



# The Possible Link Between Vitamin D Levels and Exudative Age-related Macular Degeneration

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## ARTICLE INFO

### Article history:

Received: 18 October 2018

Accepted: 18 March 2019

### Online:

DOI 10.5001/omj.2020.01

### Keywords:

Age-Related Macular Degeneration; Vitamin D;

Vitamin D Deficiency.

## ABSTRACT

**Objectives:** We sought to evaluate the possible correlation between serum vitamin D levels and exudative age-related macular degeneration (AMD). **Methods:** We conducted a cross-sectional study including 95 patients with exudative AMD and 95 healthy age- and sex-matched controls. The participants' serum 25-hydroxyvitamin D3 (25(OH)D3) levels were measured, and the results were classified into three categories: deficient (< 20.0 ng/mL), insufficient (20.1–29.9 ng/mL), and sufficient (>30.0 ng/mL). We compared serum 25(OH)D3 levels between the two study groups and the AMD ratio between the patients with deficient serum 25(OH)D3 levels and those with levels in the sufficient and insufficient ranges. **Results:** The median 25(OH)D3 levels were significantly lower in patients with AMD compared to the control subjects ( $p = 0.042$ ). The frequencies of patients with AMD among the vitamin D categories were statistically significant ( $p = 0.043$ ). Subgroup analysis showed that the frequency of patients with AMD and deficient vitamin D levels was significantly higher than that found in the patients who had sufficient and/or insufficient ranges of vitamin D levels (55.0% vs. 36.0%,  $p = 0.043$ , respectively). **Conclusions:** Serum 25(OH)D3 levels may have an impact on the neovascular type of AMD. As 25(OH)D3 levels decrease, the frequency of AMD increases.

Age-related macular degeneration (AMD), a chronic, late-onset disease resulting in degeneration of the macula, is the leading cause of irreversible vision loss in adults in developed countries.<sup>1</sup> Although the pathogenesis of AMD is not fully understood, it is well-established that angiogenesis has a major role in the development and progression of AMD.<sup>2,3</sup> Recently, inflammation has received attention as a potential risk factor for this disease.<sup>4–6</sup> Immune components including immunoglobulins, complement factors, and fibrinogens have been observed to be associated with drusen. Additionally, there is an association with immune cell involvement and oxidative stress.<sup>7–9</sup>

Several in vitro and in vivo studies have suggested an anti-inflammatory role of 25-hydroxyvitamin D3 (25(OH)D3).<sup>10,11</sup> Currently, there is evidence that 25(OH)D3 deficiency and insufficiency exists among individuals worldwide, and there is a negative relationship between 25(OH)D3 levels and several

chronic conditions associated with inflammation.<sup>12,13</sup> It has been shown that 25(OH)D3 reduces the proliferation of cells of the immune system.<sup>14,15</sup> Furthermore, it was recently shown that 25(OH)D3 was a potent inhibitor of angiogenesis by its effects on endothelial cells and by interrupting the signaling pathways that are key to angiogenesis, specifically in tumorigenesis.<sup>16–18</sup>

Based on this association and the involvement of 25(OH)D3 in processes underlying several diseases with an inflammatory or immune component, we hypothesized that 25(OH)D3 might play a role in the pathophysiology of AMD and neovascular AMD. The primary purpose of this study was to evaluate the relationship between serum 25(OH)D3 levels and AMD.

## METHODS

Our study population consisted of 95 adults with exudative type AMD and 95 age- and sex-matched

controls without AMD. Informed consent was obtained from all participants. The protocol was reviewed and approved by the Institutional Review Boards at Ondokuz Mayıs University and conformed to the tenets of the Declaration of Helsinki. The control subjects comprised of patients admitted to our clinic for a routine examination whose fundus examination revealed normal results. Both the study group and the control group were selected from patients who were admitted to the clinic between May and August 2017 to minimize the possible impact seasonal variations to vitamin D levels.

All participants underwent a complete ophthalmological examination. The patients with AMD were selected from the retina department who had the neovascular form of AMD in at least one eye. This was defined by subretinal hemorrhage, submacular choroidal neovascular membrane, fibrosis or presence of neovascularization, or leakage from the vascularity of the membrane at any phase of fluorescein angiography. The diagnosis of macular degeneration was confirmed by optical coherence tomography.

