Acta Clin Croat 2016; 55:203-208 Original Scientific Paper

doi: 10.20471/acc.2016.55.02.04

ASSOCIATION BETWEEN SERUM VITAMIN D LEVEL AND GLAUCOMA IN WOMEN

Željka Vuković Arar[1], Marijana Knežević Praveček[2], Blaženka Miškić[2], Zoran Vatavuk[3], Jadranka Vuković Rodriguez[4] and Sandra Sekelj[1]

[1]Department of Ophthalmology and Optometry, [2]Department of Internal Medicine, Dr Josip Benčević General Hospital, Slavonski Brod; Teaching Unit of the School of Medicine, Josip Juraj Strossmayer University, Osijek; [3]Clinical Department of Ophthalmology, Sestre milosrdnice University Hospital Center; [4]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

SUMMARY – The aim of the study was to determine whether serum vitamin D level is lower in female patients with glaucoma as compared with control group. The mechanism by which vitamin D reduces intraocular pressure is not fully clarified. Almost all tissues possess vitamin D receptor (VDR). The mice lacking VDR (VDR knockout mice) have greatly contributed to the understanding of the general vitamin D physiologic function. VDR has been found in some ganglion layer cells, external and internal nuclear layers of retina, and in retinal pigment epithelium, while VDR epitopes have also been found in the ciliary body epithelium, pointing to the role of this protein in eye physiology. The 1,25(OH)2D3 modulates expression of the genes involved in the regulation of intraocular pressure in non-human primates. Extracellular matrix can be remodeled by 1,25(OH)2D3 treatment. Actin disruption can lead to cell morphology alteration, trabecular meshwork relaxation and intraocular pressure reduction. This observational cross-sectional study included 90 female glaucoma subjects aged 45-55 and 50 glaucoma free female subjects as control group. Results of a pilot study conducted in 20 glaucoma subjects and 20 control subjects are presented below. All study subjects underwent history taking, complete ophthalmologic examination and serum vitamin D determination. The mean serum vitamin D level was 32.31 nmol/L in glaucoma patients and 64.17 nmol/L in control subjects. Serum vitamin D level was statistically significantly lower in glaucoma patients as compared with control group ($p<0.05$).

Key words: *Glaucoma; Intraocular pressure; Vitamin D; Receptors, calcitriol*

Introduction

Glaucoma is a multifactorial optic neuropathy characterized by acquired loss of optic nerve fibers. Current hypotheses on the potential pathogenic mechanisms in the onset of glaucomatous optic neuropathy include exotoxic lesion due to excessive retinal glutamate release, reduction in neuronal growth factor, peroxynitrite toxicity due to the increased activity of nitric monoxide synthetase, immune mediated optic nerve lesion, and oxidative stress.

There are no generally accepted guidelines on adequate vitamin D (1,25(OH)2D3) level. Serum vitamin D levels of 50-75 nmol/L (20-30 ng/mL) are considered as vitamin D insufficiency, with adverse effects on bone mineral density and possibly on muscle and physical ability[1-4]. The levels <50 nmol/L are considered as vitamin D deficiency and those <30 nmol/L as severe vitamin D deficiency, whereas vitamin D levels <20 nmol/L cause serious impairment of bone metabolism, i.e. rickets or osteomalacia, depending on age, as well as myopathy associated with physical inability[4,5]. As a steroid hormone, vitamin D directly or

Correspondence to: *Željka Vuković Arar, MD, MS*, Department of Ophthalmology and Optometry, Dr Josip Benčević General Hospital, Andrije Štampara 42, HR-35000 Slavonski Brod, Croatia
E-mail: zvarar@net.hr

