Serum vitamin D status is associated with the presence but not the severity of primary open angle glaucoma

Aurélien Goncalves[a], Dan Milea[a,b,c,d], Philippe Gohier[a], Ghislaine Jallet[a], Stéphanie Leruez[a], Mani Baskaran[b,c,d,e], Tin Aung[b,c,e], Cédric Annweiler[f,g,*]

[a] Department of Neuroscience, Division of Ophthalmology, Angers University Hospital, Angers, France
[b] Singapore Eye Research Institute, Singapore, Singapore
[c] Singapore National Eye Centre, Singapore, Singapore
[d] Duke-NUS, Singapore, Singapore
[e] Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[f] Department of Neuroscience, Division of Geriatric Medicine, Angers University Hospital, University Memory Clinic of Angers, UPRES EA 4638, University of Angers, UNAM, Angers, France
[g] Robarts Research Institute, Department of Medical Biophysics, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

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ABSTRACT

Objectives: Vitamin D is involved in visual health and function. Our objective was to determine whether age-related vitamin D insufficiency was associated with the presence and the severity of primary open angle glaucoma (POAG) in a case-control study of older adults.
Study design: Case-control study.
    Main outcome measures. One hundred fifty cases diagnosed with moderate-to-severe POAG (mean, 75.1 ± 8.5 years; 42.0% female) and 164 healthy controls (mean, 73.0 ± 7.9 years; 59.8% female) were included. POAG diagnosis was based on classical diagnostic criteria of optic nerve cupping and/or RNFL thinning, measured with optical coherence tomography. Severe POAG was defined as Humphrey visual field mean deviation (MD) worse than -12 dB. Vitamin D insufficiency was defined as serum $25OHD \leq 75$ nmol/L. Age, gender, mean arterial pressure, vitamin D supplementation, visual acuity, and intraocular pressure were used as potential confounders.
Results: POAG cases had lower mean serum 25OHD concentration than controls (42.9 ± 25.7 nmol/L versus 49.4 ± 29.5 nmol/L, $P = 0.039$) and a greater prevalence of vitamin D insufficiency (90.7% versus 82.3%, $P = 0.032$). Increased mean serum 25OHD concentrations were associated with lower POAG frequency, even after adjustment for potential confounders (OR = 0.89 per 10 nmol/L of 25OHD, $P = 0.045$). Similarly, vitamin D insufficiency was associated with POAG (OR = 2.09, $P = 0.034$). Among POAG cases, no 25OHD difference was observed between moderate and severe POAG cases (respectively, 39.2 ± 23.3 nmol/L versus 45.1 ± 26.7 nmol/L, $P = 0.188$); and no between-group difference regarding the prevalence of vitamin D insufficiency (88.9% versus 94.0%, $P = 0.313$).
Conclusions: Decreased serum 25OHD concentration was associated with POAG. There was no 25OHD difference between moderate and severe POAG.

1. Introduction

The primary open-angle glaucoma (POAG), a common chronic disease of the optic nerve in older adults associated with high intraocular pressure, is characterized by gradual loss of visual field due to damage of retinal ganglion cells and/or their axons [1–3]. To date, the pathogenic mechanisms of POAG are not fully elucidated.

Recent findings have suggested that the serum vitamin D status could be a biological determinant associated with visual function and retinal structure in older adults. With advancing age, a decreasing proportion of older adults are able to maintain normal vitamin D status (from 50% at 60 years of age to less than 20% after 90 years of age) [4]. It is now accepted that the role of vitamin D is not restricted to bone, but also targets a larger number of non-bone organs, including the eye [5]. Vitamin D insufficiency has

* Corresponding author at: Department of Neuroscience, Angers University Hospital, 49933 Angers Cedex 9, France. Tel.: +33 2 41 35 54 86; fax: +33 2 41 35 48 94.
    E-mail address: CeAnnweiler@chu-angers.fr (C. Annweiler).

been associated with reduced visual acuity in older adults [6], due to various causes, including reduced macular health [7]. In non-human primates, vitamin D eye drops resulted in a reduction of intraocular pressure by 20%, raising the question of a possible link between vitamin D status and POAG [8]. The objectives of this case-control study were to determine whether (i) POAG patients had lower serum 25-hydroxyvitamin D (25OHD) concentrations compared to healthy controls, and (ii) if POAG severity was associated with lower serum 25OHD concentrations.

