Original Article

Evaluation of Static Pupillary Parameters in Pediatric Patients with Vitamin B_{12} Deficiency

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Abstract

Background: Automatic pupillometry device enables important information about pupillary function. Neurological manifestations such as autonomic dysfunction and ophthalmological disorders are observed in vitamin B_{12} deficiency ($VB_{12}D$). **Aim:** To assess the static pupillary functions using a pupillometry device in pediatric patients with $VB_{12}D$. **Methods:** The study included 40 children with $VB_{12}D$ and 40 healthy children in the control group. The measurements were made with an automated pupillometry under static conditions. The static measurements were performed at scotopic, mesopic, and photopic light intensities. The differences between the patient and control groups were analyzed with static measurements. **Results:** Pupillary diameters of the patient and control groups under scotopic, mesopic, and photopic conditions were observed and there was a statistically significant difference under the mesopic and photopic conditions. Under mesopic conditions: The mean pupil diameter was found to be 5.92 ± 0.69 mm in the $VB_{12}D$ group and 5.18 ± 0.91 mm in the control group (P = 0.003). Under photopic conditions, the mean pupil diameter was found to be 5.13 ± 0.77 mm in the $VB_{12}D$ group and 4.53 ± 0.96 mm in the control group (P = 0.001). Under scotopic conditions, the mean pupil diameter was 6.46 ± 0.68 mm in the $VB_{12}D$ group and 6.37 ± 0.93 mm in the control group. There was no statistically significant difference between the patient and control groups in the measurements under scotopic conditions (P = 0.63). **Conclusion:** Pediatric patients with $VB_{12}D$ have significantly larger pupil diameters in photopic and mesopic conditions than healthy children. The results suggest that there is an autonomic system dysfunction in children with $VB_{12}D$, especially when the parasympathetic system is negatively affected. Pupillary contraction is reduced in children with $VB_{12}D$. Decreased pupillary myosis function is observed. Pupillary size is larger in vitamin B_{12}-deficient children under photopic and mesopic conditions.

Keywords: *Autonomic nervous system, automatic pupillometry, pupillary functions, vitamin B_{12} deficiency*

Introduction

Vitamin B_{12} (cobalamin) is a water-soluble vitamin that acts as a coenzyme.[1] It is a major mediator in the conversion of methyl malonyl-CoA to succinyl-CoA and the conversion of homocysteine to methionine in enzymatic reactions.[2] It takes part in the transfer of methyl groups and nucleotide synthesis.[3] Vitamin B_{12} is important in the central nervous system because it is the core molecule in the production of axonal myelin sheath.[4] Being effective in myelinization, synaptogenesis, and neurotransmitter synthesis, it contributes to cognitive development.[3]

Eating fewer animal products in the diet, malabsorption, and using proton pump inhibitors for

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a long time are risk factors that cause vitamin B_{12} deficiency ($VB_{12}D$).[5]

It was reported that inadequate intake of vitamin B_{12} during pregnancy and early childhood causes cognitive development disorders in children.[6] Increased occurrence of neurodevelopmental disorders and convulsions was detected based on neuroimaging.[6] $VB_{12}D$ may be presented in subclinical presentations, as well as a disease form in which hematological (pancytopenia, macrocytic anemia), neurological (peripheral neuropathy, subacute combined degeneration, hypotonia, autonomic dysfunction), and neuropsychiatric manifestations can be observed.[3] Developmental regression and cerebral atrophy were reported in infants.[7] It was reported that $VB_{12}D$, which was involved in the production of adrenaline from noradrenaline, might cause baroreceptor dysfunction in the sympathetic nervous system in adolescents.[8] The succinyl-CoA synthesis is impaired and therefore it causes the loss of vision secondary to optic neuropathy and dry eye disease.[9,10] $VB_{12}D$ has been in one of the subcategories of the metabolic optic neuropathy. In metabolic optic neuropathies, visual acuity decreased and bilateral symmetrical vision was impaired.[11] It is necessary to treat B_{12} deficiency to prevent permanent vision loss.[12]

The physiological function of the eyes is regulated by the autonomic nervous system. The eyes are innervated by sympathetic, parasympathetic, and trigeminal nerve fibers.[13] Pupil dilation and constriction depend on autonomic innervation.[14] Light induces pupil contraction via the activation of the parasympathetic pathway, while the sympathetic system only has a tonic role. When the parasympathetic pathway is activated, pupillary myosis develops. In the sympathetic pathway, pupil dilatation occurs.[15] $VB_{12}D$ may result in changes in the autonomic nervous system in the studies.[16] However, no sufficient studies were found about the autonomous changes that B_{12} deficiency may cause in the pupil.