Received October 20, 2015, accepted April 8, 2016

indirectly regulates 3% of the human genome function[6]. Favorable vitamin D effects have been demonstrated in many diseases, e.g., in the prevention of heart attack and stroke, diabetes mellitus type 1 and 2, infections and chronic respiratory diseases, autoimmune diseases, etc. Vitamin D receptor (VDR) has been found in some ganglion layer cells, external and internal nuclear layers of retina, and in retinal pigment epithelium, while VDR epitopes have also been found in the ciliary body epithelium, pointing to the role of this protein in eye physiology. Vitamin D exerts autocrine and paracrine action *via* VDR[6,7]. The mechanism by which vitamin D reduces intraocular pressure (IOP) has not yet been fully clarified. The 1,25(OH)2D3 modulates expression of the genes involved in IOP regulation in non-human primates. Extracellular matrix can be remodeled by 1,25(OH)2D3 treatment, resulting in increased fluid flow along with reduced flow resistance by disruption of cellular adhesions and relaxation of contractile molecules[8]. Nitric oxide (NO), a free radical produced by vascular endothelium induced by the endothelial nitric monoxide synthetase (eNOS) enzyme activity, is the key signaling molecule for local regulation of vascular tone. Some studies showed that increased NO levels increased the flow rate and thus reduced IOP[9,10], whereas others report that the increased NO level led to peroxynitrite toxicity and development of glaucomatous optic neuropathy. The 1,25(OH)2D3 inhibits nitric oxide inducible synthetase (iNOS) expression and reduces NO production in stimulated macrophages. The macrophage production of 1,25(OH)2D3 has protective effect on oxidative lesion caused by NO burst[11].

The main hypotheses of the study were prompted by the vitamin D immunomodulatory action on the above mentioned parameters. A sufficient vitamin D status is associated with a lower risk of rheumatoid arthritis, multiple sclerosis, diabetes mellitus type 1, autoimmune diseases and neurodegenerative diseases such as glaucoma. Despite the growing knowledge about the biological and clinical role of vitamin D, considerable prevalence of vitamin D deficiency is reported in the populations all over the world, including Croatia[6,12,13]. As VDR has been demonstrated in many structures of human eye, this type of research offers an opportunity for studies at the molecular level and for a novel diagnostic and therapeutic approach in both systemic and local vitamin D administration (autocrine and paracrine vitamin D action). If the results of this study indicate that glaucoma patients aged 45-55 have a decreased serum vitamin D level, it will provide a basis for additional clinical trials.

Patients and Methods

Patients

This pilot study included 20 female patients with open-angle glaucoma and 20 control female subjects. Blood samples for vitamin D determination were obtained in late autumn and early spring. Women with any bone metabolism disorder, primary hyperparathyroidism, and those on vitamin D supplementation therapy were not included in the study. Excluded were also women with potentially altered serum vitamin D level for any reason (e.g., suntan studio, work in the open, diet, etc.).

The study will assess vitamin D effect on glaucoma as a disease using the following procedures: serum vitamin D level will be determined in glaucoma patients and control group; serum vitamin D levels in these two groups will be compared and differences calculated; and association of serum vitamin D level with other study parameters of glaucoma development (IOP and optic nerve head lesions) will be assessed.

Methods

History data were obtained and complete ophthalmologic examination performed in study patients. Ophthalmologic examination included visual acuity testing by the subjective method using standard Snellen chart according to the international tables; anterior eye segment biomicroscopy on a biomicroscope (Haag-Streit BM 900, Switzerland); IOP measurement on a Goldmann's applanation tonometer (in mm Hg); fundus examination by direct or indirect ophthalmoscopy; pachymetry (measurement of corneal thickness, in μm); gonioscopy (chamber angle examination); and field of vision analysis by computerized static perimetry according to Humphrey using SITA fast 24-2 (Carl Zeiss Meditec, Dublin, Ca, USA) software.

Venous blood samples (≤5 mL) for determination of serum vitamin D levels were obtained in all study subjects. Total calcium (Ca) was determined on an Au 680 autoanalyzer. Parathyroid hormone (PTH) and vitamin D in plasma were determined by the electrochemiluminescence method using respective reagents.

Results were analyzed by nonparametric and parametric statistical methods (Student's t-test and F-test), with the level of statistical significance set at $p<0.05$ and 95% confidence interval (95% CI).

