Patterns of Vitamin D Levels and Exposures in Active and Inactive Noninfectious Uveitis Patients

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Purpose: To compare serum vitamin D levels and patterns of ultraviolet light and dietary exposure among patients with active and inactive noninfectious uveitis and population controls.

Design: Prospective case-control study. All participants (n = 151) underwent serum 25-hydroxy vitamin D measurement and completed a questionnaire on vitamin D intake and ultraviolet light exposure. Serum 25-hydroxy vitamin D levels were compared between active and inactive uveitis groups and with local population estimates.

Participants: Adult patients with active and inactive noninfectious uveitis were recruited from 2 Victorian tertiary hospitals and 1 private ophthalmic practice.

Methods: Serum 25-hydroxy vitamin D levels were compared between patients with active and inactive uveitis and population-based estimates of serum 25-hydroxy vitamin D levels, stratified by geographic region and season. Vitamin D intakes and exposures based on questionnaire results, including vitamin D supplementation and sunlight exposures on weekdays and weekends, were compared between active and inactive uveitis groups.

Main Outcome Measures: Serum vitamin D levels, intake of vitamin D, and exposure to sources of vitamin D.

Results: The median level of serum vitamin D in those with active uveitis (n = 74) was 46 nmol/l (interquartile range [IQR], 29–70 nmol/l), significantly lower than in the inactive control group (n = 77) at 64 nmol/l (IQR, 52–79 nmol/l; P < 0.001). The active uveitis group also showed lower median serum vitamin D levels than the local population median of 62 nmol/l (IQR, 46–77 nmol/l). Vitamin D supplementation also was associated significantly with uveitis inactivity (P = 0.026, Kendall’s τ test). In a subanalysis of vitamin D–deficient participants, sun exposure was associated significantly with uveitis inactivity (P = 0.014 for weekday and weekend analyses).

Conclusions: Participants with active uveitis showed significantly lower serum 25-hydroxy vitamin D levels than inactive uveitis patients and local population-based estimates. Vitamin D supplementation was found to be associated with decreased uveitis activity, as was sun exposure in those with vitamin D deficiency. These results suggest that vitamin D supplementation should be studied as an option for the prevention of uveitis relapse in at-risk patients.

Uveitis, defined as intraocular inflammation, is the fifth leading cause of vision loss in the United States, causing 10% to 15% of visual impairment in the Western world.1 Because it commonly affects young people, uveitis has a profound impact on quality-adjusted life-years and a disproportionately high socioeconomic burden.2 Noninfectious, immune-mediated uveitis is the most common form of uveitis in developed countries.3 Clinically, uveitis can be classified as active or inactive. Recurrent and chronic uveitis are the most problematic and are associated with higher rates of vision loss and morbidity.4 Treatment of recurrent and chronic uveitis remains suboptimal. Although the current mainstays of treatment are effective in decreasing inflammation, few are curative. Furthermore, these treatments themselves have systemic, and vision-threatening ocular side effects.1 Vitamin D is best known for its effects on calcium homeostasis. Vitamin D deficiency also has been found increasingly to be associated with the development of autoimmune diseases.5 This relationship is particularly strong with multiple sclerosis (MS). There are links between MS disease prevalence and low vitamin D levels at the population level and increasing evidence that low vitamin D levels are related to MS relapses.6 Furthermore, early studies have demonstrated a potential effect of vitamin D on preventing MS relapses.7–9 Importantly, MS and immune-mediated uveitis are closely related, both experimentally and clinically.10–13 In addition, vitamin D deficiency has been linked with multiple other autoimmune diseases that are related closely to uveitis, including spondyloarthritis, inflammatory bowel disease, and juvenile idiopathic arthritis, as well as systemic lupus erythematosus.
and Sjögren’s syndrome. As such, this has formed the basis for our further research into vitamin D deficiency in immune-mediated uveitis.

There remains a paucity of evidence surrounding uveitis and vitamin D deficiency. Th17 cells, a class of proinflammatory CD4-positive T-helper cells, are suppressed indirectly by calcitriol. Calcitriol also has the potential to reverse already-developed experimental autoimmune uveitis. Furthermore, patients with uveitis have been found to have lower levels of vitamin D than healthy controls. A comprehensive literature search revealed that no prior studies have examined the relationship between vitamin D levels in active compared with inactive uveitis states to determine the effect of vitamin D levels on uveitis relapses. Thus, we aimed to study vitamin D levels and patterns of sunlight exposure in patients with active uveitis when compared with patients with inactive disease and population-based estimates from the same season and geographic region.

