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Evaluation of pupillary functions in adult vitamin D deficiency patients

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Abstract

Purpose To evaluate the pupillary dynamics in patients with serum vitamin D (25(OH)-D) levels below normal and compared with healthy controls.

Methods This study included 132 patients and 76 healthy controls. Serum 25(OH)-D concentrations within the range of 10 to 30 ng/mL were classified as vitamin D insufficiency (VDI, Group 1), while those at or below 10 ng/mL were categorized as vitamin D deficiency (VDD, Group 2). The static phase of pupillometry, including scotopic, mesopic, and photopic pupil diameters, was evaluated. Additionally, in the dynamic phase of pupillometric evaluation, pupil dilation velocity (mm/sec) was calculated. These values were compared between patient and control groups.

Results In static pupil diameters, only photopic pupil diameters were significantly different between Groups 1 and 2 compared to the control group ($p=0.012$ and $p=0.008$, respectively). In dynamic measurements, the pupil diameter values showed a statistically significant difference between the patient and control groups ($p=0.003$). In intragroup comparison, the mean pupil diameter was 3.42 ± 0.81 mm in group 1 and 3.94 ± 0.96 mm in group 2 ($p=0.029$). Mean pupil dilation velocity was significantly slower in the patient group ($p<0.001$). In intragroup comparison, the mean pupil dilation velocity was 0.162 ± 0.049 mm/sec in group 1 and 0.088 ± 0.032 mm/sec in group 2 ($p<0.001$).

Conclusion We demonstrated that VDD alters pupillary functions with objective measurements. Our study may shed light on the role of vitamin D in multisystemic diseases since it is expressed in many tissues and has multiple functions. Multicenter studies with a large number of participants are needed in the future.

Keywords Automatic pupillometry, Automatic nervous system, Pupillary function, Vitamin D

Introduction

Vitamin D deficiency (VDD) is a major health problem affecting more than one billion people worldwide [1]. The average daily intake of vitamin D in a large proportion of the population is generally insufficient to maintain normal vitamin D levels [2]. Studies have reported that many cells have vitamin D receptors and that vitamin D also has effects on non-skeletal tissues [3]. Furthermore, VDD has been associated with numerous acute and chronic diseases including neurologic disorders, autoimmune disorders, cardiovascular diseases, malignancies and diabetes mellitus [1].

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Autonomic nervous system dysfunction is a clinical condition that may lead to various symptoms resulting from unbalanced functioning of the sympathetic and parasympathetic nervous systems [4]. Vitamin D is a hormone that provides balance in the autonomic nervous system and has a regulatory role in the neuronal system [4]. Although there is no definite information on the relationship between VDD and autonomic dysfunction, literature studies provide important evidence related to autonomic dysfunctions by reporting gastrointestinal and cardiac dysfunctions in VDD [5].

Direct and indirect pupillary reflexes are regulated by sympathetic and parasympathetic systems. A robust anatomical structure and a functional autonomic system are needed for healthy miosis and mydriasis [6]. Pupillary reflexes can be evaluated subjectively by the clinician and can be measured objectively with new generation automatic pupillometry devices [7]. Clinically unobservable changes in pupil diameter can be measured with pupillometry devices.

In our study, pupillometric examinations were performed in adult patients with vitamin D (25(OH)-D) levels below normal and compared with healthy controls.

Methods

Ethical approval

This prospective, case-control study was conducted between January 2024 and September 2024 by the Departments of Ophthalmology and Internal Medicine at Niğde Ömer Halisdemir University. The study protocol was approved by Niğde Ömer Halisdemir University Clinical Research Ethics Committee (2024/57). Written informed consent was obtained from all participants before the study. The study was conducted in accordance with the Declaration of Helsinki.

Power analysis

Using the G Power program, the number of samples to be included in this study was determined as 63 in each group with type I error ($\alpha = 5\%$), confidence ($1\text{-}\alpha = 95\%$), type II error ($\beta = 15\%$), test power ($1\text{-}\beta = 85\%$) and effect size ($d = 0.5\%$).