Results

Both study group and control group consisted of 20 female subjects each. The mean age of glaucoma patients and control subjects was 51.8 and 49.45 years, respectively. The mean serum vitamin D level was 32.31 nmol/L in glaucoma patients and 64.17 nmol/L in control subjects (Figs. 1 and 2). The mean Ca and PTH levels were within the normal range and served only to exclude primary hyperparathyroidism, which was not diagnosed in any of study subjects.

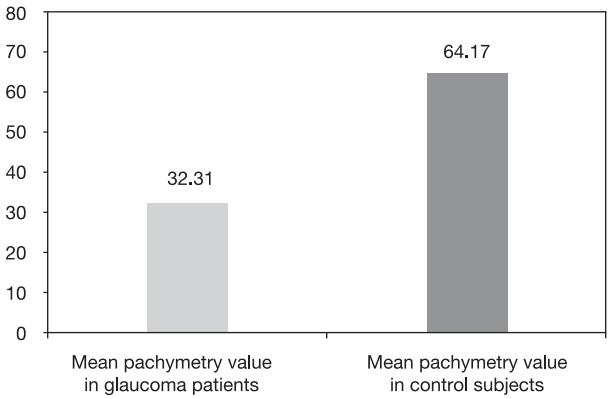


Fig. 1. Relationship of vitamin D levels in glaucoma patients and control subjects.

Visual acuity examined by use of Snellen chart was 1.0 with or without correction in both groups. In glaucoma patients, the mean corneal thickness measured by use of a pachymeter was 548.85 μm and 549.85 μm on the right and left eye, respectively. In control group, the respective figures were 552.60 μm and 551.90 μm (Fig. 3). Fundus lesions were found in 17 (85%) glaucoma patients and three (15%) control subjects.

In glaucoma patients, the mean IOP value with therapy was 16.9 mm Hg and 17.2 mm Hg on the right and left eye, respectively, *versus* 15.85 mm Hg measured on both eyes in control group, yielding no statistically significant between-group difference (Figs. 4 and 5). Intraocular pressure higher than 21 mm Hg was not found in any of the control subjects. Pathologic field of vision with glaucoma associated lesions was recorded in eight (40%) glaucoma patients and two (10%) control subjects.

The mean value of the optic nerve cup-to-disc (c/d) ratio measured on the right and left eye was 0.423 and 0.417 in glaucoma patients *versus* 0.340 and 0.350 in control subjects, respectively; t-test yielded a statistically significant between-group difference at the level of $p<0.05$.

Coefficient of variation (CV) for vitamin D levels measured in glaucoma patients was higher compared to control subjects (32.32% *vs.* 28.33%). This relative variability was even more important considering that the mean serum vitamin D level was by 49.96% lower in glaucoma patients compared to control group. Upon calculating standard deviation of these two arithmetic means, followed by their comparison and calculation

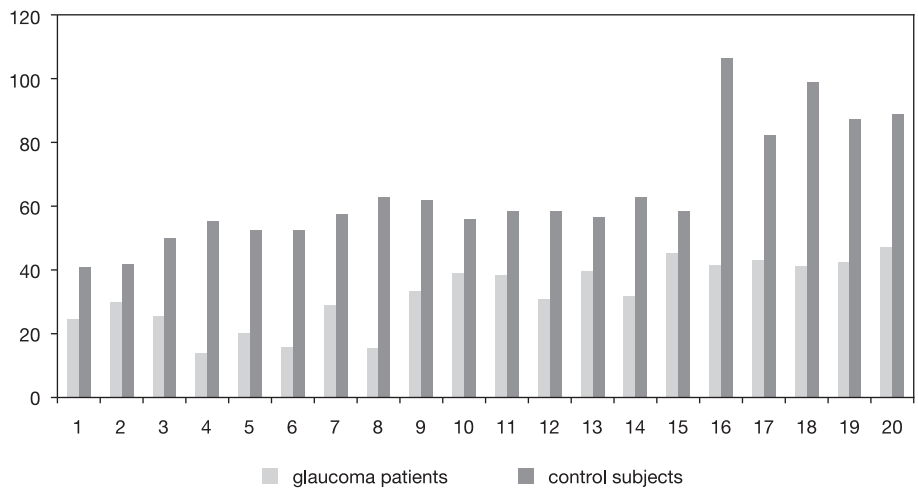


Fig. 2. Vitamin D levels measured in glaucoma group and control group.

