Original Communication

Vitamin D Status and Cytokine Levels in Patients with Crohn's Disease

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Received: July 28, 2010; Accepted: November 22, 2010

Abstract: *Objective:* There is growing evidence that vitamin D may have immunomodulatory properties in Crohn's disease (CD). The aim of this study was to determine if serum 25-hydroxy-vitamin D [25(OH)D] was associated with inflammatory cytokines, IL-10, and TNF-alpha levels in patients with inactive CD. *Methods:* This was a prospective study of 75 adults with quiescent CD. Serum 25(OH)D was measured by radioimmunoassay and serum IL-10 and TNF-alpha by ELISA. Disease activity was assessed by the Crohn's disease activity index (CDAI) and C-reactive protein (CRP). *Results:* IL-10 levels were significantly lower in patients with vitamin D insufficiency compared with the vitamin D replete group (mean and SE 2.48 ± 0.51 v 6.77 ± 2.49 pg/mL, $p < 0.001$). There were, however, no differences in serum TNF-alpha or CRP levels based on vitamin D status. The use of a vitamin D supplement at a low dose (200 IU) did not significantly influence IL-10 levels. *Conclusion:* Circulating levels of IL-10, but not TNF-alpha, were significantly lower in CD patients with inadequate serum 25(OH)D. This suggests that poor vitamin D status may be linked to reduced anti-inflammatory capacity in this group.

Keywords: Vitamin D, 25-hydroxyvitamin D, Interleukin-10, Crohn's disease, Inflammatory Bowel Disease, Nutrition

Introduction

Crohn's disease (CD) is a life-long, remitting and relapsing inflammatory disease of the gastrointestinal tract characterized by abdominal pain, diarrhea, and fever. This disease, along with a related condition, ulcerative colitis, is collectively referred to as Inflammatory Bowel Disease (IBD). Crohn's disease is associated with increased morbidity, hospitalizations, surgery, and several medical and nutritional complications including malnutrition and osteoporosis.

Vitamin D has an established role in the promotion of bone health in people with Crohn's disease [1, 2]. For more than a decade, consensus guidelines have highlighted the importance of identifying and treating vitamin D deficiency and have recommended vitamin D supplements during corticosteroid treatment for active disease [1]. Vitamin D, however, appears to have implications for Crohn's disease beyond bone health, with growing evidence from animal models and *in vitro* studies that this vitamin exhibits immune-modulating properties [3–5]. In animal models of IBD, vitamin D

DOI 10.1024/0300–9831/a000066

deficiency is reported to accelerate the development of experimental colitis in interleukin-10 (IL-10) knockout mice [6]; whereas treatment with dietary vitamin D and calcium appear to protect against the development of inflammation [4]. There are few human studies published on vitamin D status and inflammation in patients with Crohn's disease. Bartels *et al.* [7] reported anti-inflammatory effects of vitamin D in T-cells derived from patients with Crohn's disease: In these studies of 1,25-dihydrohyvitamin D3 increased IL-10 production and reduced interferon gamma (IFNγ). This suggests that patients with Crohn's disease, who are vitamin D-deficient, may have lower production of the anti-inflammatory cytokine IL-10.

Vitamin D deficiency is common in people with Crohn's disease [8,9] especially in winter [10], in countries of Northern latitudes where there is insufficient sunlight to stimulate vitamin D production by the skin. Generally, sunlight or oral vitamin D supplements would be required to prevent deficiency. Diet alone is unlikely to maintain adequate serum vitamin D, as few foods, apart from oily fish and fortified foods, are rich sources of this vitamin.

Epidemiological studies show a North-South gradient in the prevalence of Crohn's disease, with a higher prevalence in countries of Northern latitudes [11] mirroring sunlight exposure and most probably vitamin D levels. Similarly, rheumatoid arthritis, which share common inflammatory cytokines with Crohn's disease, has been linked to vitamin D deficiency [12], with women living in more Northerly latitudes in the US reportedly at greater risk for the disease [13]. In Crohn's disease, genetic studies further suggest an association between risk of Crohn's disease and single nucleotide polymorphisms in the vitamin D receptor (VDR) gene [14, 15]. There are growing lines of evidence to propose that vitamin D may be implicated in either the pathogenesis or progression of Crohn's disease, but much remains to be proven. Importantly, Jørgensen *et al.* [16] recently showed a trend for reduced relapse rates when patients with Crohn's disease were supplemented with oral vitamin D. Although, the mechanisms by which this occurs, either via IL-10 or otherwise, have yet to be fully investigated.

