,25-D.^{36, 37} Of the nine patients, five showed elevated levels of C-reactive protein while none showed elevated creatinine kinase levels.

Of 43 patients with chronic fatigue syndrome/fibromyalgia^{38, 39} ten had < 50 nmol/L of 25-D

while 38 had > 110 pmol/L of 1,25-D. Eight patients had elevated levels of C-reactive protein while only one had an elevated level of creatinine kinase.

Post Treatment Lyme Disease Syndrome⁴⁰ patients may well represent a subcategory of CFS. Of 12 patients tested three showed 25-D levels < 50 nmol/L. 9 showed 1, 25-D levels > 110 pmol/L. 2 patients had C reactive proteins > 5 mg/L while 3 had above normal creatinine kinase levels.

In six patients with osteoarthritis (OA) and degenerative disc disease (DDD),⁴¹ only 1 patient had < 50 nmol/L 25-D. However all 6 patients had > 110 pmol/L of 1,25-D. None had elevated levels of C-reactive protein. Two had elevated levels of creatinine kinase.

Discussion

These findings show that vitamin D deficiency was not as common as speculated in Canadian adults living in the Pacific Northwest and was not found in the majority of patients with diseases

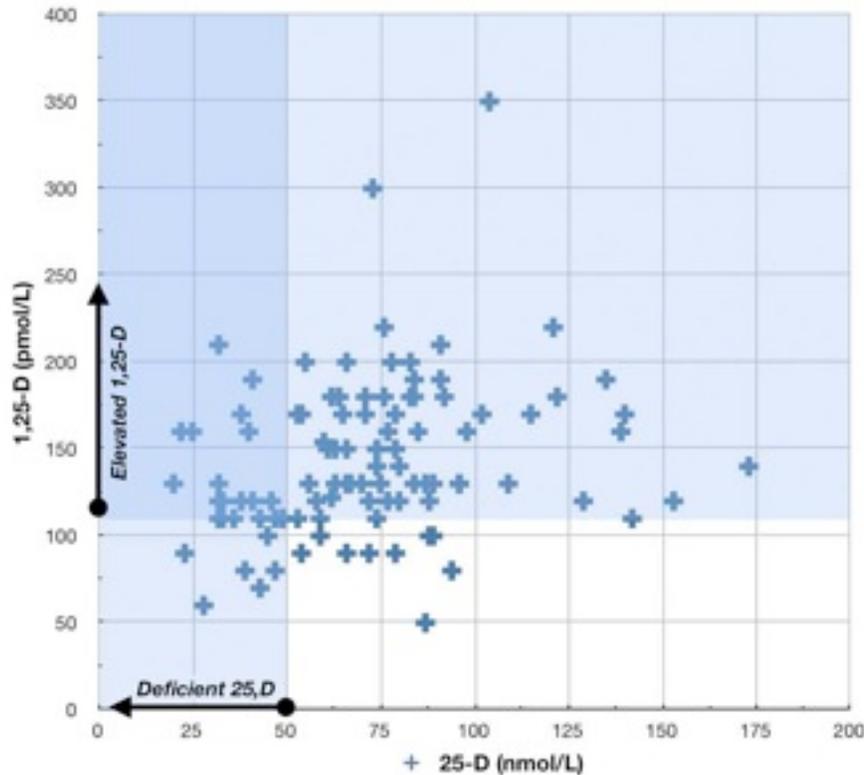


Figure 2. 25-D vs. 1,25-D in a cohort of 100 patients

previously associated with vitamin D deficiency. However, elevated levels of serum 1,25-D were found in 85% of patients examined.

Technically, high levels of 1,25-D down-regulate, via the PXR nuclear receptor, the amount of vitamin D converted into 25-D, resulting in low levels of 25-D in patients with autoimmune disease.⁴² That 25-D levels remained above range in the majority of our cohort suggests that subjects were supplementing with vitamin D or simply eating any number of a plethora of foods that are now artificially fortified with the secosteroid/hormone. So long as this confounding variable continues to exist, reliable data on how 25-D may be affected by the disease process itself and is likely to remain inconclusive.