Methods

Ethics committee approval was obtained from The Alfred Hospital and the Royal Victorian Eye and Ear Hospital Human Research Ethics Committee, and all work was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment in the study.

Participants

Consecutive patients with active and inactive noninfectious anterior uveitis, intermediate uveitis, posterior uveitis, or panuveitis, as diagnosed by a uveitis fellowship-trained ophthalmologist (A.J.H. and L.L.L.), were recruited prospectively from the Alfred Hospital General Ophthalmology Clinic, the Royal Victorian Eye and Ear Hospital Ocular Immunology Clinic, and the private practice of 2 authors (L.L.L. and A.J.H.) from January through August 2017, inclusive. All patients with suspected or confirmed infectious, traumatic, or drug-induced uveitis were excluded.

Uveitis was defined as active if slit-lamp examination revealed uveitic activity in the 30 days preceding the vitamin D blood test. An anatomic breakdown was used: anterior uveitis was defined as more than 0.5+ anterior chamber cells or 1+ flare, intermediate uveitis was defined as more than 0.5+ vitreous cells, and posterior uveitis was defined as active chorioretinal inflammation. Disease data including anatomic description, whether it was an initial presentation, presence of an identifiable uveitic syndrome, and any associated systemic disease were recorded. Dates and season of presentation and vitamin D collection as well as relevant demographic data were recorded.

All participants completed a vitamin D exposure questionnaire with the primary recruiter and underwent a single serum 25-hydroxy vitamin D measurement through an external pathology service. Vitamin D levels were measured in 1 main and 3 additional pathology laboratories. Methods of measurement are outlined in Table S1 (available at www.aaojournal.org).

The population-based estimates of serum 25-hydroxy vitamin D levels were obtained from the Australian Bureau of Statistics (ABS) National Nutrition Survey 2011 through 2012, controlled for geographic location (Victoria) and divided and assessed by season of collection. This was a random subsample of the population of Australian permanent residents used by the National Health Survey. These aggregated values provide a local population context for comparison of vitamin D levels in people with and without uveitis and were chosen because of the ability to control for location and season of collection.

Exposure Measurement

Information regarding patterns of vitamin D exposure was obtained through the completed questionnaire. This was derived from the validated questionnaire from the A Quantitative Assessment of Solar UV Exposure for Vitamin D Synthesis in Australian Adults (AusD) study. In our questionnaire, participants were required to answer multiple-choice questions on skin-tanning capabilities (dark tan, medium tan, light tan, or practically no tan); night shift work in the previous month (yes or no); length of time spent outside on each of weekdays and weekends (never, <15 minutes, 15–30 minutes, 30–45 minutes, 45–60 minutes, more [specify hours]); frequency of use in summer of each of hats, long-sleeved shirts, long trousers, sunglasses, and sunscreen (never/rarely, less than half the time, more than half the time, almost always/always); and frequency of intake of the past month of each of milk, yogurt, cheese, ice cream, vitamin D tablets, calcium tablets (none, 1–2 per week, 1 per day, more than 1 per day).

Statistical Analysis

All statistical analyses were performed using Stata/IC software version 15 for Windows (StataCorp, College Station, TX). The Mann—Whitney U test was used to test for differences between the active uveitis group and the inactive group. Vitamin D deficiency was defined as a serum 25-hydroxy vitamin D concentration of less than 50 nmol/l, in accordance with the National Endocrine Society guidelines. Each question in the questionnaire was analyzed separately. The Kendall rank correlation coefficient was used to test questions with hierarchical options, and the Fisher exact test was used for questions with dichotomous answer options. To test demographic data for similarities, the Mann—Whitney U test was used for continuous data and the Fisher exact test was used for discrete data.

Results

Serum Analysis

Of the 245 participants enrolled, 83 were excluded because they did not complete their serum 25-hydroxy vitamin D measurement and a further 11 were excluded because of a later finding of infectious uveitis. A total of 151 participants completed the serum 25-hydroxy vitamin D measurement; among these were 74 patients with active uveitis and 77 participants with inactive uveitis. The median age of included participants was 43 years (interquartile range [IQR], 33–55 years). Participants with active uveitis were significantly younger (P = 0.025) and less likely to take vitamin D supplements (P = 0.032) than those with inactive disease (Table 2). All other baseline characteristics were comparable, and the anatomic classification of the uveitis was broadly similar. Uveitic syndromes reported include Behçet’s disease, Eale’s disease, Fuchs’ heterochromic iridocyclitis, and Vogt–Koyanagi–Harada disease. Systemic diseases documented include HLA-B27–related disease (ankylosing spondylitis, juvenile idiopathic arthritis, psoriatic arthritis, and HLA-B27–positive participants), inflammatory bowel disease (Crohn’s disease and ulcerative colitis), multiple sclerosis, and sarcoidosis. Aggregated serum 25-hydroxy vitamin D values from the local general population were obtained from 594 participants (43.3% men; median age, 52 years [IQR, 39–65 years]).