The hypothesis of the present study was that poor vitamin D status would be associated with lower systemic IL-10 and higher tumor necrosis factor (TNF)-alpha levels in patients with Crohn's disease. Specifically, this study set out to investigate the association between vitamin D insufficiency, as defined by serum 25(OH)D levels, and the inflammatory cytokines IL-10 and TNF-alpha in a group of patients with quiescent Crohn's disease.

Subjects and methods

Study population

This was a prospective cross-sectional study of 75 adult (≥18 years) patients with confirmed Crohn's disease. The study was approved by St. James's Hospital and the Adelaide & Meath Hospital, incorporating the National Children's Hospital Dublin, Research Ethics Committee. Written informed consent was obtained from all participants. Patients were recruited from the IBD specialized outpatients clinic at the Adelaide and Meath Hospital (Dublin, Ireland). Clinical and demographic information were recorded at interview for all patients. Disease activity was assessed by the Crohn's disease activity index (CDAI) [17] and by measuring C-reactive protein (CRP). A fasting blood sample was taken from all patients and stored at −80°C until analysis.

Vitamin D status

Serum D 25(OH)D was measured by radioimmunoassay (DiaSorin Inc, Minnesota, USA) at the Department of Biochemistry, St. James's Hospital Dublin. Serum vitamin D status was classified using the criteria described by Lips [18] where levels below 50 nm/L were considered inadequate and termed vitamin D-insufficient and levels above this cut-off were deemed vitamin D-replete. Information on vitamin D supplementation usage was recorded at patient interview.

Cytokine assays

Serum IL-10 and TNF-alpha were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems Europe Ltd. (UK) and Biosource (Belgium) respectively. All assays were performed in duplicate and in accordance with the manufacturers' protocols.

Statistics

Data were analyzed using SPSS (version 14). Comparisons between groups were performed using the two-tailed Student's *t*-tests for parametric data and Mann-Whitney tests for non-parametric data. Probability $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

Patients were a mean age of 36 years and over half (61 %) were female. The group was predominantly in clinical remission as defined by a CDAI of less than 150 points. Characteristics of the study group are summarized in Table I.

Table I: Characteristics of the Crohn's disease study group

Age mean years ± SE	36.3 ± 1.3
Gender % female (n)	61 (46)
Years since diagnosis mean ± SE	7.68 ± 0.76
Disease Location % (n)	
Small Bowel	49 (37)
Large Bowel	30 (23)
Small and Large Bowel	20 (15)
Disease activity	
CDAI mean ± SE	113 ± 10
CRP median (inter-quartile range) mg/L	3.7 (2.9–8.0)
Current treatment % (n)	
5-aminosalicylic acid	77 (58)
Immunosuppressant	45 (34)
Biologics (infliximab)	13 (10)
Steroid use in past year	23 (17)
Previous surgery for CD	27 (20)

SE – standard error; CDAI - Crohn's disease activity index; CRP – C-reactive protein.

Vitamin D status and inflammatory cytokines

Vitamin D insufficiency was common in Crohn's disease, being documented in 64 % (n = 48) of patients. The remaining 36 % (n = 27) were considered to have adequate levels and classified as vitamin D-replete. IL-10 levels were significantly lower in patients with vitamin D insufficiency compared with the replete group, as shown in Figure 1(a).

TNF-alpha levels did not differ based on vitamin D status. Mean and standard error for TNF-alpha levels for patients classed as vitamin D-insufficient and replete were 6.13 ± 0.34 pg/mL and 6.19 ± 0.38 pg/mL (p = 0.89), respectively. CRP values did not differ based on vitamin D status: median CRP values for the vitamin D-insufficient and replete groups were 2.9 mg/L (percentile 2.9–9.4) compared with 4.4 (percentile 2.9–10.1), respectively (p = 0.79, Mann-Whitney test).

