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Higher serum vitamin D levels are associated with protective serum cytokine profiles in patients with ulcerative colitis

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ABSTRACT

Background & Aims: Vitamin D has immune modulating effects on cytokines. Serum vitamin D levels are associated with the risk of relapse in patients with ulcerative colitis (UC), through unknown mechanisms. We tested the hypothesis that this beneficial role of vitamin D on UC is mediated through anti-inflammatory serum cytokine profiles.

Methods: Serum samples from a prospective cohort of seventy UC patients in clinical remission were collected and baseline histological and endoscopic scores were recorded at enrollment. Clinical relapse events were recorded over the 12-month follow-up period. Serum vitamin D and cytokines levels (IL-6, IL-8, IL-17A, TNF-α, IFN-γ, IL-4, IL-10) were quantified using ELISA. Linear regression was used to determine correlation between vitamin D and cytokine profiles. Logistic regression models were used to determine the association between serum cytokine profiles and baseline histologic mucosal healing and clinical relapse.

Results: Higher serum vitamin D levels positively correlated with higher ratios of IL-4 + IL-10/IL-17A + TNF-α ($r = 0.37$, $P < .01$), and IL-4 + IL-10/IL-6 + TNF-α ($r = 0.32$, $P < .01$). In multivariate analysis, IL-4 + IL-10/IL-17A + TNF-α ratios at baseline were associated with the presence of histologic mucosal healing (O.R. 1.29, 95% CI 1.02–1.62, $P = .03$). A higher ratio of serum IL-4 + IL-10 to IL-6 + TNF-α was associated with a reduced risk of clinical relapse (O.R. 0.72, 95% CI 0.58–0.89, $P = .003$), and longer time to relapse ($p = .03$), over the 12-month follow-up period. This ratio during remission had an AUC of 0.7 in predicting later clinical relapse.

Conclusions: Vitamin D is associated with anti-inflammatory serum cytokine profiles. Anti-inflammatory cytokine patterns may mediate the protective effects of higher serum vitamin D levels in patients with ulcerative colitis.

1. Introduction

Cytokines are important mediators of inflammation. An imbalance between anti-inflammatory and proinflammatory cytokines underlies the pathogenesis of various inflammatory disorders [1–3]. Ulcerative colitis (UC) and Crohn's disease are inflammatory bowel diseases (IBD) that have been shown to have dysregulated systemic and intestinal cytokine responses [4–6]. Emerging therapeutic strategies are aimed at targeting cytokines or shifting the cytokine response to favor a more anti-inflammatory phenotype (e.g. Ustekinumab, Tofacitinib).

Vitamin D has been increasingly recognized for its immunomodulatory role and has been implicated in the pathogenesis of autoimmune disorders [7,8]. Vitamin D has been shown to have numerous and dynamic effects on the immune system, ranging from altering T cell responses [9,10] to influencing dendritic cell differentiation and protecting epithelial mucosal barriers against inflammation [11,12]. Vitamin D has also been shown to regulate cytokine pathways. In one *in vitro* study [13] involving peripheral blood mononuclear cells, vitamin D induced a dose-dependent, down regulation of proinflammatory cytokines IL-6, TNF-α, IL-17, and IFN-γ, while increasing the anti-inflammatory cytokine IL-10. Similar studies [14–16] demonstrated that vitamin D inhibited the production of proinflammatory cytokines (IL-6, TNF-α, IL-17, IL-21, IFN-γ) by macrophages and T cells. In another study [17], vitamin D promoted IL-10 production in human B cells.

Clinical studies have previously evaluated the effects of vitamin D

Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis, Simple Colitis Clinical Activity Index, SCCAI; OR, Odds Ratio; ROC, receiver operating characteristic; AUC, area under the curve; NSAIDs, nonsteroidal anti-inflammatory drugs, 5ASA, 5-aminosalicylic acid; 6MP/AZA, 6-mercaptopurine/azathioprine; anti-TNF-α, anti-tumor necrosis factor-α, IL, interleukin; IFN-γ, interferon-gamma; RCT, randomized controlled trial

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on serum cytokine production in patients. In one randomized, controlled trial (RCT) involving healthy adult patients [18], supplemental vitamin D increased the serum concentration of IL-10 and IFN-γ in patients with initially low serum vitamin D. In two other RCTs [19,20], vitamin D supplementation resulted in increased serum levels of IL-10 in congestive heart failure patients, while TGF-β was increased in multiple sclerosis patients. Clinical studies evaluating the effect of vitamin D and cytokine profiles in IBD patients are lacking. One prospective study [21] in patients with Crohn's disease demonstrated that circulating levels of IL-10 were lower in patients with lower serum vitamin D levels, but did not link these to clinical outcomes. No current studies exist exploring vitamin D status and serum cytokine profiles in patients with ulcerative colitis.

The clinical impact of vitamin D status on IBD patients, especially in patients with ulcerative colitis has recently attracted significant attention. One recent prospective study [22] demonstrated that low vitamin D is associated with higher morbidity, disease severity, and healthcare utilization in IBD patients. In another study [23], serum vitamin D was found to be inversely associated with mucosal inflammation in UC patients. Our group recently demonstrated that low serum vitamin D during remission increases the risk of UC clinical relapse independent of endoscopic or histologic grade [24]. Despite the increasing evidence of the benefits of vitamin D on ulcerative colitis, a mechanistic link between vitamin D and its immunoprotective effects in ulcerative colitis patients is not well understood.