Patients whose only exudative finding was retinal pigment epithelium (RPE) detachment were excluded from the study. We also excluded patients with signs of pathological myopia, presumed ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, any hereditary retinal diseases other than AMD, and previous laser treatment due to retinal conditions. Participants taking any supplementary therapy including 25(OH)D3 were also excluded. We collected morning venous blood from the participants to measure serum 25(OH)D3 levels. The serum 25(OH)D3 levels were

studied according to the standard protocol of the biochemistry department, and classified into three categories: deficient (< 20.0 ng/mL), insufficient (20.1–29.9 ng/mL), and sufficient (> 30.0 ng/mL).<sup>19</sup> Serum 25(OH)D3 levels were compared between the study and control subjects. The AMD ratio was also compared between the patients with deficient serum 25(OH)D3 levels and those with levels in the sufficient and insufficient ranges.

Continuous variables are given as median (min-max), and the categorical variables as frequencies and percentages. The Mann-Whitney U test was used for comparisons of the continuous variables, and the Pearson's chi-squared test to compare the categorical variables. A *p*-value of less than 0.050 was considered statistically significant.

## RESULTS

The characteristics of the participants are shown in Table 1. There was no statistically significant difference in terms of age ( $p = 0.756$ ) and sex ( $p = 0.773$ ) ratios between the patients with AMD and the control subjects. The median 25(OH)D3 levels were significantly lower in the patients with AMD compared to the control subjects ( $p = 0.042$ ). The status of the serum 25(OH)D3 levels of the patients and control subjects are also shown in Table 2. The frequencies of patients with AMD among the vitamin D categories were significantly different ( $p = 0.043$ ). Subgroup analysis showed that the frequency of patients with AMD and deficient vitamin D levels was significantly higher than in those with sufficient or insufficient levels (55.0% vs. 36.0%,  $p = 0.043$ , respectively).

**Table 1:** Characteristics of patients with AMD and control patients.

Characteristics	AMD (n = 95)	Control (n = 95)	<i>p</i> -value
Age, mean ± SD, years	73.6 ± 7.8	73.3 ± 7.8	0.756
Sex (M/F)	55/40	53/42	0.773
25(OH)D3 level, median (min-max), ng/mL	11.7 (4.0–34.0)	17.0 (4.2.0–37.0)	0.042

AMD: age-related macular degeneration; SD: standard deviation; M: male; F: female; 25(OH)D3: 25-hydroxyvitamin D3.

**Table 2:** Serum 25-hydroxyvitamin D3 levels in patients with AMD and control group patients.

Category	AMD (n = 95)	Control (n = 95)	<i>p</i> -value
Deficient	77 (81.1)	63 (66.3)	0.043
Insufficient	11 (11.6)	23 (24.2)	0.043
Sufficient	7 (7.4)	9 (9.5)	0.043

Data presented as n (%). AMD: age-related macular degeneration.

## DISCUSSION

AMD is the most common and rapidly increasing cause of blindness in the Western world. The major risk factors are having a first degree relative with AMD and smoking.<sup>1</sup> Overweight and obesity due to excessive food intake are also significant risk factors for AMD.<sup>20</sup> Accumulating evidence suggests that low plasma levels of micronutrients, particularly zinc, lutein, and carotenoids accelerate AMD progression, while on the other hand, increased antioxidant intake protects against AMD progression.<sup>21</sup>

Chronic local inflammation and complement cascade activation are held responsible in the pathogenesis of AMD.<sup>8,22</sup> Complement system proteins, complement activators, and complement regulatory proteins are also identified as molecular constituents of geographic atrophy and choroidal neovascularization associated with advanced AMD.<sup>23,24</sup> Inflammatory response within the Bruch's membrane and the choroid causes injury to the RPE and choriocapillaris, which then leads to the formation of an abnormal extracellular matrix (ECM). This abnormal ECM results in altered RPE-choriocapillaris behavior, leading ultimately to atrophy of the retina, RPE and choriocapillaris, and choroidal new vessel growth, which is an abnormal angiogenic process modulated by growth factors including the vascular endothelial growth factor (VEGF).<sup>25</sup> Associations between AMD and inflammation markers such as C-reactive protein have also been shown.<sup>26</sup>

Vitamin D is provided by some foods and is generated endogenously by exposure to sunlight. Several studies have reported that 25(OH)D3 decreases the proliferation of T-helper cells,<sup>10</sup> T-cytotoxic cells, natural killer cells, and enhances T-suppressor cell activity.<sup>11,27</sup> Other studies have reported that 25(OH)D3 also decreases the production of proinflammatory agents such as IL-2, IL-8, IL-6, and IL-12.<sup>14,15,28,29</sup> One recent study demonstrated that 25(OH)D3 intake reduces C-reactive protein, a marker of systemic inflammation.<sup>30</sup> In physiological concentrations, 25(OH)D3 has also been shown to protect cell proteins and membranes from oxidative damage. Vitamin D has also been demonstrated to inhibit angiogenesis by interrupting signaling pathways throughout the endothelial cells that are key in angiogenesis, particularly in tumorigenesis. VEGF

expression was downregulated in tumor cells treated with 25(OH)D3.<sup>16-18</sup>