2. Materials and methods

2.1. Participants

We studied consecutive patients followed in a glaucoma clinic at the Department of Ophthalmology, University Hospital of Angers, France, and recruited in the Glaucogen study between 17 December 2013 and 15 July 2014. The Glaucogen study is a case-control study designed to identify genes of polymorphism as susceptibility factors for POAG, which included patients aged ≥60 years, diagnosed with moderate-to-severe POAG, but no other associated intraocular conditions. Controls were selected from an elderly population (age ≥60 years), fulfilling the following inclusion criteria: visual acuity ≥20/50 and no intraocular pathology, except moderate cataract. Exclusion criteria in the control group were personal or family history of glaucoma, of ocular hypertension or any other intraocular pathology, including retinal conditions. For the present analysis, we included only participants who underwent a serum vitamin D assay at the time of the inclusion. Thus, four participants without blood testing were excluded from the analysis. Finally, 150 POAG cases and 164 controls were included in our analysis.

Participants were included after having given their written informed consent for research. The study was conducted in accordance with the ethical standards set forth in the Helsinki Declaration (1983). The Glaucogen protocol was approved by the University of Angers Ethical Review Committee (CPP Ouest 2−24/10/2013).

In addition to a full medical examination and blood test for 25OHD, all included participants underwent an ophthalmic evaluation, including best-corrected visual acuity, intraocular pressure, pachymetry, slit-lamp examination, fundoscopy, and high-definition optical coherence tomography (HD-OCT) using the Cirrus device (Carl Zeiss Meditech, Dublin, CA).

2.2. Primary open-angle glaucoma

The diagnosis of chronic POAG was based on consensual criteria, i.e. intraocular pressure >21 mmHg, glaucomatous optic nerve damage with a cup/disc ratio >0.3, open anterior chamber angle, and progressive visual field loss without signs of secondary glaucoma or nonglaucomatous cause of optic neuropathy [9].

Standard automated perimetry (Humphrey field analyser, Carl Zeiss, Dublin, CA) using the 24-2 SITA-standard algorithm was performed in the glaucoma group, and the mean defect (MD) was used to grade the severity of POAG. The reliability indices retained to were false positives or false negative under 15% and fixation losses under 20%. No repetition of the standard automated perimetry was performed. In our sample with moderate-to-severe POAG, severe POAG was defined using the classic threshold MD of worse than −12 dB [9].

2.3. Serum 25-hydroxyvitamin D concentration

Venous blood was collected from resting participants. Serum 25OHD concentration, an effective indicator of vitamin D status [4–6,8,10], was measured by radioimmunoassay (DiaSorin corp.,

Stillwater, MN). Intra- and interassay precisions were, respectively, 5.2% and 11.3%. Vitamin D insufficiency was consensually defined as 25OHD concentrations ≤75 nmol/L (to convert to ng/mL, divide by 2.496) [4]. All measurements were performed locally at the University Hospital of Angers, France.

2.4. Covariables

Age, gender, mean arterial pressure (MAP), vitamin D supplementation, visual acuity, and intraocular pressure were used as potential confounders in our analysis.

The best-corrected visual acuity was measured using decimal Monoyer charts and converted into logMAR units for statistical analysis purposes. Intraocular pressure was measured using Goldmann applanation tonometer. For the analysis, we included data from the most affected eye in POAG patients and from the right eye, in healthy controls. Blood pressure was measured at rest in a quiet environment by trained nurses according to a standardized protocol [11] using a sphygmomanometer placed on the brachial artery with arm at heart level. The MAP (i.e., the average BP over the entire course of the BP cycle) was calculated in mmHg from systolic (SBP) and diastolic blood pressures (DBP) using the following formula: MAP = (SBP + 2 DBP)/3 [12]. The regular use of vitamin D supplements was reported by direct inquiry, whatever the dosage schedule or route of administration, and regardless of the date of commencement.

2.5. Statistical analysis

The participants' characteristics were summarized using means and standard deviations (SD) or frequencies and percentages, as appropriate. Normality of data distribution was checked using skewness–kurtosis test. As the number of observations was higher than 40, comparisons were not affected by the shape of the error distribution and no transform was applied [12]. First, comparisons between cases with POAG and controls were performed using Student's t-test or the Chi-square test, as appropriate. Second, univariate and multiple logistic regressions were used to examine the association between serum 25OHD (independent variable) and POAG (dependent variable), while adjusting for potential confounders. Third, a t-test and a Chi-square test were used among cases to compare the serum 25OHD concentration and the prevalence of vitamin D insufficiency between those with severe POAG (i.e., MD worse than −12 dB) and those with moderate POAG (i.e., MD better than −12 dB). A Pearson correlation was also used to determine whether the serum 25OHD correlated with MD among cases. P-values <0.05 were considered significant. All statistics were performed using SPSS (v19.0, IBM Corporation, Chicago, IL).