Pupillometry provides important information on the balance between the sympathetic and parasympathetic nervous systems.[17] Our study aims to investigate the effect of $VB_{12}D$ on pupillary responses in children and to study the differences in static pupillary parameters between the $VB_{12}D$ group and the healthy control group.

Materials and Methods

The study was carried out in Niğde Omer Halisdemir University Training and Research Hospital in Niğde province in the Central Anatolia region of Turkey. The study was performed between August 2023 and December 2023. The case-control study was started after obtaining written and verbal informed consent from the parents and children. The study was approved by the Niğde Omer Halisdemir University Clinical Research Ethics Committee in Niğde province in Turkey (decision date 2023, Ethical report number 52). The study was conducted in accordance with the Declaration of Helsinki. Since the region where the study was conducted has a low socioeconomic status and the incidence of nutritional deficiencies is high, vitamin B_{12} levels are checked for standard practice. Forty children aged 6–18 who were brought to the Pediatrics Clinic for routine check-ups and were incidentally found to have $VB_{12}D$ from laboratory testing were included in the study group. Another age-matched 40 children who attended the Pediatrics Clinic and who had normal serum vitamin B_{12} levels aged 6–18 were included in the control group. The pediatrician designed the study, detected the vitamin B_{12} levels of the children, calculated the body mass index (BMI), and took information about demographic characteristics. Information was obtained about demographic characteristics such as age, gender, and place of residence. The age range was between 6 and 18 years because the children under the age of 6 years could not adapt to pupillometric measurements. The case and control groups were matched between those with low B_{12} levels and those with normal B_{12} levels in similar age groups and in normal BMI in the determined time period. The $VB_{12}D$ group did not have any symptoms, or anemia in our study. The children with $VB_{12}D$ were in subclinical presentation. Those whose serum vitamin B_{12} level was <200 pg/ml were considered the $VB_{12}D$ group and those with >200 pg/ml were the healthy control group.[3] The collected data was taken from the hospital data system. Children's BMI was calculated by dividing the weight in kg by the square of the height in meters (kg/m^2). BMI values between the 15th and 85th percentiles are considered normal.[18] The children who were found to have $VB_{12}D$ in the pediatrics clinic and those in the healthy control group who had normal vitamin B_{12} levels were directed to the ophthalmology clinic subsequently. Detailed eye examinations were conducted in the ophthalmology clinic. The inclusion criteria were being between the ages of 6 and 18, having no previously detected eye pathology, having no chronic disease, having a normal BMI, and having a low vitamin B_{12} level. The children had the best corrected visual acuity. Those who had prior eye surgery, strabismus or amblyopia, frontal anterior or posterior segment pathology, myopia or hypermetropia more than 3 D, and those who had astigmatism more than 1 D were excluded from the study. The measurements were performed within the first 1 week after the patients were diagnosed with $VB_{12}D$ before starting their treatment. The measurements were carried out using automated pupillometry (Sirius Topographer, Costruzione Strumenti Oftalmici, Florence, Italy). Automated pupillometry is not a routine equipment in our clinic. We decided to use this device because it allows us to examine the invisible changes in pupil functions caused by pathologies at a micro level. Data from the only right eye measurements of the $VB_{12}D$ group and control group were used in the analysis. There was a high correlation between the right and left eye data, only the right eye data were taken in order not to compromise the objectivity of the data, to avoid doubling the number of data, and to avoid giving incorrect results. Pupillometry is a medical device designed to measure the size of the pupil with reflected light. An automated pupillometer is a portable, handheld device that provides a reliable and objective measurement of pupillary size, symmetry, and reactivity through the measurement of the pupillary light reflex.[19] Investigator ophthalmologists made the pupillometric investigation and they interpreted the results of automatic pupillometry. The ophthalmologists performed three consecutive measurements for each child and average values were selected for data analysis. The vitamin B_{12}-deficient children's and healthy control group children's pupil diameters were measured automatically under static conditions, and their pupillary reflexes were evaluated. The static measurements were performed under three environmental luminance conditions, scotopic (0.4 lux), mesopic (4 lux), and photopic (40 lux). Scotopic responses were measured when the only visible light source was the light-emitting diode (LED) source in the device (0.4 lux). Mesopic responses were assessed under the ambient light intensity of 4 lux, and photopic responses were assessed under the ambient light intensity of 40 lux. Luminance levels are determined automatically on the Sirius Topographer device. Both the $VB_{12}D$ group and healthy control group's measurements were made under scotopic, mesopic, and photopic conditions. Both vitamin B_{12}-deficient children and healthy control children were asked to stay away from caffeinated products such as Coke for 24 h before the measurements. Parents were informed that their children should not consume caffeinated foods. What they eat is monitored under the control of their families, and then confirmation is obtained from the families. The static pupillometry measurement data and pupillary diameters were compared between the vitamin B_{12}-deficient group and the healthy control group.