A possible role of 25(OH)D3 in ocular functions is supported by evidence that the 25(OH)D3 receptor (VDR) is located in vertebrate retinal tissue and expressed in human cultured retinal endothelial cells.<sup>31</sup> We hypothesized that 25(OH)D3 levels might be decreased in patients with exudative AMD compared to non-AMD subjects. The median 25(OH)D3 levels were significantly lower in patients with AMD compared to the control subjects. We found a significant association between 25(OH)D3 levels and AMD, and this association was stronger at the deficient levels of 25(OH)D3 and proven in the subgroup analysis. We found significantly higher frequencies of patients with AMD who had deficient levels of vitamin D compared to those with sufficient or insufficient levels.

The association between serum 25(OH)D3 concentrations and AMD has been examined within cross-sectional studies. The first study to evaluate the association between serum of 25(OH)D3 levels and AMD prevalence in a large, cross-sectional study showed a correlation between reduced serum vitamin D3 levels and risk of early AMD. However, they failed to demonstrate a significant association with advanced AMD.<sup>32</sup> Increased serum 25(OH)D3 concentrations were associated with decreased odds of early AMD in women younger than 75, and the authors suggested that high serum 25(OH)D3 concentrations may be protective.<sup>33</sup> Recently, the finding of lower dietary 25(OH)D3 intakes in monozygotic twins with severe AMD than in monozygotic co-twins with less-severe AMD raised the idea that 25(OH)D3 deprivation could exacerbate the development of AMD and result in advanced stages of the disease.<sup>34</sup> In a cross-sectional study of 1045 AMD patients and 8124 non-AMD subjects whose vitamin D levels were taken as a part of routine examinations, the mean 25(OH)D3 level was  $24.1 \pm 9.41$  ng/mL (range 0.8–120) for the AMD patients and  $24.13 \pm 9.50$  ng/mL (range 0.0–120) in the control patients. They did not find any association between 25(OH)D3 levels and the presence of AMD. In their study, they assessed patients with both nonexudative and exudative AMD.<sup>35</sup>

Although previous studies mainly focused on early AMD, the association between serum hypovitaminosis D and the advanced stages of

AMD has been studied little. We only included the exudative type of AMD, which may be meaningful in reflecting the antiangiogenic properties of 25(OH)D3. Another strength of our study was that we excluded subjects taking any vitamin supplements (25(OH)D3 in particular) in both the study and control groups. The results of other retrospective and cross-sectional studies could be influenced by a large percentage of the patients who were taking supplements of 25(OH)D3 for other medical conditions (e.g., osteomalacia, osteoporosis).

### CONCLUSION

We investigated the association between serum 25(OH)D3 levels and neovascular AMD. The 25(OH)D3 25- levels were found to be reduced in patients with AMD when compared to healthy subjects. Besides, the frequencies of patients with AMD showed an association among the 25(OH)D3 categories ( $p = 0.043$ ). Subgroup analysis showed that the frequency of patients with AMD and deficient vitamin D levels was significantly higher than that found in the subjects who had sufficient or insufficient levels. Therefore, patients with 25(OH)D3 deficiency may have a higher risk of neovascular AMD and vice versa. We think that 25(OH)D3 levels may impact the neovascular type of AMD, meaning, the more decrease in 25(OH)D3 levels, the more increase in AMD frequency. Such studies may have important implications for the prevention or treatment of neovascular AMD by regulation of modifiable lifestyle factors that influence levels of the vitamin. More studies are needed to verify this association prospectively.

#### Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

#### REFERENCES

- Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* 2001 Oct;119(10):1417-1436.
- Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration. *Prog Retin Eye Res* 2009 Jan;28(1):1-18.
- Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N Engl J Med* 2008 Jun;358(24):2606-2617.
- Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res* 2000 Apr;70(4):441-449.
- Hutchinson AK, Grossniklaus HE, Capone A. Giant-cell reaction in surgically excised subretinal neovascular membrane. *Arch Ophthalmol* 1993 Jun;111(6):734-735.
- Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res* 2001 Nov;20(6):705-732.
- Dastgheib K, Green WR. Granulomatous reaction to Bruch's membrane in age-related macular degeneration. *Arch Ophthalmol* 1994 Jun;112(6):813-818.
- Anderson DH, Radeke MJ, Gallo NB, Chapin EA, Johnson PT, Curletti CR, et al. The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. *Prog Retin Eye Res* 2010 Mar;29(2):95-112.
- Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000 Sep-Oct;45(2):115-134.
- Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells in vivo are due in part to the control of integrin-mediated T lymphocyte homing. *Eur J Immunol* 2004 Apr;34(4):1068-1076.
- Thomasset M. [Vitamin D and the immune system]. *Pathol Biol (Paris)* 1994 Feb;42(2):163-172.
- Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004 Mar;79(3):362-371.
- Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG; Iowa Women's Health Study. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* 2004 Jan;50(1):72-77.
- Manolagas SC, Provvedini DM, Murray EJ, Tsoukas CD, Deftos LJ. The antiproliferative effect of calcitriol on human peripheral blood mononuclear cells. *J Clin Endocrinol Metab* 1986 Aug;63(2):394-400.
- Müller K, Gram J, Bollerslev J, Diamant M, Barington T, Hansen MB, et al. Down-regulation of monocyte functions by treatment of healthy adults with 1 alpha,25 dihydroxyvitamin D3. *Int J Immunopharmacol* 1991;13(5):525-530.
- Bernardi RJ, Johnson CS, Modzelewski RA, Trump DL. Antiproliferative effects of 1alpha,25-dihydroxyvitamin D(3) and vitamin D analogs on tumor-derived endothelial cells. *Endocrinology* 2002 Jul;143(7):2508-2514.
- Shokravi MT, Marcus DM, Alroy J, Egan K, Saornil MA, Albert DM. Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma. *Invest Ophthalmol Vis Sci* 1995 Jan;36(1):83-87.
- Iseki K, Tatsuta M, Uehara H, Iishi H, Yano H, Sakai N, et al. Inhibition of angiogenesis as a mechanism for inhibition by 1alpha-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 of colon carcinogenesis induced by azoxymethane in Wistar rats. *Int J Cancer* 1999 May;81(5):730-733.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011 Jul;96(7):1911-1930.
- Johnson EJ. Obesity, lutein metabolism, and age-related macular degeneration: a web of connections. *Nutr Rev* 2005 Jan;63(1):9-15.
- Hogg R, Chakravarthy U. AMD and micronutrient antioxidants. *Curr Eye Res* 2004 Dec;29(6):387-401.
- Augustin AJ, Kirchhof J. Inflammation and the pathogenesis of age-related macular degeneration. *Expert Opin Ther Targets* 2009 Jun;13(6):641-651.

23. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. *Exp Eye Res* 2001 Dec;73(6):887-896.
24. Despret DD, Klaver CC, Wittteman JC, Bergen AA, Kardys I, de Maat MP, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA* 2006 Jul;296(3):301-309.
25. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Arch Ophthalmol* 2004 Apr;122(4):598-614.
26. Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between C-reactive protein and age-related macular degeneration. *JAMA* 2004 Feb;291(6):704-710.
27. Hayes CE, Nashold FE, Spach KM, Pedersen LB. The immunological functions of the vitamin D endocrine system. *Cell Mol Biol (Noisy-le-grand)* 2003 Mar;49(2):277-300.
28. Takahashi K, Horiuchi H, Ohta T, Komoriya K, Ohmori H, Kamimura T. 1 alpha,25-dihydroxyvitamin D3 suppresses interleukin-1beta-induced interleukin-8 production in human whole blood: an involvement of erythrocytes in the inhibition. *Immunopharmacol Immunotoxicol* 2002 Feb;24(1):1-15.
29. D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998 Jan;101(1):252-262.
30. Lefebvre d'Helencourt C, Montero-Menei CN, Bernard R, Couez D. Vitamin D3 inhibits proinflammatory cytokines and nitric oxide production by the EOC13 microglial cell line. *J Neurosci Res* 2003 Feb;71(4):575-582.
31. Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM* 2002 Dec;95(12):787-796.
32. Parekh N, Chappell RJ, Millen AE, Albert DM, Mares JA. Association between vitamin D and age-related macular degeneration in the Third National Health and Nutrition Examination Survey, 1988 through 1994. *Arch Ophthalmol* 2007 May;125(5):661-669.
33. Millen AE, Voland R, Sondel SA, Parekh N, Horst RL, Wallace RB, et al; CAREDS Study Group. Vitamin D status and early age-related macular degeneration in postmenopausal women. *Arch Ophthalmol* 2011 Apr;129(4):481-489.
34. Seddon JM, Reynolds R, Shah HR, Rosner B. Smoking, dietary betaine, methionine, and vitamin D in monozygotic twins with discordant macular degeneration: epigenetic implications. *Ophthalmology* 2011 Jul;118(7):1386-1394.
35. Golan S, Shalev V, Treister G, Chodick G, Loewenstein A. Reconsidering the connection between vitamin D levels and age-related macular degeneration. *Eye (Lond)* 2011 Sep;25(9):1122-1129.