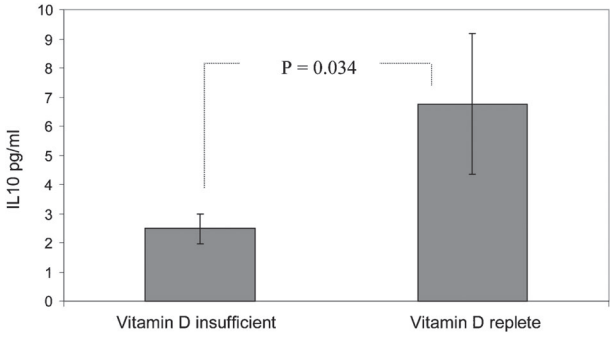

Figure 1(a): IL-10 levels in patients with Crohn's disease according to vitamin D status (a) and vitamin D supplementation (b)

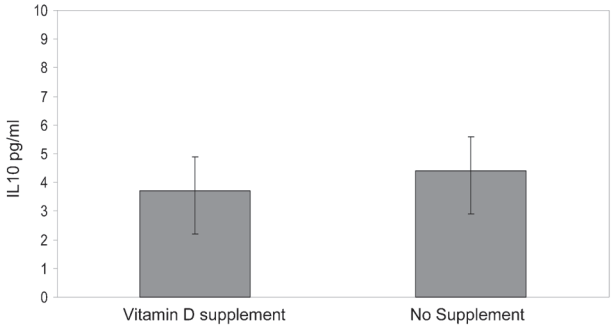

Figure 1(b)

The clinical characteristics, disease activity, and medication use of patients with vitamin D insufficiency were similar to the replete group and unlikely, in themselves, to fully explain the differences in IL-10 levels between the groups. For the vitamin D-insufficient group compared with the replete group, age was 36.8 ± 1.5 compared with 35.7 ± 2.0 years (p = 0.65); CDAI score was 115.1 ± 13.4 compared with 111.0 ± 14.7 (p = 0.84) and years since diagnosis was 7.8 ±0.8 compared with 7.7 ± 1.2 (p = 0.96). The most common current medication in both groups was a 5-aminosalicylic acid (5-ASA) compound.

Vitamin D supplement use and IL-10

We sought to determine if the use of a vitamin D supplement by patients was associated with IL-10 levels. Overall, 44 % (n = 33) of patients reported taking a vitamin D-containing supplement. As illustrated in Figure 1 (b), however, mean IL-10 levels were similar in patients who used a vitamin D-containing and non-users (p = 0.755). Oral vitamin D supplements, however, were predominantly taken in the form of a

multivitamin, which provided a low dose of 200 IU/day of vitamin D. Equally, this level of supplementation did not significantly influence the rate of vitamin D insufficiency (59 % vs. 67 %, NSχ^2 supplement-users and non-users respectively).

Discussion

This study shows that patients with Crohn's disease with low levels of vitamin D had significantly lower serum IL-10 levels than patients with adequate vitamin D. Other inflammatory markers, namely TNF-alpha or C-reactive protein, were not associated with vitamin D status in this study. This may fit with the hypothesis from experimental studies that 25(OH)D is associated with production of the anti-inflammatory cytokine IL-10 [7, 19] in Crohn's disease.

To date, few published human studies have investigated the role of low 25(OH)D status and cytokines in patients with Crohn's disease in the context of inflammation. In a cohort of Crohn's disease with inactive disease, we showed that patients with higher vitamin D levels had correspondingly higher serum IL-10 levels. In line with this, Bartels *et al.* [7] reported that 1,25-dihydrohyvitamin D3 increased IL-10 production in T-cells derived from patients with Crohn's disease and reduced interferon-gamma (IFNγ) production. In the present study, we found no differences in TNF-alpha levels based on vitamin D status, although IFNγ levels were not assessed. The lack of association with TNF levels may reflect the quiescent disease state of patients studied. Data from animal models support an anti-inflammatory role for vitamin D [3–5]. Deficiency of vitamin D has been reported to accelerate the development of inflammation in the colon of IL-10 knock-out mice [6], while dietary vitamin D and calcium suppressed inflammation [4].

