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Vitamin D deficiency in Crohn's disease and healthy controls: A prospective case–control study in the Netherlands☆

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Abstract

Background and aims: Vitamin D deficiency has been observed in a wide range of medical conditions including Crohn's disease (CD). We aimed to assess whether CD patients have lower vitamin D levels than healthy controls, and to determine risk factors for vitamin D deficiency.
Methods: 25(OH)D was measured by chemiluminescent immunoassay in serum obtained from 101 CD patients and 41 controls. Demographics, sunlight exposure, dietary vitamin D intake, comorbidities and medication were recorded using validated questionnaires. In CD patients the Harvey–Bradshaw index, Montreal classification and surgical resections were also evaluated. 25(OH)D levels of >75 nmol/L, between 50 and 75 nmol/L and <50 nmol/L were considered as normal, suboptimal and deficient, respectively.
Results: Vitamin D levels were rather low but comparable among CD patients and controls (mean 25(OH)D 51.6 nmol/L(±26.6) in CD, and 60.8 nmol/L(±27.6) in controls. Multivariate regression analysis revealed BMI, sun protection behaviour, non-Caucasian ethnicity, no use of tanning beds, and no holidays in the last year as significantly associated with serum 25(OH)D levels in CD patients (R = 0.62). In the control group no statistically significant factors were identified that had an impact on 25(OH)D serum levels.
Conclusions: Vitamin D deficiency is common in CD patients, but also in healthy controls. Appropriate vitamin D screening should be advised in patients with CD. Moreover, the positive

Abbreviations: CD, Crohn's disease; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1.25-dihydroxyvitamin D; PTH, parathyroid hormone; UV, ultraviolet; HBI, Harvey–Bradshaw Index; CRP, C-reactive protein; IQR, inter quartile range; MS, multiple sclerosis; IL, interleukin; LC–MS/MS, liquid chromatography–tandem mass spectometry.

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effect of sunlight on the vitamin D status should be discussed with CD patients, but this should be balanced against the potential risk of developing melanomas, especially in patients using thiopurines.

1. Introduction

Vitamin D has recently become a topic of scientific attention. Vitamin D belongs to the group of fat-soluble secosteroids, which humans mainly obtain from ultraviolet B radiation from sunlight exposure. In small amounts it is acquired from the diet, mainly fatty fish like salmon or mackerel, fish oil, fortified milk/orange juice, margarine, and egg yolk. Vitamin D from both the skin and diet is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D, calcidiol) and subsequently in the epithelial cells in the proximal tubule of the kidneys to the active form, 1.25-dihydroxyvitamin D ($1,25(OH)_2D$, calcitriol).[1,2]

The classic role of vitamin D is related to bone homeostasis.[1,3,4] Lately, however, vitamin D was also shown to be involved in various forms of cancer such as colorectal, prostate and breast cancer,[5] and it exerts anti-inflammatory and immunomodulating effects.[1,6,7] Vitamin D deficiency is more common in populations living at higher latitudes because of more limited sunlight exposure in these areas, in elderly and in women during pregnancy or lactation.[1] Finally, there is a seasonal variance in vitamin D levels, with lowest levels during the winter period.[8–10]

Hypovitaminosis D is common in patients with Crohn's disease (CD),[8,10–20] most probably due to a combination of different mechanisms such as malabsorption of vitamin D due to short gut syndrome (after surgery) or small bowel inflammation, intestinal loss due to protein losing enteropathy, decreased dietary intake, and limited exposure to sunlight.[1,2,21] Vitamin D is mostly absorbed in the small intestine and is subject to the enterohepatic circulation.[19,22,23]

Interestingly, the incidence of CD varies geographically with an increased incidence of CD with latitudes farther north and south of the equator. CD patients with less sunlight exposure have been reported to have lower serum 25(OH)D levels which is associated with more active disease, although a causal relationship has not been established.[2,24,25]

There has been an extensive discussion with regard to which cut-off values should be used for 'normal' and 'low' concentrations of vitamin D in standard and diseased populations. Vitamin D deficiency is often defined as a serum level of 25(OH)D that can lead to evident pathology, such as osteoporosis or rickets disease.[26,27] Vitamin D insufficiency refers to a serum level of 25(OH)D leading to increased parathyroid hormone (PTH) levels, which eventually leads to osteomalacia.[27] Previous studies on cut-off values however are based on bone mineral density instead of autoimmune diseases,[28,29] and laboratory target values vary greatly due to the lack of consensus on 25(OH)D ranges and different laboratory tests of measuring vitamin D serum levels.[2,30–32] Vitamin D levels are monitored as serum 25(OH)D, since it has a relatively long circulating half-life of approximately 10–15 days, and because it represents stores of vitamin D more reliably than active calcitriol.[33,34] The World Health Organization defines vitamin D deficiency as a 25(OH)D serum level below 50 nmol/L.[1,35,36] Levels $\leq$25 nmol/L are defined as 'severe deficiency' and levels $\leq$75 nmol/L as suboptimal.[20,27,35,37,38] In this article, we use the term "deficiency" to indicate at least suboptimal levels of $\leq$75 nmol/L.