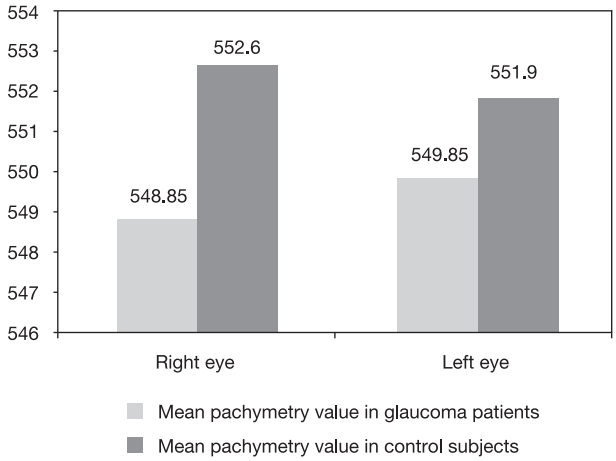


Fig. 3. Pachymetry values on the right eye and left eye in glaucoma group and control group.

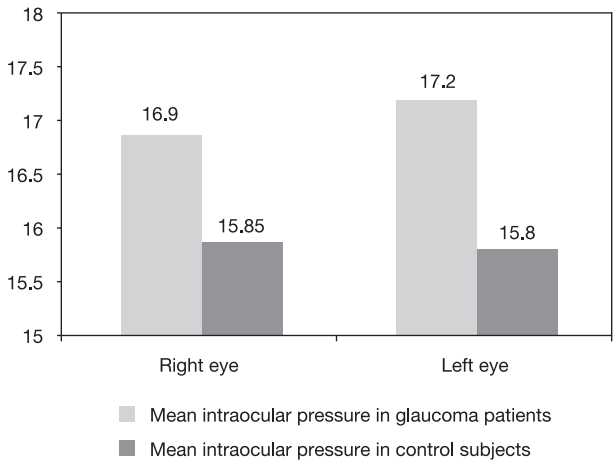


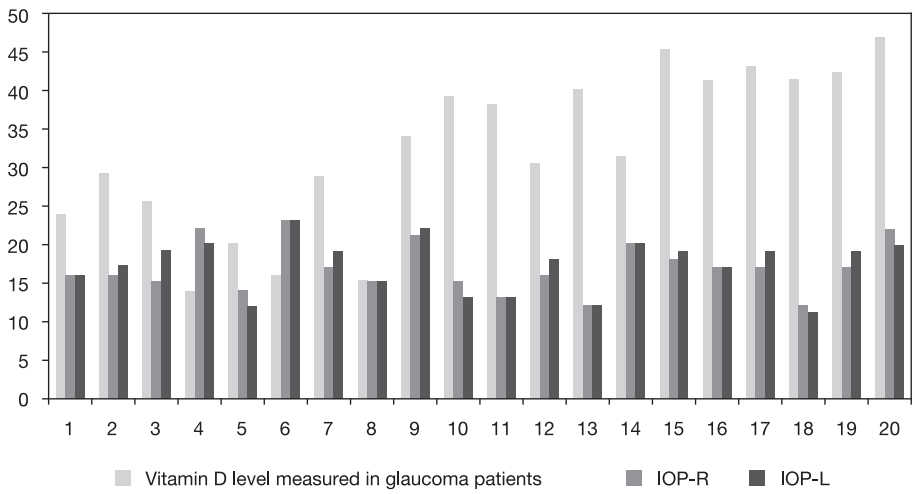
Fig. 4. Intraocular pressure values on the right eye and left eye in glaucoma group and control group.

of common standard deviation of two arithmetic means, statistical significance of the vitamin D standard deviations of the mean was calculated by F-test, which yielded a statistically significant difference between these two arithmetic means at the level of $p<0.05$.