Considering the effect that vitamin D has on cytokine production by circulating immune cells, and the imbalance in cytokine responses that underlie the pathogenesis of ulcerative colitis, we hypothesized that higher serum vitamin D is associated anti-inflammatory serum cytokine profiles and that this shift towards a more anti-inflammatory cytokine phenotype protects against clinical relapse. In this prospective study, we thus sought to determine the association between serum cytokine profiles and vitamin D levels during a period of clinical remission and their impact on presence of histologic mucosal healing at enrollment and subsequent risk of clinical relapse in ulcerative colitis patients.

2. Materials and methods

2.1. Study design and patient enrollment

We conducted a prospective study of patients with UC in clinical remission, defined by a Simple Clinical Colitis Activity Index (SCCAI) ≤2 [25,26], who were recruited after a surveillance colonoscopy from the Inflammatory Bowel Disease Center at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School, Boston, MA from 2009 to 2012. The study was approved by the BIDMC institutional review board (IRB) under protocol # 2009P000314. A total of seventy patients who had baseline serum samples collected at the time of index colonoscopy were enrolled in the study. After the 12-month follow-up period was completed, blinded investigators (JG, SM) measured serum vitamin D and serum cytokine levels in these de-identified samples. Age, gender, ethnicity, smoking status, creatinine, duration of disease, extent of disease, relevant medications (current NSAIDs, current 5-ASA, current 6MP/AZA, current anti-TNF-α, steroids in the past year, and vitamin D supplementation), and season of enrollment were recorded for each patient. Baseline laboratory values (white blood cell count, hematocrit, erythrocyte sedimentation rate, and C-reactive protein) were also obtained. (Table 1). Since this was an observational cohort study, we did not actively supplement patients with vitamin D. Furthermore, we did not assess for baseline dietary vitamin D intake or initiation of vitamin D supplementation anytime during the follow-up periods.

2.2. Assessment of baseline endoscopic and histologic inflammation and clinical relapse

Each enrolled patient had a clinically-indicated surveillance colonoscopy. During the index colonoscopy, endoscopic activity was classified using the sigmoidoscopy sub-score of the Mayo activity index based on the most inflamed segment of the colon. [27] Histological activity in all segments was classified using the Geboes score by a GI pathologist blinded to endoscopic scores. [28] For each patient, a total Geboes score was assigned to biopsies from each colonic segment and the highest score (most inflamed segment) was used as the cumulative histologic score. In our study, we defined endoscopic inflammation as a Mayo endoscopic score (MES) ≥2. Histologic inflammation was defined as a Geboes histologic score ≥3. Histologic mucosal healing was defined by Geboes histologic score < 3. "Clinical relapse" during the follow-up periods (at 3, 6, and 12 months) was defined as a SCCAI score > 2, medication intensification, or UC-related hospitalization at any time during our follow-up period of 12 months. Medication intensification was defined by an increase in dose of current regimen, addition of another medication, or change in class of medication due to symptom relapse. Of note, physicians assessing for clinical relapse were blinded to baseline endoscopic and histologic grade, serum vitamin D levels, and serum cytokine profiles.

Table 1
Baseline clinical characteristics of cohort of ulcerative colitis patients (N = 70 patients).

Clinical characteristic	Number of subjects (Percentage)
Demographics	
Average age (years)	48.6 (± 15.2)
Female gender	45 (64.3)
Caucasian ethnicity	65 (92.9)
Smoking (current)[a]	2 (2.9)
Average creatinine (mg/dL)	0.86 (± 0.19)
Season of enrollment (Low sunlight) [b]	15 (21.4)
Ulcerative colitis characteristics	
Disease duration (average years)	12.0 (± 13.6)
Left-sided colitis	27 (38.6)
Extensive colitis	37 (52.9)
Duration of remission ≥6 months	57 (81.4)
Medications	
Current NSAIDs	7 (10)
Current 5ASA	52 (74.3)
Current 6MP/AZA	13 (18.6)
Current anti-TNF-α	4 (5.7)
Steroids in past year	8 (11.4)
Vitamin D supplement [c]	42 (60)
Baseline lab values (Mean ± SD)	
White blood cell (K/uL)	6.7 (± 2.3)
Hematocrit (%)	41.0 (± 5.0)
Erythrocyte sedimentation rate (mm/hr)	9.6 (± 9.3)
C-reactive protein (mg/L)	3.2 (± 4.7)
Serum vitamin D (ng/mL) [d]	44.0 (± 29)
Baseline inflammation	
Endoscopic inflammation [e]	9 (12.9)
Histologic inflammation [f]	32 (45.7)

[a] There were only two patients who were current smokers at start of study, no patients were former smokers.
[b] Low sunlight season in Massachusetts (September to February), high sunlight season (March to August).
[c] Includes patients who were on baseline Vitamin D supplements (including Multi-Vitamin) at time of enrollement in study.
[d] Baseline serum Vitamin D level at time of enrollement in study.
[e] Endoscopic Inflammation defined as Mayo Endoscopic Score ≥2.
[f] Histologic Inflammation defined as Geboes histologic score of ≥3.

2.3. Vitamin D measurement

Serum vitamin D levels (25(OH)D) were measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Calbiotech, San Diego, CA) according the manufacturer's instructions. From our previous study [24], we determined that a serum vitamin D cutoff of ≤35 ng/mL had the greatest association with clinical relapse.