In this prospective study, we aimed to assess whether CD patients have decreased levels of vitamin D compared to controls in an outpatient setting. Secondly, we investigated factors potentially contributing to vitamin D deficiency in both groups.

2. Methods

2.1. Study population

Adult patients ($\geq$18 years of age) with established CD were prospectively recruited at the Inflammatory Bowel Disease outpatient clinic of the Academic Medical Center, Amsterdam, The Netherlands (a tertiary referral center), during the fall (September–December 2012). Controls were healthy hospital employees visiting the department of occupational health, safety and environment and patients with functional oesophageal disorders without other bowel disorders. Subjects were only asked to participate if routine blood examination withdrawal was performed for other reasons. Subjects using vitamin D medication or multivitamin supplements were excluded from this study. No institutional ethical permission was required based on Dutch legislation.

2.2. Questionnaires

Subjects enrolled in this study received a standardized questionnaire, containing demographic data, sunlight exposure, dietary vitamin D intake, smoking habits and medication use. The sunlight exposure questions were based on a consensus-based set of questions on ultraviolet (UV) radiation exposure outlined by Glanz et al.[39] The skin colour (of the inner side of the upper arm, ranging in 5 steps between very fair and very dark), sunny or active holidays in the past year, tanning bed use, time spent outside in the summer between 10 am and 4 pm during the week and weekend on a normal day, and the use of sun protection during a regular sunny day (using sunscreen, wearing a T-shirt with long sleeves, covering of the head, staying in the shadow and wearing sunglasses) were studied. It has been shown that these self-reports of sun exposure are statistically significant valid measurements of UV exposure.[40] Each answer to the five questions on the sun protection behaviour could range between one (not at all using protection) to 5 (always using protection) points; therefore, the total score could range from 5 to 25 points. In addition, medical comorbidities, pregnancy and lactation were recorded. With the help

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of dedicated dieticians we created a table of all vitamin D containing food ingredients in order to measure the dietary vitamin D intake. Participants had to document their entire nutritional intake during the previous 3 days before their visit at the outpatient clinic. In patients with CD, we also determined clinical disease activity based on the Harvey–Bradshaw index (HBI). Moreover, the Montreal classification was documented as well as intestinal resections, if applicable.

2.3. Laboratory assessments

Serum concentrations of 25(OH)D, C-reactive protein (CRP), calcium, phosphate, alkaline phosphatase and albumin were measured in the routine clinical chemistry laboratory at our hospital. Determination of 25(OH)D was performed using the chemiluminescent immunoassay technology (DiaSorin Liaison, Minnesota, USA). For routine practice, the normal vitamin D levels are based on blood samples from almost 400 healthy blood donors collected in different seasons and different geographical regions (manufacturing procedure DiaSorin). Therefore, based on available literature and routine practice, we considered 25(OH)D levels >75 nmol/L to be normal, 50–75 nmol/L to be suboptimal[1,35,36] and <50 nmol/L to reflect deficiency.

2.4. Statistical analysis

Data were analysed using SPSS (version 20.0). Descriptive data were given as mean and standard deviation (SD), or for non-parametric data as median and interquartile range (IQR). A sample size of 98 CD patients and 43 controls will provide a 90% power in a two-sided test model with an alpha error of <0.05. We assumed the mean normal vitamin D level in CD patients to be 50 nmol/L and in the control group at least 65 nmol/L.[8,41,42] An independent *t*-test or Mann–Whitney *U* test was performed on continuous demographic data. A chi-square test was used to compare the vitamin D status (< or >50 nmol/L, and < or >75 nmol/L between the two groups). Linear regression analysis was done to determine possible risk factors associated with serum 25(OH)D levels. Statistically significant values were used to run a multivariate linear regression model. A two-tailed *P* value <0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

Hundred and one CD patients and 41 controls participated in the study, with a median age of 41 and 28 years, respectively. Most of the participants were female (69% and 80% in the CD and control group, respectively). Baseline and biochemical characteristics are shown in Table 1. Disease-specific characteristics are shown in the supplementary data. Approximately half (51%) of the CD patients had undergone one or more bowel resections. The vitamin D status was comparable between CD patients and controls (51.6 ± 26.6 nmol/L vs. 60.8 ± 27.6 nmol/L, Fig. 1). 81% of CD patients and 73% in the control group had suboptimal levels of vitamin D (<75 nmol/L). When lowering the lowest limit of normal of 25(OH)D to 50 nmol/L, meaning they were at least insufficient, both groups remained comparable (54 vs. 44%, respectively).