Discussion

The objective of this cross-sectional study was to determine whether serum vitamin D level is lower in female patients with glaucoma as compared with control group. Results of a pilot study that included 40 female subjects (20 in glaucoma group and control group each) are reported. Glaucoma group consisted of female patients with open-angle glaucoma, while control group included glaucoma-free female subjects. Glaucoma subjects were recruited from the Glaucoma Registry of the Brod-Posavina County. The male to female ratio in the Glaucoma Registry is 30% to 70%. In contrast, most studies worldwide report no sex predominance for glaucoma (e.g., Baltimore, Beaver Dam, and Roscommon studies). The Barbados Eye Study found men to have a higher age-adjusted risk of glaucoma than women, the Rotterdam study reports on a three-fold higher risk of glaucoma in men, whereas Dalby study found the risk of glaucoma to be higher in women[14-18].

Our study subjects were aged 45-55. In this age group, vitamin D deficiency generally is not as pronounced as in >60 age group. A study conducted by Laktašić-Žerjavić *et al.* suggested vitamin D to vary



IOP-R = intraocular pressure – right eye;
IOP-L = intraocular pressure – left eye

Fig. 5. Multifunctional correlation of vitamin D and intraocular pressure for the right eye and left eye in glaucoma group and control group.

with age, sex, skin type, and season[19]. Advanced age, female sex, dark skin type, less sun exposure and poor dietary habits lacking vitamin D rich food have been associated with vitamin D deficiency[12,19-21]. The 25(OH)D level depends on its intradermal formation because dietary intake of vitamin D is low, and the capacity of vitamin D formation is weakened in the elderly due to skin thinning. Laktašić-Žerjavić *et al*. report on the 25(OH)D level to decline most significantly at the age 59 and the prevalence of all grades of vitamin D deficiencies to rise significantly with age. A very high prevalence (23.8%) of severe vitamin D deficiency (25(OH)D <30 nmol/L) was recorded in female subjects aged >65[21]. Therefore, on recruiting our study subjects according to age, we tended to avoid age-dependent vitamin D deficiency.

The mean serum level of vitamin D was 32.31 nmol/L in glaucoma patients and 64.17 nmol/L in control subjects, yielding a statistically significant difference at the level of $p<0.05$. There was no statistically significant difference in the IOP values between glaucoma patients and control subjects (16.9 mm Hg/17.2 mm Hg on the right and left eye in glaucoma patients and 15.85 mm Hg on both eyes in control subjects), suggesting good therapeutic regimen in the former. There was no statistically significant between-group difference in the mean pachymetry values (548.85/549.85 μm and 552.6/551.9 μm for the right/left eye in glaucoma patients and control subjects, respectively). Thus, there was no statistically significant between-group difference in the effect on IOP either.

There are only few studies on this subject. In 2012, Krefting *et al*. published their study on IOP regulation in non-human primates with topical application of 1α,25 dihydroxy vitamin D(3) (1α25-(OH)2D3) or its analogs. Micro-field analysis demonstrated that vitamin D regulated the genes known to be involved in IOP regulation[22]. Yoo *et al*. were the first to report on the association of vitamin D and primary open-angle glaucoma in 2014. Their study that included 6094 subjects showed vitamin D deficiency to be associated with a potential risk of open-angle glaucoma development[23]. Several months later, however, Krefting *et al*. reported opposite findings. Their study conducted in healthy subjects failed to demonstrate any association between serum vitamin D level and IOP. In subjects with low vitamin D level, vitamin D administration had no effect on IOP[8].

Conclusion

The mean serum vitamin D level was lower in study subjects suffering from glaucoma as compared to control subjects (32.31 nmol/L *vs*. 64.17 nmol/L), yielding a statistically significant between-group difference ($p<0.05$). This pilot study has justified continuation of the research. If the pilot study results be confirmed, then vitamin D may be considered a marker in the early diagnosis and prevention of glaucoma.