Inclusion and exclusion criteria

Patients with complete blood count and serum biochemical data in the hospital data system within the last 1 month were included in this study. Patients with vitamin deficiency other than serum 25(OH)-D, with concomitant systemic disease, taking systemic medication for any reason, smoking and/or drinking alcohol, breastfeeding or pregnancy were excluded. All participants underwent a complete ophthalmologic examination. Patients with any significant anterior or posterior segment pathology, history of previous ophthalmic surgery, significant refractive error (myopia or hyperopia greater than 3.00 D and astigmatism greater than 1.00 D), strabismus, amblyopia and caffeine intake in the last 24 h prior to measurement were excluded. The findings were compared with those of healthy volunteers with similar age and gender distribution to the patient group.

Determination of vitamin D level

Vitamin D level was determined by measuring serum 25-hydroxy vitamin D (25(OH)-D) level from peripheric venous samples. In our study, values of 30 ng/mL and above were considered normal. Patients with serum 25(OH)-D levels of 150 ng/mL and above were excluded from the study due to potential toxicity. Serum 25(OH)-D levels between 10 and 30 ng/mL were defined as insufficient (VDI, group 1), and levels of 10 ng/mL or less were defined as deficient (VDD, group 2).

Collection of study data

Each patient underwent a complete ophthalmologic examination. Best corrected visual acuity (BCVA) according to Snellen chart and intraocular pressure was measured with a pneumatic tonometer (Topcon TRK-2P, Topcon, Tokyo, Japan). Anterior and posterior segment findings were examined with slit-lamp biomicroscope. A dilated fundus examination with indirect ophthalmoscopy was performed after the pupillometric examination. Axial length measurements were performed with IOL-Master 500 (Carl ZeissMeditec, Jena, Germany). Pupillary light reflexes, relative afferent pupillary defect (RAPD), and color vision were evaluated. Optic nerve pathologies were excluded by Humphrey visual field examination (HFA II-i, Carl Zeiss) and retinal nerve fiber layer thickness measurements (Cirrus HD-OCT, Carl Zeiss Meditec Inc., Dublin, CA, USA).

Pupillometric measurements

Measurements were obtained within a one-week period post-diagnosis of vitamin D insufficiency or deficiency and prior to the initiation of vitamin D supplementation. Pupil function was evaluated quantitatively through the automated pupillometry capabilities of Sirius Topographer (Costruzione Strumenti Oftalmici, Florence, Italy). The static phase of pupillometry consists of three stages: scotopic, mesopic and photopic (0.4 lx, 4 lx and 40 lx, respectively). The dynamic phase of pupillometry starts at 500 lx illumination. Miosis is created at this level of illumination. Then the illumination is decreased, and the pupil diameter increases. In the dynamic phase of pupillometric evaluation in our study, the first 10 s were evaluated (Fig. 1). Pupil dilation velocity (mm/sec) was calculated by subtracting the pupil diameter (mm) at the tenth second from the pupil diameter (mm) at the first moment and dividing by 10. Patients were asked to look