3. Results

One hundred fifty cases with POAG (mean ± standard deviation, 75.1 ± 8.5 years; 42.0% female;) and 164 controls (73.0 ± 7.9 years, 59.8% female) were included in the study. The design of the study imposed inclusion of moderate-to-severe POAG cases, explaining the visual field impairment (mean MD, −17.2 ± 7.9 dB). There were more cases with severe POAG ($n = 99$, 66% with MD > −12 dB) than moderate POAG. Overall, the mean 25OHD concentration was 46.3 ± 27.9 nmol/L; vitamin D insufficiency was found in 271 of the included participants (86.3%). As illustrated in Table 1, POAG cases had lower serum 25OHD concentration than controls (42.9 ± 25.7 nmol/L versus 49.4 ± 29.5 nmol/L, $P = 0.039$). Vitamin D insufficiency was more frequently present in POAG cases than in controls free of POAG (90.7% versus 82.3%, $P = 0.032$). POAG cases were older ($P = 0.025$), with a higher MAP ($P = 0.005$), and with a greater proportion of males ($P = 0.002$), than controls.

Table 1

Characteristics and comparison[*] of cases with primary open-angle glaucoma ($n = 150$) and matched controls without primary open-angle glaucoma ($n = 164$).

	Total sample ($n = 314$)	Primary open-angle glaucoma		P-value[*]
		Yes ($n = 150$)	No ($n = 164$)	
Demographical and clinical measures				
Age (years)	74.0 ± 8.2	75.1 ± 8.5	73.0 ± 7.9	**0.025**
Female gender, n (%)	161 (51.3)	63 (42.0)	98 (59.8)	**0.002**
Mean arterial pressure (mmHg)	100.0 ± 12.6	102.2 ± 12.4	98.1 ± 12.6	**0.005**
Vitamin D supplementation, n (%)	98 (31.2)	45 (30.0)	53 (32.7)	0.606
Season of evaluation				0.202
Spring, n (%)	121 (38.5)	49 (32.7)	72 (43.9)	
Summer, n (%)	33 (10.5)	19 (12.7)	14 (8.5)	
Fall, n (%)	8 (2.5)	4 (2.7)	4 (2.4)	
Winter, n (%)	152 (48.4)	78 (52.0)	74 (45.1)	
Ophthalmic measures				
Visual acuity[†] (logMAR)	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.4	0.597
Axial length[†] (μm)	23.6 ± 1.2	23.9 ± 1.2	23.4 ± 1.1	**0.001**
Intraocular pressure[†] (mmHg)	14.5 ± 3.6	14.0 ± 4.3	15.0 ± 2.8	**0.020**
Macular thickness[†] (μm)	265.0 ± 63.1	267.0 ± 81.6	262.7 ± 29.3	0.601
RNFL thickness[†] (μm)	71.5 ± 16.5	60.6 ± 9.2	88.7 ± 8.9	**<0.001**
Cup-to-disc ratio[†] (%)	68.9 ± 20.9	81.8 ± 10.2	47.6 ± 16.0	**<0.001**
Primary open-angle glaucoma				
Right eye most affected, n (%)	–	66 (44.0)	–	–
Disease duration since diagnosis (years)	–	14.9 ± 8.8	–	–
Mean defect[†]				
Mean (dB)	–	-17.2 ± 7.9	–	–
Severe[‡], n (%)	–	99 (66.0)	–	–
Serum measures				
Mean 25-hydroxyvitamin D concentration (nmol/L)	46.3 ± 27.9	42.9 ± 25.7	49.4 ± 29.5	**0.039**
Vitamin D insufficiency[#], n (%)	271 (86.3)	136 (90.7)	135 (82.3)	**0.032**

Data presented as mean ± standard deviation where applicable. RNFL: retinal nerve fibre layer.

[*] Comparisons between participants with and without primary open-angle glaucoma based on t-test or Chi-square, as appropriate.

[†] Measure on most affected eye among cases with primary open-angle glaucoma, and on right eye among controls without primary open-angle glaucoma.

[‡] Mean defect worse than -12 dB.

[#] Serum 25-hydroxyvitamin D ≤ 75 nmol/L; P-value significant (i.e., $P < 0.05$) indicated in bold.

Unsurprisingly, POAG cases had significantly thinner RNFL on OCT measurements ($P < 0.001$) and higher cup-to-disc ratio ($P < 0.001$), as well as lower intraocular pressure ($P = 0.020$) than controls. In contrast, there were no between-group differences regarding the season of inclusion ($P = 0.202$), the use of vitamin D supplements (30.0% vs 32.7%, $P = 0.606$), the visual acuity ($P = 0.597$) and the macular thickness ($P = 0.601$).