All participants were grouped according to uveitis activity status and season of serum vitamin D measurement (Table 3). Recruitment
occurred across 3 seasons: summer (January–February), fall (March–May), and winter (June–August). A continuous analysis for significance was conducted only for active versus inactive participants, because continuous data were not available from the ABS. The median serum vitamin D level in the active group was 46 nmol/l (IQR, 29–70 nmol/l), compared with 64 nmol/l (IQR, 52–79 nmol/l) in the inactive group ($P < 0.001$). Notably, the median of the active group was within the deficient range, as defined by the National Endocrine Society ($< 50$ nmol/l).\textsuperscript{27}

There were statistically significant slightly reduced odds of active uveitis compared with inactive uveitis as serum vitamin D levels increased (odds ratio, 0.98; 95% confidence interval, 0.96–1.00).

### Table 3. Serum Vitamin D Levels by Season of Collection in Patients with Active Uveitis Compared with Patients with Inactive Uveitis and Local General Population Estimates

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Continuous Analysis</th>
<th>Deficiency Analysis ($&lt;50$ nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (Interquartile Range)</td>
<td>P Value*</td>
</tr>
<tr>
<td>All seasons</td>
<td>151</td>
<td>59 (38–76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All uveitis</td>
<td>74</td>
<td>46 (29–70)</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>77</td>
<td>64 (52–79)</td>
<td></td>
</tr>
<tr>
<td>Local population</td>
<td>594</td>
<td>62 (46–77)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>All uveitis</td>
<td>10</td>
<td>60 (44–91)</td>
</tr>
<tr>
<td>Active</td>
<td>7</td>
<td>59 (44–91)</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>3</td>
<td>61 (32–101)</td>
<td></td>
</tr>
<tr>
<td>Local population</td>
<td>79</td>
<td>68 (56–76)</td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>All uveitis</td>
<td>63</td>
<td>63 (42–78)</td>
</tr>
<tr>
<td>Active</td>
<td>29</td>
<td>58 (34–77)</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>34</td>
<td>66 (38–80)</td>
<td></td>
</tr>
<tr>
<td>Local population</td>
<td>317</td>
<td>65 (53–79)</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>All uveitis</td>
<td>78</td>
<td>55.5 (36–71)</td>
</tr>
<tr>
<td>Active</td>
<td>38</td>
<td>40 (22–63)</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>40</td>
<td>60 (49–76.5)</td>
<td></td>
</tr>
<tr>
<td>Local population</td>
<td>198</td>
<td>53 (37–71)</td>
<td></td>
</tr>
</tbody>
</table>

Boldface indicates statistical significance.

*Wilcoxon rank-sum test for difference between active and inactive uveitis patients.

\textsuperscript{1}Fisher exact test for difference between active, inactive, and all uveitis patient groups compared with local general population estimate.
0.96–0.99). These analyses were performed in all participants (n = 151), participants with diseases not related to inflammatory bowel disease or sarcoidosis (n = 142), participants whose serum sampling occurred at a single main pathology laboratory (n = 105), and participants with single laboratory results and diseases not related to inflammatory bowel disease or sarcoidosis (n = 100). This relationship was not changed appreciably after adjusting for age, vitamin D supplementation use, or season of sampling.

In the local population data from ABS, vitamin D deficiency affects 28.6% of the population. Our participants with active uveitis showed higher rates of vitamin D deficiency than the local population (54.1% vs. 28.6%; P < 0.001; Table 3) across all seasons measured. In contrast, inactive uveitis participants (in total and across all seasons) recorded similar percentages of inadequate vitamin D levels compared with ABS controls (23.4% in the inactive group compared with 28.6% in the local population; P = 0.418).

To ensure that supplemental vitamin D intake would not have a significant effect on our results, a subanalysis excluding all participants who were taking supplemental vitamin D was undertaken. As outlined in Table S4 (available at www.aaojournal.org), results once again were comparable with our original analysis.