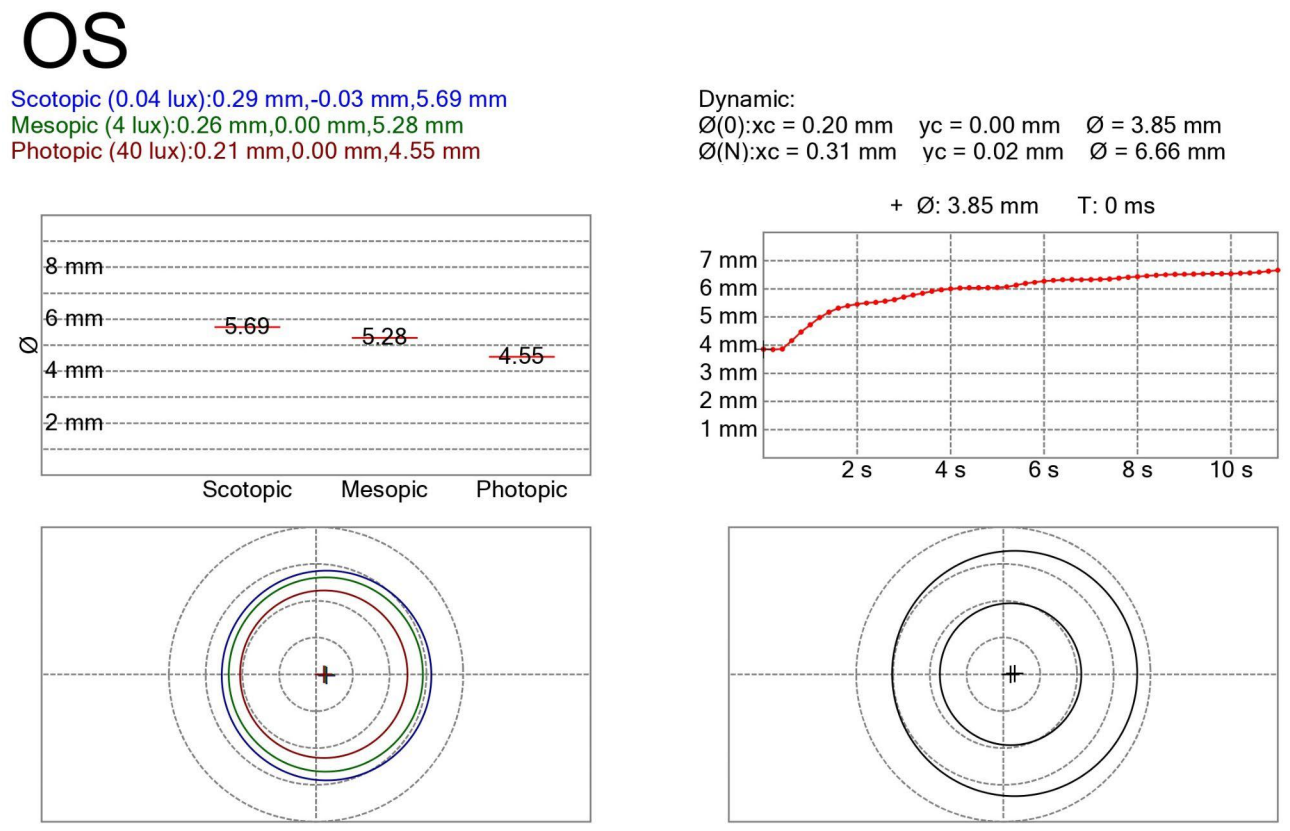

Fig. 1 Static and dynamic pupillometer results

at the light source 3 m away with their non-examined eye to prevent accommodation during the measurement. All participants rested in a dark room for at least 5 min before pupillometry imaging. All measurements were performed by the same investigator between 10 and 11 am to minimize the effects of diurnal rhythm on the pupil. All data were calculated by averaging three consecutive measurements. The evaluated eyes were selected randomly.

Statistical analysis

The study data were analyzed using the Statistical Package for the Social Sciences (SPSS v25.0.1, IBM Co., NY, USA). Normally distributed data are shown as mean ± standard deviation (minimum– maximum). Categorical data are expressed as numbers (n) and percentages (%).The conformity of the data to normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. T-test was used for pairwise comparisons of numerical data and one-way ANOVA was used for comparisons of more than two groups. Chi-square statistics was used to analyze non-numerical data. For all comparisons, $p < 0.05$ was considered significant.

Results

This study included 132 patients and 76 healthy controls. BCVA levels were ≥ 20/20 (logMAR 0.0) and intraocular pressures were 12–20 mm Hg in both groups. The mean age of the patient group was 37.4 ± 10.3 (22–68) years and the control group was 35.1 ± 8.4 (20–70) years and there was no significant difference between the groups ($p = 0.167$). There were 74 females (56.1%) in the patient group and 46 females (60.5%) in the control group and the groups were similar in terms of gender distribution ($p = 0.394$). (Table 1)

There was no statistically significant difference in the static measurements of pupillometry, including scotopic and mesopic conditions between patient and control groups ($p = 0.131$ and $p = 0.354$, respectively). Similarly, intra-group comparisons of static pupillometric parameters (scotopic, mesopic, and photopic) between VDI and VDD groups revealed no significant differences ($p > 0.05$). (Table 2) Conversely, significant differences were observed in photopic pupil diameter, with VDI and VDD patients demonstrating significantly larger pupil sizes compared to the control group ($p = 0.012$ and $p = 0.008$, respectively). (Table 2)

In the dynamic phase of pupillometry, pupil diameter at zero second (mm) and mean dilation velocity (mm/sec) data were evaluated. According to the test results, the