Acknowledgment

The authors are thankful to all the colleagues having contributed to this study.

References

1. Laktašić-Žerjavić N, Koršić M, Crnčević-Orlić Ž, Anić B. Vitamin D: vitamin from the past and hormone of the future. Lijec Vjesn. 2011;133(5-6):194-204.
2. Willems HM, van den Heuvel EG, Carmeliet G, Schaafsma A, Klein-Nulend J, Bakker AD. VDR dependent and independent effects of 1,25-dihydroxyvitamin D3 on nitric oxide production by osteoblasts. Steroids. 2012;77(1-2):126-31, http://dx.doi.org/10.1016/j.steroids.2011.10.015
3. Kutuzova GD, Gabelt BT, Kiland JA, Hennes-Beann EA, Kaufman PL, DeLuca HF. 1α,25-Dihydroxyvitamin D(3) and its analog, 2-methylene-19-nor-(20S)-1α, 25-dihydroxyvitamin D(3) (2MD), suppress intraocular pressure in non-human primates. Arch Biochem Biophys. 2012;518(1):53-60, http://dx.doi.org/10.1016/j.abb.2011.10.022
4. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of Vitamin D in skeletal muscle: form, function, and metabolism. Endocr Rev. 2013;34(1):33-83, http://dx.doi.org/10.1210/er.2012-1012
5. Zittermann A, Gummert JF. Nonclassical vitamin D action. Nutrients. 2010;2(4):408-25, http://dx.doi.org/10.3390/nu2040408
6. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10(4):482-96, http://dx.doi.org/10.1016/j.coph.2010.04.001
7. Holick MF. Vitamin D: extraskeletal health. Rheum Dis Clin North Am. 2012;38(1):141-60, http://dx.doi.org/10.1016/j.rdc.2012.03.013
8. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med. 2008;29(6):361-8, http://dx.doi.org/10.1016/j.mam.2008.08.008
9. Agarwal R, Gupta SK, Agarwal P, Saxena R, Agrawal SS. Current concepts in the pathophysiology of glaucoma. Indian J Ophthalmol. 2009;57(4):257-66, http://dx.doi.org/10.4103/0301-4738.53049

10. Molinari C, Uberti F, Grossini E, Vacca G, Carda S, Invernizzi M, Cisari C. 1α,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cell Physiol Biochem.2011;27(6):661-8,http://dx.doi.org/10.1159/000330075
11. Chang JM, Kuo MC, Kuo HT, Hwang SJ, Tsai JC, Chen HC, Lai YH. 1-alpha,25-Dihydroxyvitamin D3 regulates inducible nitric oxide synthase messenger RNA expression and nitric oxide release in macrophage-like RAW 264,7 cells. J Lab Clin Med. 2004;143(1):14-22, http://dx.doi.org/10.1016/j.lab.2003.08.002
12. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008; 87(4):1080S-6S.
13. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73-8, http://dx.doi.org/10.1016/j.annepidem.2007.12.001
14. Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, Javitt J. Racial variations in the prevalence of primary open angle glaucoma. JAMA. 1991;266(3):369-74, http://dx.doi.org/10.1001/jama.1991.03470030069026
15. Klein BEK, Klein R, Sponsel WE, Franke T, Cantor LB, Martone J, Menage MJ. Prevalence of glaucoma. The Beaver Dam Eye Study. Ophthalmology. 1992;99(10):1499-504, http://dx.doi.org/10.1016/S0161-6420(92)31774-9
16. Coffey M, Reidy A, Wormald R, Xian WX, Wright L, Courtney P. Prevalence of glaucoma in the west of Ireland. Br J Ophthalmol. 1993;77(1):17-21, http://dx.doi.org/10.1136/bjo.77.1.17
17. Leske MC, Connell AMS, Schachat AP, Hyman L. The Barbados Eye Study – prevalence of open angle glaucoma. Arch Ophthalmol. 1994;112(6):821-9, http://dx.doi.org/10.1001/archopht.1994.01090180121046
18. Dielemans I, Vingerling JR, Wolfs RCW, Hofman A, Grobbee DE, de Jong PT. The prevalence of primary open angle glaucoma in a population based study in The Netherlands. Ophthalmology. 1994;101(11):1851-5, http://dx.doi.org/10.1016/S0161-6420(94)31090-6
19. Laktasic-Zerjavic N, Korsic M, Crncevic Orlic Z, Kovac Z, Polasek O, Soldo-Juresa D. Vitamin D status, dependence on age and seasonal variations in the concentration of vitamin D in Croatian postmenopausal women initially screened for osteoporosis. Clin Rheumatol. 2010;29(8):861-7, http://dx.doi.org/10.1007/s10067-010-1409-3
20. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J; IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20 (11):1807-20, http://dx.doi.org/10.1007/s00198-009-0954-6
21. Lips P. Vitamin D status and nutrition in Europe and Asia. J Steroid Biochem Mol Biol. 2007;103(3-5):620-5, http://dx.doi.org/10.1016/j.jsbmb.2006.12.076
22. Krefting EA, Jorde R, Cristoffersen T, Grimnes G. Vitamin D and intraocular pressure – results from a case-control and an intervention study. Acta Ophthalmol. 2014;92(4):345-9, http://dx.doi.org/10.1111/aos.12125
23. Yoo TK, OH E, Hong S. Is vitamin D status associated with open-angle glaucoma? A cross-sectional study from South Korea. Public Health Nutr. 2014;17(4):833-43, http://dx.doi.org/10.1017/S1368980013003492