Table 2 shows univariate and multiple logistic regressions between serum 25OHD concentration and the diagnosis of POAG. Increased serum 25OHD concentration was associated with less frequent POAG diagnosis (unadjusted OR = 0.92 per 10 nmol/L of 25OHD, $P = 0.041$), even after adjustment for all potential confounders (OR = 0.89 per 10 nmol/L of 25OHD, $P = 0.045$). Similarly, vitamin D insufficiency was associated with POAG diagnosis (OR = 2.09 [95% confidence interval: 1.06–4.12], $P = 0.034$).

Regarding POAG severity, 99 cases (66.0%) with moderate POAG (i.e. mean MD better than -12 dB) exhibited a mean serum 25OHD concentration of 45.1 ± 26.7 nmol/L, while severe POAG patients (MD worse than -12 dB) had a mean 25OHD of 39.2 ± 23.3 nmol/L ($P = 0.188$). There was no between-group difference regarding the prevalence of vitamin D insufficiency (88.9% in cases with severe POAG versus 94.0% in cases with moderate POAG, $P = 0.313$). Finally, the serum concentration of 25OHD did not correlate with MD among POAG cases ($r = -0.11$, $P = 0.200$), neither with predictors for worsening of POAG like intraocular pressure ($r = -0.36$, $P = 0.531$) and vertical cup-to-disc ratio ($r = -0.034$, $P = 0.604$).

4. Discussion

The main finding of the present case-control study is that the group of POAG patients had 15-percent lower serum 25OHD concentrations, and exhibited more often vitamin D insufficiency than controls. The association of POAG with decreased serum 25OHD concentration remained significant after adjustment for all measured potential confounders. In contrast, there was no correlation between serum 25OHD concentration and the POAG severity.

These findings are consistent with the growing epidemiological evidence on the deleterious effect of vitamin D insufficiency on eye health and function [6,7,13,14]. While previous research was primarily focused on macular conditions such as AMD [15,16], only one clinical study focused specifically on the association between vitamin D and POAG [17]. Yoo et al. showed among 6,094 adults an inverse association between POAG ($n = 290$; mean, 63.3 ± 10.7 years; 53.1% female) and serum 25OHD concentration (OR = 0.98 with $P = 0.04$ for 1 ng/mL increase in 25OHD) [17]. However, various limitations of this study, including its cross-sectional design, precluded any conclusion regarding an involvement of vitamin D insufficiency in POAG. Also, vitamin D supplementation, an important potential confounder, was not taken into account in the mentioned study. It is yet well-recognized that supplementation is the main exogenous source of vitamin D in older adults due to decreased level of 7-dehydrocholesterol in the skin with aging and reduced digestive absorption [10]. Unlike the latter study, our study was based on a case-control design, and analyses were adjusted for the use of vitamin D supplements, making our results robust. Despite these methodological differences, both studies consistently found that lower 25OHD concentration was associated with POAG. However, unlike Yoo et al., we found no significant correlation between 25OHD concentration and predictors for POAG worsening like mean defect, intraocular pressure or vertical cup-to-disc ratio. Thus, our data suggests that serum 25OHD level may rather be a marker associated with the presence rather than the severity of POAG.

Table 2
Univariate and multiple logistic regression models examining the cross-sectional association between serum 25-hydroxyvitamin D (25OHD) concentration (independent variable) and primary open-angle glaucoma (dependent variable), adjusted for potential confounders (n = 314).

| | Primary open-angle glaucoma | | | |
| | Unadjusted model | | Fully adjusted model | |
	OR [95%CI]	P-value	OR [95%CI]	P-value
Serum 25OHD concentration*	0.92 [0.84–0.99]	**0.041**	0.89 [0.80–0.99]	**0.045**
Age	1.03 [1.00–1.06]	**0.026**	1.02 [0.98–1.05]	0.331
Female gender	0.49 [0.31–0.77]	**0.002**	0.55 [0.32–0.94]	**0.029**
Mean arterial pressure	1.03 [1.01–1.05]	**0.006**	1.03 [1.01–1.05]	**0.010**
Vitamin D supplementation	0.88 [0.55–1.42]	0.606	1.19 [0.61–2.29]	0.611
Visual acuity	1.14 [0.70–1.88]	0.596	1.22 [0.70–2.13]	0.490
Intraocular pressure	0.93 [0.87–0.99]	**0.022**	0.88 [0.81–0.96]	**0.004**

CI: confidence interval; OR: odds ratio.