Table 1 Demographic and clinic data of the patient and control group

	Patient						Control		
	Overall		Group 1		Group 2				*p*
N	**132**		**69**		**63**		**76**		
Age (years)	37.4 ± 10.3 (22–68)		38.1 ± 12.5 (23–68)		36.6 ± 14.9 (22–65)		35.1 ± 8.4 (20–70)		0.167
Male (n, %)	58	43.9	31	44.9	27	42.9	30	39.5	0.394
Female (n, %)	74	56.1	38	55.1	36	57.1	46	60.5	
IOP (mmHg)	15.1 ± 3.5 (12–20)		14.9 ± 4.4 (13–18)		15.4 ± 4.9 (13–20)		15.2 ± 4.1 (12–20)		0.798
AXL (mm)	23.1 ± 2.1 (20.4–24.9)		23.2 ± 2.2 (20.4–24.9)		23.0 ± 1.5 (20.7–24.0)		22.5 ± 1.2 (20.8–24.3)		0.342
SE (D)	-1.03 ± 0.66 (-2.00-+1.00)		-1.09 ± 0.69 (-1.75-+1.00)		-1.04 ± 0.58 (-2.00-+0.50)		-0.97 ± 073 (-2.25-+1.25)		0.077
RNFL (µm)	105.4 ± 9.1 (90–120)		106.1 ± 8.8 (92–115)		104.8 ± 9.5 (90–120)		105.3 ± 7.7 (85–120)		0.112

IOP: Intraocular pressure, AXL: Axial length, SE: Spherical equivalent, RNFL: Retinal nerve fiber layer

Table 2 Measurements of the static phase of pupillometry

	Patients			Control	
	Overall	Group 1	Group 2		*p*
N	**132**	**69**	**63**	**76**	
Scotopic (mm)	5.43 ± 0.86 (3.50–7.22)	5.46 ± 0.92 (3.52–7.18)	5.41 ± 0.89 (3.50–7.22)	5.61 ± 0.46 (3.46–7.21)	0.131
Mesopic (mm)	4.38 ± 0.79 (2.53–5.74)	4.40 ± 0.87 (2.59–5.72)	4.37 ± 0.93 (2.53–5.74)	4.45 ± 0.59 (2.68–5.73)	0.354
Photopic (mm)	3.59 ± 0.62 (2.20–4.69)	3.61 ± 0.79 (2.24–4.69)	3.69 ± 0.74 (2.20–4.47)	3.42 ± 0.53 (2.15–4.74)	0.021

Table 3 Measurements of the dynamic phase of the pupillometer

	Patient			Control	*p*
	Overall	Group 1	Group 2		
N	**132**	**69**	**63**	**76**	
Pupil diameter (mm)	3.67 ± 0.68 (2.19–4.75)	3.42 ± 0.81 (2.19–4.49)	3.94 ± 0.96 (2.44–4.75)	3.32 ± 0.64 (2.06–4.58)	0.003
Dilation velocity (mm/sec)	0.127 ± 0.029 (0.068–0.184)	0.162 ± 0.049 (0.140–0.184)	0.088 ± 0.032 (0.068–0.113)	0.211 ± 0.058 (0.150–0.272)	< 0.001

pupil diameter values showed a statistically significant difference between the patient and control groups. The mean pupil diameter was 3.67 ± 0.68 (2.19–4.75) mm in the patient group and 3.32 ± 0.64 (2.06–4.58) mm in the control group ($p = 0.003$). In intragroup comparison, the mean pupil diameter was 3.42 ± 0.81 (2.19–4.49) mm in group 1 and 3.94 ± 0.96 (2.44–4.75) mm in group 2. This difference between the groups was statistically significant ($p = 0.029$). Mean pupil dilation velocity was significantly slower in the patient group compared to controls. The mean pupil dilation velocity was 0.127 ± 0.029 (0.068–0.184) mm/sec in the patient group and 0.211 ± 0.058 (0.150–0.272) mm/sec in the control group ($p < 0.001$). In intragroup comparison, the mean pupil dilation velocity was 0.162 ± 0.049 (0.140–0.184) mm/sec in group 1 and 0.088 ± 0.032 (0.068–0.113) mm/sec in group 2. This difference between the groups was statistically significant ($p < 0.001$). (Table 3)