3.2. Predicting factors associated with 25(OH)D levels in CD patients and controls

The univariate linear regression model including all investigated factors showed that high BMI, non-Caucasian ethnicity, and never using a solarium were associated with serum levels of 25(OH)D in both groups, as shown in Table 2. In CD

Table 1 Baseline clinical and biochemical characteristics.

Characteristic	CD	Controls	*p*-value
Age (IQR)	41 (30–50)	28 (24–39)	$p = 0.002$
Gender (% male)	31	20	NS
BMI (SD)	23.5 (4.2)	22.0 (2.7)	$p = 0.01$
Ethnic origin (% Caucasian)	83	88	NS
Born in northern Europe (%)	87	88	NS
Time spent outside, hours/week (SD)	16.2 (10.3)	12.8 (7.8)	$p = 0.04$
Current smoker (% yes)	25	18	NS
Tanning bed use (% never)	81	80	NS
Skin colour (mode)	Practically colourless (51%)	Light coloured (49%)	NS
Total sun protection behaviour (SD)	15 (3.5)	15 (2.5)	NS
Dietary vitamin D intake (IQR, μg/day)	7.4 (4.45–12.80)	6.1 (4.55–10.65)	NS
CRP (IQR, mg/L)	2.1 (0.9–5.5)	1.1 (0.4–1.9)	$p = 0.001$
Calcium (SD, mmol/L)	2.3 (0.09)	2.4 (0.07)	NS
Phosphate (SD, mmol/L)	1.00 (0.18)	1.05 (0.16)	NS
Alkaline phosphatase (SD, U/L)	77 (27.1)	62 (22.0)	NS
Albumin (SD, g/L)	43.8 (3.81)	45.7 (2.47)	$p = 0.03$
25(OH)D (SD, nmol/L)	51.6 (26.6)	60.8 (27.6)	NS

SD: standard deviation, IQR: inter quartile range, CRP: C-reactive protein, NS: not statistically significant.
Median and quartiles are shown for non-parametric data.

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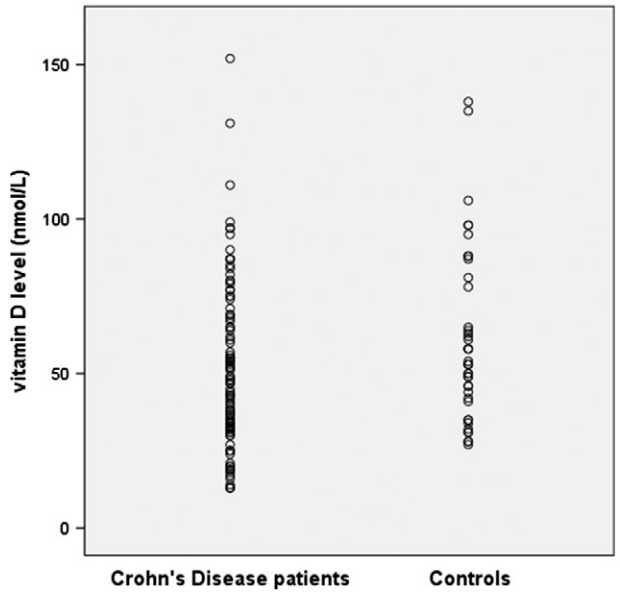

Figure 1 Serum vitamin D levels in CD patients and controls (in nmol/L).

patients the following factors were associated with low 25(OH)D serum levels: born outside Europe, no sunny or active holiday in the previous year, never visiting a solarium, more sun protection use, and azathioprine use. Remarkably there was no association with other thiopurines such as 6-mercaptopurin; however, patients who used azathioprine had a significantly higher sun protection behaviour (17 versus 15 points, p = 0.009). From the CD patients who never used a tanning bed, more were on azathioprine, although this just failed to reach significance (92% of the azathioprine users never used a tanning bed versus 78%, of the non-azathioprine users, p = 0.055). Statistically non-significant trends were observed for serum 25(OH)D levels and overweight (CD: p = 0.07, controls: p = 0.08). Factors related to disease course (CRP serum level, HBI, Montreal classification, current therapy, bowel resections) did not show an association with 25(OH)D levels. Among these factors associated with vitamin D levels in CD patients, multivariate regression analysis revealed that BMI, sun protection behaviour, non-Caucasian ethnicity, never using a tanning bed, and no holiday during the previous year were significantly associated with serum 25(OH)D levels (R = 0.62, p = 0.01, 0.00, 0.04, 0.01, and 0.01 respectively). For controls, no statistically significant factors were identified.