* Per 10 nmol/L of serum 25-hydroxyvitamin D; OR significant (i.e., P-value <0.05) indicated in bold.

However, it is not clear whether the association between lower vitamin D and POAG is causal. Alternatively, it can be argued that older individuals with POAG and visual dysfunction are more likely to have impaired functional abilities [10,18,19], leading subsequently to reduced vitamin D intakes, sun exposure and consequently lower 25OHD concentrations. However, no dedicated functional score is available in the studied cohort to support this assumption. Alternatively, it has been speculated that decreased 25OHD concentration might increase the risk for POAG. In line with a potential role of vitamin D in the pathophysiology of POAG, are the following arguments:

(i) Vitamin D may play a role in the regulation of the intraocular pressure [20]. In an animal study, vitamin D eye drops in non-human-primates resulted in a reduction of intraocular pressure by 20%, mainly by increasing the uveoscleral outflow [8]. On the opposite, a randomized controlled intervention trial involving humans found no IOP reduction after high-dose vitamin D3 supplementation (20,000 IU twice per week) during 6 months [20]. In our study, POAG cases were exhibiting lower IOP compared to controls (Table 1), probably due to appropriate POAG treatments (the vitamin D supplementation being similar in both groups).

(ii) A second speculative approach is to consider that vitamin D insufficiency is linked to generalized neurodegeneration, including POAG. Such tight interconnections between brain and retina have prompted several authors to consider the eye as a privileged window to the brain, allowing early diagnosis and monitoring of neurodegenerative disease [21]. It is nowadays accepted that vitamin D insufficiency is associated with all-cause dementia and Alzheimer disease [22,23], a condition in which retinal ganglion cell loss and RNFL thinning can be detected with OCT [21,24]. Indeed, a common mechanism has been proposed to explain both retinal degeneration and Alzheimer disease [25]. Various neuroprotective effects of vitamin D in the CNS and the eye may explain these associations. In vitro, vitamin D increases the synthesis of neurotrophic agents such as the Nerve Growth Factor (NGF) and the Glial cell line-Derived Neurotrophic Factor (GDNF), and regulates neuronal differentiation and maturation [26,27]. It also accelerates neuronal growth in a dose-dependent way in rat hippocampal cell cultures [28]. Parallel, the anti-inflammatory properties of vitamin D may be involved as there is evidence for a pivotal role of vitamin D in the immune system [29,30] by modulating effect on the immune cells that produce vitamin D and express the VDR. Finally, the antioxidant properties of vitamin D against oxidative stress of reactive oxygen and nitrogen species in the central nervous system [31] should also be mentioned.

The strengths of the present study include (i) the originality of the research question in POAG, which is a highly common, potentially blinding condition, (ii) the detailed description of the participants' characteristics allowing the use of regression models to measure adjusted associations, and (iii) the standardized collection of data from a single research centre. In addition, enrolling patients in a single centre allowed to centralize vitamin D assays in a single laboratory and to avoid inter-laboratory variability. Regardless, our study also has some limitations. First, the case-control design of our study is less robust than a prospective longitudinal cohort study. Second, the study cohort was restricted to 314 older patients, who might be unrepresentative of the population of glaucomatous patients. Third, although we excluded from the control group the participants with corneal and retinal conditions, controls had mild cataract, which may limit the interpretation of results.

In conclusion, we found that decreased 25OHD concentration was directly associated with the presence of POAG, but not its severity. This result suggests that vitamin D may be linked to POAG. Although the present findings meet most of Hill's criteria of causation including strength, dose–response relationship, consistency, plausibility, consideration of alternate explanations, experiment, and coherence [32], we could not provide answers to the issues of temporality (which is an essential criterion of causation) and specificity (which is a minor criterion). To firmly establish causation, prospective observational cohorts and randomized clinical intervention trials are needed on a variety of adult acute care units. Before such evidence is available, our findings provide a rationale for measuring and correcting, if necessary, vitamin D levels in patients with POAG.

Contributors

Contributions of the authors:

- AG has full access to all of the data in the study, takes responsibility for the data, the analyses and interpretation, and the conduct of the research, and has the right to publish any and all data, separate and apart from the attitudes of the sponsor.
- Study concept and design: CA.
- Acquisition of data: AG, PG, GJ and SL.
- Analysis and interpretation of data: AG, DM, MB, TA and CA.
- Drafting of the manuscript: CA and AG.
- Critical revision of the manuscript for important intellectual content: DM, PG, GJ, SL, MB and TA.
- Obtained funding: Not applicable.
- Statistical expertise: CA.
- Administrative, technical, or material support: DM.
- Study supervision: CA and DM.

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