Discussion

Vitamin D is a neuroactive steroid hormone that plays a very important role in the central nervous system and autonomic balance [8]. Pupillary functions are an important clinical finding in which the balance of sympathetic and parasympathetic nervous systems can be monitored. Objective and reproducible results can be obtained by measuring pupil diameter with automatic pupillometers developed in recent years [7]. In our study, the effects of VDI and VDD on pupillary reflexes in adults were examined with an automatic pupillometer. Accordingly, no significant difference was observed in mesopic and scotopic pupil diameters in VDI and VDD compared to healthy controls, but dynamic functions and photopic diameters were found to be impaired.

Studies have reported that vitamin D is an important component of cerebral activity in both embryonic and adult periods. Many neurologic and psychiatric diseases

including sleep disorders, schizophrenia, Alzheimer's, multiple sclerosis and Parkinson's disease have been associated with low vitamin D levels [9]. In these studies, the neuroprotective functions of vitamin D are mentioned [10]. Vitamin D supplementation during embryonic and neonatal periods has also been reported to reduce the risk of some cerebral diseases [11].

In the literature it is reported that VDI and VDD are associated with autonomic dysfunctions. Maser et al. reported that VDD was associated with decreased parasympathetic function in a study conducted in patients with type 2 diabetes mellitus [12]. Vitamin D is involved in the regulation of the enteric nervous system and affects gastric emptying [4, 13]. Calcitriol treatment of a patient with postural orthostatic tachycardia syndrome improved orthostatic intolerance and palpitations [14]. Current studies suggest that vitamin D has important regulatory functions in the autonomic nervous system and therapeutic benefits.

Pupillometric studies have been performed in patients with vitamin A deficiency as vitamin deficiency [15, 16]. Palmer et al. reported that carotenoid supplementation regulated pupillary functions in a study conducted in pediatric vitamin A deficiency patients [15]. However, to our knowledge, there is no example of a study examining the effect of VDD on pupillary functions in adults. However, Biçer et al. reported significant losses in pupillary functions in pediatric VDD patients compared to healthy controls [5]. According to the results of our and their study, under photopic conditions, VDD patients had a larger pupil diameter compared to healthy controls. This suggests that dysautonomia affecting especially the parasympathetic nervous system may be observed due to VDD.

In our study, there was a significant difference in the dynamic phase of pupillometry in patients compared to healthy controls. An insufficient miosis is observed in the dynamic phase measurements in the patient group. In addition, pupil dilatation velocity is slower in the patient group. Especially in group 2 (VDD), these effects are more pronounced compared to group 1 (VDI).

It has been reported that miosis is related to the parasympathetic nervous system and pupil dilatation velocity is related to the parasympathetic and sympathetic nervous systems [17]. Therefore, it is thought that changes may occur in both the parasympathetic and sympathetic nervous systemsin adults with VDD. However, when compared with the literature data, the differences in patient age groups may have altered the effects on the autonomic nervous system.

Our study is the first study to investigate the effect of VDD on pupillary functions in adults. In addition to this advantage, it has some limitations. Since the pupillometry device did not have the capability of visualizing miosis second by second, we could not obtain results related to miosis velocity. In addition, 10 s was taken as a reference in the calculation of pupil dilatation velocity and longer test times may be more informative. Due to the fact that pupillometric assessments were performed before the subjects received vitamin supplements, the research did not include the evaluation of any possible alterations after the treatment.

In conclusion, we demonstrated that VDD alters pupillary functions with objective measurements. Our study may shed light on the role of vitamin D in multisystemic diseases since it is expressed in many tissues and has multiple functions. Multicenter studies with a large number of participants are needed in the future.

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Not applicable.

Author contributions
ZB, ÖÖ, and LD reviewed literature, collected the data, drafted, and critically revised the manuscript. ÖÖ, LD, and ZB wrote the manuscript. ES, ESG, and PE collected data and critically revised the manuscript. ÖÖ and LD critically revised the manuscript. ZB made the statistical evaluation.

Funding
No funding was received to conduct the study.
Data availability.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by Niğde Ömer Halisdemir University Clinical Research Ethics Committee (2024/57). Written informed consent was obtained from all participants before the study. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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