Sažetak

POVEZANOST SERUMSKE KONCENTRACIJE VITAMINA D I GLAUKOMA KOD ŽENA

Ž. Vuković Arar, M. Knežević Praveček, B. Miškić, Z. Vatavuk, J. Vuković Rodriguez i *S. Sekelj*

Cilj istraživanja bio je utvrditi je li kod ispitanica oboljelih od glaukoma snižena serumska koncentracija vitamina D u odnosu na kontrolnu skupinu. Mehanizam kojim vitamin D snižava očni tlak nije jasno poznat. Gotovo sva tkiva posjeduju receptor za vitamin D (VDR). Velik doprinos razumijevanju globalne fiziološke funkcije vitamina D dobiven je od miša kojemu nedostaje VDR (engl. *knockout mice for VDR*). VDR je pronađen u nekim stanicama ganglijskog sloja, u vanjskom i unutarnjem nuklearnom sloju mrežnice i u retinalnom pigmentnom epitelu. Epitopi za VDR su pronađeni i u epitelu cilijarnog tijela. To sve govori o važnosti tog proteina u fiziologiji oka. 1,25 (OH)2D3 modulira ekspresiju gena koji su uključeni u regulaciju očnog tlaka u nehumanih primata. Nakon liječenja pomoću 1,25(OH)2D3 može se remodelirati ekstracelularni matriks. Disrupcija aktina može dovesti do promjene stanične morfologije, relaksacije trabekularne mreže i sniženja očnog tlaka. Provedeno je opažajno presječno istraživanje s kontrolnom skupinom. U istraživanje je bilo uključeno 90 ispitanica oboljelih od glaukoma u dobi od 45-55 godina i 50 ispitanica u kontrolnoj skupini. Prikazuju se rezultati probnog ispitivanja u 20 ispitanica oboljelih od glaukoma i isto toliko u kontrolnoj skupini. Ispitanicama je uzeta anamneza i kompletan oftalmološki status te je određen vitamin D u serumu. Koncentracija vitamina D u serumu ispitanica s glaukomom bila je 32,31 nmol/L u odnosu na kontrolnu skupinu gdje je bila 64,17 nmol/L. U zaključku, kod ispitanica oboljelih od glaukoma bila je snižena serumska koncentracija vitamina D u odnosu na kontrolnu skupinu na statistički značajnoj razini od $p<0,05$.

Ključne riječi: *Glaukom; Intraokularni tlak; Vitamin D; Receptori za kalcitriol*