Vitamin D Intake and Exposure Analysis

Of the 151 participants who completed the blood test, 139 participants fully completed the questionnaire and 1 participant partially completed the questionnaire. Of these, there were 68 active and 71 inactive participants. We compared the vitamin D intake and sun exposure questionnaire responses between the active and inactive participants (Fig 1). Here, patients with active uveitis showed lower rates of vitamin D supplementation than those with inactive uveitis, demonstrating that vitamin D supplementation showed a small but significant association with uveitis inactivity (P = 0.026). Within a subgroup of participants with vitamin D deficiency, we compared the vitamin D intake and exposure responses between active and inactive uveitis groups (Fig 2) and found 35 active and 16 inactive participants. Participants with active uveitis reported significantly decreased sunlight exposure than inactive uveitis, both in a weekday analysis (P = 0.014) and a weekend analysis (P = 0.014). Intake of calcium sources (milk, yogurt, cheese, and ice cream) were included in our questionnaire because they were included in the validated AusD questionnaire. However, in this article, we have limited our analyses to the vitamin D– and sun-related questions.

Discussion

This study is, to the best of our knowledge, the first to investigate a difference in vitamin D levels and uveitis activity. The results demonstrated that active uveitis patients showed lower serum vitamin D levels than inactive uveitis patients and population controls. These findings are consistent with previous literature reporting a relationship between uveitis and low vitamin D levels.22,23,27

Vitamin D is produced primarily from cholesterol in the skin, through a photolytic process when exposed to ultraviolet B light. Although fatty fish, fish liver oil, and egg yolk contain higher concentrations of vitamin D than other foods, it is rare to find large sources of vitamin D in foods.28,29 Notably, vitamin D intake through supplementation is the most potent mechanism of increasing vitamin D levels.26,30 In our assessment of vitamin D intakes and exposures, vitamin D supplementation showed a significant impact on uveitis disease activity. This supports the hypothesized relationship between higher levels of vitamin D and uveitis inactivity.

Within the subgroup of participants with deficient serum vitamin D levels, increased time in sunlight showed the strongest association with uveitis inactivity. Because the current literature suggests that ultraviolet radiation exposure is much less potent than vitamin D supplementation in increasing serum vitamin D levels,26,30 this leads to a hypothesis that in patients not taking supplemental vitamin D (only 8 participants in this analysis were taking supplemental vitamin D), direct sunlight was the most potent mechanism of increasing vitamin D levels, and therefore most related to decreased uveitis activity. This finding thus further strengthens the potential for vitamin D supplementation as a preventative treatment option for chronic and recurrent uveitis.

This study has several key strengths. We were able to assess large numbers of active and inactive patients and perform vitamin testing at the time of uveitis activity or inactivity. To the best of our knowledge, this is the first prospective study to examine vitamin D intake and exposures specifically in the context of autoimmune disease association. Furthermore, recruitment of consecutive uveitis patients aimed to eliminate a significant selection bias inherent in retrospective studies: for vitamin D levels to have been measured previously, there may have been a reason to suspect deficiency, so vitamin D deficiency may be overrepresented.

There are limitations to our observational study design. First, cases and controls were not matched demographically. Nevertheless, the main disparity found was in age, with the active group significantly younger than the inactive group. Because vitamin D levels usually are found to decrease with age,31 our current misdistribution of participants likely would have resulted in a bias toward the active group showing higher serum vitamin D levels compared with the inactive group (the opposite of what we found). A subsequent regression analysis demonstrated that this relationship was not changed appreciably after adjusting for age. Furthermore, an analysis excluding patients with inflammatory bowel disease and sarcoidosis, both linked with uveitis and vitamin D deficiency, demonstrated similar findings. These analyses are outlined in Table S5 (available at www.aaojournal.org).

It is possible that patients with active uveitis were less likely to spend time outside in the sun because of photophobia, a common feature of active disease. This would lead to patients with active uveitis demonstrating lower serum vitamin D levels as a consequence of, rather than as a contributor to, uveitis. Nevertheless, a significant difference in ultraviolet light exposure between active and inactive groups as a whole was not seen in our study (Fig 1), leading to the conclusion that uveitis activity may not have decreased sun exposure significantly in our cohort. It remains possible that sun and ultraviolet light exposure were different between the active uveitis patients and the inactive patients, but that we were not able to demonstrate
Figure 1. Bar graphs showing the relationships among dietary, environmental, and behavioral vitamin D exposure and uveitis inactivity status (n = 138 complete and 1 incomplete respondents). P values from Kendall’s τ test for association between questionnaire response and uveitis activity status. Act = active uveitis group; In = inactive uveitis group.
Figure 2. Bar graphs showing the relationships among dietary, environmental, and behavioral vitamin D exposure and uveitis inactivity status in questionnaire respondents with vitamin D deficiency (<50 nmol/l; n = 51 respondents). P values from Kendall’s τ test for association between questionnaire response and uveitis activity status. Act = active uveitis group; In = inactive uveitis group.
this with our questionnaire. It also must be acknowledged that a significant limitation of any questionnaire is that it ultimately is subjective and has potential for recall bias. However, the question still can be raised as to whether all patients, both active and inactive, were avoiding the sun because of uveitis. However, it was reassuring that patients with inactive uveitis showed similar serum vitamin D levels as the local population, thereby decreasing the likelihood that this would have affected results.