Vitamin D supplementation leads to the formation of 25-D rather than 1,25-D. High levels of 25-D do not appear to prevent inflammatory disease as 34 out of the 100 patients tested had 25-

D levels above 80 nmol/L. This is consistent with other poor results of vitamin D supplementation in the treatment of inflammatory disease, particularly over long periods of time. Certain studies have documented a therapeutic effect of vitamin D over the short term; Lappe *et al* showed that consumption of vitamin D appears to be protective against colorectal cancer in a four-year trial.⁴³ However, a similar study done on a larger cohort that lasted only an additional three years found no such effect.⁴⁴ In fact, studies in which patients had been consuming vitamin D over the course of decades have shown a negative effect of vitamin D supplementation.^{45, 46}

Also, it is well-documented that those who supplement with vitamin D are qualitatively different than those who don't, having higher socioeconomic status,⁴⁷ better education, stronger interest in health education,⁴⁶ and presumably access to higher quality health care. This should

give one further pause when assessing observational epidemiological studies on vitamin D supplementation.

In contrast, 1,25-D appears to be a highly sensitive clinical marker both in diagnosis of autoimmune and associated diseases. It may be fruitful to consider why levels of 1,25-D are elevated in patients with autoimmune diagnoses. One possibility is that the VDR becomes dysregulated when exposed to sufficient quantities of substances created by bacteria that antagonize or otherwise inhibit the VDR. One such substance is the sulfonolipid ligand, Capnine.⁴⁸ The protease, caspase-3, which is up-regulated by *P. aeruginosa*⁴⁹ and *H. pylori*,⁵⁰ has a similar effect on the VDR, effectively inactivating it by cleaving it.⁵¹ The persistent and difficult-to-culture bacteria that create these substances may play a role in the pathogenesis of autoimmune and related diseases.

As bacterial ligands compromise the activity of the VDR, the receptor is prevented from expressing CYP24, an enzyme that breaks the 1,25-D down into its inactive metabolites.⁴² This allows 1,25-D levels to rise without a feedback system to keep them in check, resulting in the elevated levels of the hormone as observed in our cohort. Acquired hormone resistance has also been recognized with insulin, thyroid, steroid, and growth hormone releasing hormone.^{52, 53} Elevated levels of hormones are seen in some of these conditions.⁵⁴

Consistent with the hypothesis is that although 85 out of 100 patients in our cohort had a 1,25-D higher than 110 pmol/L and a significant number (38) had levels greater than 160 pmol/L, there were no apparent clinical manifestations of hypercalcemia.

This suggests that although 1,25-D rises in inflammatory disease, it is unable to actually bind to the VDR and drive the expression of genes associated with calcium absorption. This could result because the Receptor is already antagonized by bacterial ligands.

This model is supported by data collected from a trial in which patients with autoimmune diagnoses used a VDR agonist to restore VDR activity. Over the course of therapy, 1,25-D levels dropped into a normal range as inflammation decreased. This suggests that tracking 1,25-D levels may also serve as a valuable clinical marker of therapeutic response and efficacy of treatment modalities for autoimmune disease.

Given the potential benefits of serum 1,25-D as a clinical marker both in the diagnosis and monitoring of treatment response, further research is warranted. If larger, controlled studies continue to associate elevated levels of 1,25-D with an inflammatory disease state, it could be used as a reliable marker of the autoimmune disease process.

Due to the length of this paper, we are unable to summarize all of the cutting edge issues that surround this research. For this reason, we refer to the following recent literature on this subject.⁵⁵⁻⁵⁹

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* For guidelines detailing the antibiotics, dosages and pulsing schedules used, contact Foundation@AutoimmunityResearch.org.

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