Poor vitamin D status was highly prevalent in Crohn's disease (64 %) as reported by others [8, 9]. In the present study, while patients with serum 25(OH)D above 50 nm/L had significantly higher IL-10 than those with 25(OH)D less than 50 nm/L, arguably, levels in excess of 50 nm/L may be required to translate into anti-inflammatory or clinical effects. While insufficient/inadequate vitamin D is typically defined as serum 25(OH)D of 40 or 50 nmol/L [18, 20, 21], others propose higher cut-off levels in the region of 75–80 nmol/L [20, 22]. The optimal levels of 25(OH)D that translates into measurable anti-inflammatory effects, if any, in Crohn's disease have yet to be determined. Most of the cut-off levels are based on prevention of bone disease rather than inflammation. Our data suggest that a level at least above 50 nmol/L would be required to detect changes in immune markers, while recent work [16] suggests that levels in excess of 96 nmol/L would be required translate into effects on relapse rates.

It is plausible that improvement of vitamin D status through the use of vitamin D supplements may influence cytokine status in Crohn's disease. We report no difference, however, in IL-10 levels based on vitamin D supplement usage. Although 44 % of patients reported taking a vitamin D-containing supplement, which provided a low dose of vitamin D of 200 IU/day, this low dose did not significantly influence 25(OH)D levels, nor prevent deficiency, and correspondingly did not influence IL-10 levels. Even in healthy subjects, the level of supplementation necessary to maintain optimal 25(OH)D levels is not clear. In healthy adults, Cashman *et al.* [23] showed that a vitamin D intake on the order of 1120 IU/day would be required to maintain serum 25(OH)D concentrations above 50 nmol/L, which was the serum cut-off level in our study. Bischoff-Ferrari *et al.* [24] suggest that serum concentrations of 75 nmol/L may require vitamin D intakes of over 1000 IU/day. Importantly in Crohn's disease, Jørgensen *et al.* [16] recently showed that supplementation with 1200 IU vitamin D daily resulted in a mean serum 25(OH)D level of 96 nmol/L and a trend for a reduction in relapse rates of the disease. However, whether the mechanisms underlying this possible maintenance of remission in Crohn's disease involved the promotion of higher IL-10 production remains to be elucidated.

The relationship between IL-10 and vitamin D is likely to be complex [25], as are the mechanisms by which vitamin D exerts putative anti-inflammatory effects in Crohn's disease; for example via the promotion of IL-10 [7, 19] suppression of IFNγ [7] or TNF-alpha [4], and enhancement of gut mucosal barrier integrity [26]. The finding of an association between IL-10 and vitamin D supports results from animal and experimental studies [3–5]. This study, however, was cross-sectional to investigate the association between vitamin D deficiency and inflammatory markers and does not distinguish cause and effect. Assessment of relapse rates as well as an extensive profile of immune effects, including peripheral blood mononuclear cell or intestinal biopsy tissue, may better inform future studies. A notable aspect of this study was that differences in IL-10 (but not TNF-alpha) were detectable at serum level. Furthermore, these differences were detectable among patients with quiescent or mild Crohn's disease. Future large-scale studies will

be important to better understand the role of vitamin D and the mechanisms underlying putative anti-inflammatory effects in Crohn's disease, and how 1 L-10 fits into this picture.

In conclusion, this study shows that vitamin D insufficiency was associated with lower circulating levels of IL-10, but not TNF-alpha or CRP, in patients with quiescent Crohn's disease. The results provide important patient-based data, suggesting that inadequate vitamin D levels may be linked to reduced anti-inflammatory capacity in this disease.

Acknowledgements

We acknowledge financial support from the Meath Foundation Dublin, Ireland (www.meathfoundation.com).

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