The active uveitis group included an increased proportion of first-presentation uveitis patients. If there is a particular propensity for patients to obtain blood tests if they were specifically at risk of having low vitamin D levels. Nonetheless, this bias should be equal between active and inactive uveitis patients. All baseline demographic characteristics were not statistically different between those included in the study and those excluded because of a lack of serum vitamin D, as outlined in Table S1 (available at www.aaojournal.org). Again, it is reassuring that the proportion of participants with vitamin D deficiency was similar in the inactive uveitis participant group and the population controls.

Furthermore, the use of multiple pathology laboratories may have affected results. The breakdown of this is outlined in Table S1 (available at www.aaojournal.org). Given that it is possible that different pathology laboratories calculate serum vitamin D differently, this statistically insignificant (P = 0.0761) discrepancy may have influenced our results. A supplementary analysis of participants whose samples were analyzed by the single main pathology laboratory is presented in Table S7 (available at www.aaojournal.org). Reassuringly, this analysis demonstrated similar results in the fall (active uveitis median, 47 nmol/l; inactive uveitis median, 66 nmol/l; P = 0.022) and winter (active uveitis median, 40 nmol/l; inactive uveitis median, 60 nmol/l; P < 0.01) sampling groups. Although it was not possible to prove causality or therapeutic effects in this cross-sectional observational study, these promising results have provided significant rationale for future longitudinal studies in this area and further research into the efficacy of vitamin D supplementation on decreasing uveitis relapses. We recommend further studies, including longitudinal cohort studies with prediagnostic and postdiagnostic measurements of patients who demonstrate uveitis, as well as cohort studies examining vitamin D status in the patients before and after activity.

It must be acknowledged that the role of vitamin D deficiency in autoimmune disease has been debated heavily and plagued with confusion and premature attribution of causality, with failure of therapeutic trials. There is still much work to be carried out, and we hope that our work contributes to the body of knowledge in this area.

In conclusion, this research has provided evidence to support the growing literature of vitamin D deficiency in autoimmunity, as well as novel insights into vitamin D levels in uveitis activity. To the best of our knowledge, this was the first study of its kind to evidence a link between active uveitis and vitamin D deficiency, as well as a relationship between increased vitamin D intake and decreased uveitis activity. These results could provide further insights into the causes of uveitis relapse and pave the way into further research on decreasing uveitis relapses by supplementation of vitamin D.

References


Footnotes and Financial Disclosures

Originally received: December 22, 2018.
Final revision: June 20, 2019.

Accepted: June 28, 2019.

Available online: [link].

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Financial Disclosure(s):

The author(s) have made the following disclosure(s): L.L.L.: Advisory board — Allergan, Bayer, AbbVie; Lecturer — AbbVie, Novartis, Allergan; Financial support (to institution) — Bayer, Novartis.

A.J.H.: Advisory board — Novartis, Bayer, AbbVie; Lecturer — AbbVie, Novartis; Financial support (to institution) — Novartis.

Supported by the Ophthalmology Department Special Purposes Fund, The Alfred Hospital, Melbourne, Australia (grant no.: Y3158); and the National Health and Medical Research Council, Canberra, Australia (Early Career Fellowship no.: GNT 1109330 [L.L.L.]).

The Centre for Eye Research Australia receives funding from the Australian Government. These funding organizations had no role in the design or conduct of this research.

HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at The Alfred Hospital and the Royal Victorian Eye and Ear Hospital approved the study. All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent. No animal subjects were included in this study.

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Conception and design: Chiu, Lim, Rogers, Hall

Analysis and interpretation: Chiu, Lim, Rogers, Hall

Data collection: Chiu, Lim, Rogers, Hall

Obtained funding: Lim, Hall

Overall responsibility: Chiu, Lim, Rogers, Hall

Abbreviations and Acronyms:

ABS = Australian Bureau of Statistics; IQR = interquartile range; MS = multiple sclerosis.

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