Table 3 Odds ratio model of factors independently associated with the dependent variable serum 25 (OH)D levels < or >50 nmol/L in CD. CI: confidence interval.

Independent variable	Odds ratio	95% CI
Ethnicity (non-Caucasian vs Caucasian)	8.65	1.86–40.22
No sunny/active holiday	2.7	1.02–7.11
Azathioprine use	2.86	1.07–7.63
Country of birth (outside Europe vs northern Europe)	13.14	1.64–105.46
Never using a tanning bed	5.86	1.79–19.24

3.3. Predicting factors for inadequate 25(OH)D levels in CD patients and controls

To assess the impact of the variables on vitamin D deficiency, odds ratios were calculated. In CD patients, non-Caucasian ethnicity, azathioprine use, born outside Europe, no sunny or active holiday during the previous year, and never using a tanning bed were significantly associated with levels of 25(OH)D below 50 nmol/L (Table 3). In control patients, none of these factors were predictive of low vitamin D serum levels.

Using multiple logistic regression, azathioprine treatment and never using a solarium were associated with levels of vitamin D below 50 nmol/L (OR 3.19, p = 0.034; and OR 3.85, p = 0.035, respectively).

When considering 75 nmol/L as lowest level of normal 25(OH)D, no active or sunny holiday (OR 3.25, 95% CI 1.12–9.46) and never using a tanning bed (OR 4.69, 95% CI 1.55–14.25) were significantly associated with suboptimal levels of vitamin D in CD patients. In controls, never using a tanning bed was associated with suboptimal vitamin D levels (OR 7.50; 95% CI 1.39–40.35).

Using multiple logistic regression analysis, no holiday and never using a tanning bed remained significantly associated with vitamin D levels below 75 nmol/L in CD patients (OR 3.40, p = 0.034; and OR 4.87, p = 0.01 respectively). In control subjects, the only factor that remained significantly associated with vitamin D level <75 nmol/L was never using a solarium (OR 7.68, p = 0.025).

4. Discussion

This prospective study showed that vitamin D deficiency is indeed common in CD patients but also in controls, with a

Table 2 Linear regression model of factors independently associated with the dependent variable serum 25(OH)D in CD and controls. NS: not statistically significant.

Independent variable	CD	Controls
Ethnicity (non-Caucasian vs Caucasian)	R = 0.35, p = 0.00	R = 0.31, p = 0.05
Never using a tanning bed	R = 0.35, p = 0.00	R = 0.39, p = 0.01
BMI	R = 0.21, p = 0.04	R = 0.37, p = 0.02
Country of birth (outside Europe vs northern Europe)	R = 0.30, p = 0.002	NS
Sun protection behaviour	R = 0.20, p = 0.05	NS
No sunny/active holiday (in the last year)	R = 0.45, p = 0.04	NS
Azathioprine use (n = 25)	R = 0.21, p = 0.04	–

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prevalence of 81% and 73%, respectively. Factors that were independently associated with 25(OH)D levels in CD patients were mainly related to UV exposure (i.e., never using a solarium, sun protection behaviour, no holiday during the previous year, non-Caucasian ethnicity, UV-B radiation) as well as a high BMI.

The potential importance of UV-B radiation in CD has been properly investigated in a geographic study in France,[43] where the incidence of CD is higher in areas with low sunshine exposure. In the United States, Scotland and France, there is a reproducible north-south gradient of CD incidence.[9,44,45] A similar phenomenon has been reported in patients with multiple sclerosis (MS), with a strong inverse correlation between MS prevalence and sun exposure. Moreover, increasing disability was strongly associated with lower levels of 25(OH)D and reduced sun exposure in MS patients.[46,47]

People with darker skin absorb more UV-B radiation in the melanin of their skin than people with a whiter skin. As a result, people with darker skin need more sun exposure in order to maintain similar levels of vitamin D.[48] This is in line with our finding that non-Caucasian patients had lower levels of vitamin D. The use of sunscreen reduces UV-B radiation absorption and is therefore associated with reduced vitamin D production in the skin.[49,50] For the general population, the importance of UV-B radiation should be stressed; however this has to be done with caution since there is the risk of developing melanomas due to excessive UV-B radiation. The use of azathioprine may lead to non-melanoma skin cancers; however this effect is only strengthened by UV-A radiation.[51–53] Patients using thiopurines such as azathioprine are instructed to limit sunlight exposure because of potential phototoxic skin reactions. Hence, this might explain why the CD patients in our cohort who used azathioprine had less sunlight exposure, and as a possible consequence that azathioprine use was a predictor for low vitamin D levels. There was no statistical significant correlation between azathioprine use and the Montreal classification, HBI or CRP values.

Obese individuals have lower levels of 25(OH)D than non-obese persons. Vitamin D is fat soluble and is principally stored in adipose tissue. There is a decreased bioavailability of vitamin D3 from UV-B and dietary sources in obese patients because of this deposition in body fat.[54] This is in line with our finding that BMI is a predictor for lower vitamin D levels.

In agreement with our observations, previous studies have reported vitamin D deficiency in CD patients[8,10,13–20] as well as in control patients.[10,17,19] However, in these studies the use of vitamin D containing supplements was allowed,[10,17] or the questionnaires did not record sunlight exposure.[19] We made use of elaborated, validated sun exposure questionnaires that separated week and weekend sunlight exposure. Previously, the use of sun-exposure questionnaires has been validated with the use of UV-dosimeters and was shown to reliably reflect sun exposure habits.[40] The part of the questionnaire focusing on alimentary vitamin D intake was extensive and consisted of all dietary products known to contain vitamin D. In the Netherlands, the daily required amount of vitamin D from dietary intake is 2.5 μg/day (=100 IU) for people between the age of 4 and 50, 5 μg/day (=200 IU) for people aged between 51 and 60 years, and 10 μg/day (=400 IU) in the category of 61–70 years old.[55] In our study cohort, this age-adjusted required daily amount was reached in only 25% of the CD patients and 11% of the controls. However, given the fact that every 100 IU of dietary vitamin D (=2.5 ug) can only raise serum 25(OH)D levels by 1 ng/mL, it is not surprising that the dietary intake alone did not have a profound contribution to the vitamin D status.[56]

The median age of the CD patients in our cohort was significantly higher than that of the controls. However, age was not significantly associated with serum vitamin D levels. Both groups were comparable in other parameters, except for the weekly time spent outside, but this correlated neither with vitamin D levels.

The insignificant outcome of the difference between vitamin D levels in CD patients and controls can likely be a result of a relative small sample size in the control group, given that the vitamin D levels in the controls were slightly lower than we expected. Hence, the difference could have reached a statistical significance if more controls had been included.

The measurement of 25(OH)D differs between various assays that are widely used,[57] with a mean bias of up to 25 nmol/L.[31] In 2004, the U.S. Food and Drug Administration approved the DiaSorin Liaison chemiluminescence assay for clinical measurement of total vitamin D.[58] Since then, this laboratory test is most commonly used in Dutch hospital laboratories. Another method that is being frequently used is the liquid chromatography–tandem mass spectometry (LC–MS/MS), which is considered the golden standard.[59] However, this method had a poor interlaboratory agreement as each laboratory used its own calibration method.[60] Since 2009 this became standardized, but still not every laboratory uses this standardization method.[61] The comparison between DiaSorin Liaison and LC-MS/MS is very good (r = 0.936)[62,63]; however in some studies with the non-standardized calibration method the DiaSorin Liaison gives an underestimation of 26 nmol/L of the 25(OH)D serum levels in 28% of the cases.[64] Hence, standardization of measurement methods is needed.

In conclusion, we here demonstrate that vitamin D deficiency is frequently observed in both CD patients and controls. The importance of vitamin D intake, both from UV-B exposure as from the dietary intake, should be stressed to both CD patients and the normal population. However, disadvantages of UV-B radiation with regard to potential carcinogenic and photoaging skin effects should be taken into consideration.

Conflict of interest

The authors of the manuscript entitled 'Vitamin D Deficiency in Crohn's Disease and Healthy Controls: a Prospective Case-control Study in the Netherlands' have no conflicts of interest to declare.

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All authors have made substantial contributions to the following: conception and design of the study (JdB, GD), acquisition of data (JdB, RvH, CP, GvdB, ML, AB, GF), providing patients (CP, GvdB, ML, AB, GF, GD), analysis and interpretation of data (JdB, RvH), drafting the article (JdB, RvH), and final approval of the version to be submitted (all authors).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2014.03.004.

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