Statistical analysis

The study data were analyzed using the Statistical software STATA 14.2 (StataCorp LLC, College Station, Texas, USA). The independent sample *t*-test was used in the analysis of continuous variables with normal distribution for comparison of vitamin B_{12}-deficient children and the control group. The Chi-square test was used in the analysis of categorical variables. Independent *t*-test was used to evaluate statistically significant differences in demographic and pupil parameters between the patient and control groups. *P* value < 0.05 was considered statistically significant. A hedge g value was calculated for the effect sizes of significant values. The sample size was determined using the G * Power 3.1 program, provided that alpha = 0.05, power = 0.95. Results showed that a minimum of 40 children were required to achieve a power of 0.95.

Results

This study included 80 eyes of 80 participants, comprising 40 from the $VB_{12}D$ group and 40 from the healthy control group. The mean age of the patients in the $VB_{12}D$ group was 12.55 ± 2.48 years, and the mean age of the control group was 12.87 ± 1.89 years, and there was no difference between the groups in terms of age (*P* = 0.13). There were 20 females and 20 males in the $VB_{12}D$ group and 24 females and 16 males in the control group, and the groups were similar in terms of gender (*P* = 0.36).

The mean level of vitamin B_{12} was 165.05 ± 15.07 ng/ml in the vitamin B_{12}-deficient group and 251.87 ± 33.06 ng/ml in the control group. The level of vitamin B_{12} in the $VB_{12}D$ group was significantly lower than the control group (*P* < 0.001). There was no statistically significant difference in terms of BMI between the groups. Demographic and clinical characteristics of the groups are shown in Table 1.

Table 2 shows the pupillary functions of the patient and control groups under static conditions (scotopic, mesopic, photopic). According to the results obtained, there was no difference in the scotopic measurements between the two groups (*P* = 0.09), but there were statistically significant differences in the mesopic and photopic measurements (*P* = 0.003 and *P* = 0.001, respectively). The mean pupil diameter under mesopic conditions was 5.92 ± 0.69 mm in the vitamin B_{12}-deficient group and 5.18 ± 0.91 mm in the control group, which shows insufficient contraction in the

Table 1: Demographic and clinical characteristics of groups (between 6 and 18 years)

	$VB_{12}D$ group	Control group	*P*
Age	12.55±2.48	12.87±1.89	0.13
Sex (f/m)	20/20	24/16	0.36
BMI	21.97±5.35	20.74±3.88	0.4
Level of vitamin B_{12} (mean±SD)	165.05±15.07	251.87±33.06	<0.01

$VB_{12}D$: Vitamin B_{12} deficiency, SD: Standard deviation, f/m: Female/male, BMI: Body mass index

Table 2: Comparison of the static pupillometry data between the patient $VB_{12}D$ group and control groups

	$VB_{12}D$ group (*n*=40)		Control group (*n*=40)		*P*	Effect size
	Mean	SD	Mean	SD		Cohen's *d*
Mean scotopic pupil diameter (mm)	6.46	0.68	6.37	0.93	0.633	–0.11
Mean mesopic pupil diameter (mm)	5.92	0.69	5.18	0.91	0.003	–0.80
Mean photopic pupil diameter (mm)	5.13	0.77	4.53	0.96	0.001	–0.68

SD: Standard deviation, $VB_{12}D$: Vitamin B_{12} deficiency

vitamin B_{12}-deficient group under mesopic conditions. Under photopic conditions, the mean pupil diameter was found to be 5.13 ± 0.77 mm in the vitamin B_{12}-deficient group and 4.53 ± 0.96 mm in the control group, which shows insufficient contraction in the vitamin B_{12}-deficient group. The effect size of the statistical significance under mesopic conditions was –0.80 according to Cohen's *d*.

Discussion

This study investigated the effect of $VB_{12}D$ on pupillary functions using pupillometry in pediatric patients. Under mesopic and photopic conditions, the pupil diameters of vitamin B_{12}-deficient children were found significantly larger than those of healthy children.

Evaluating the pupils in terms of size, shape, symmetry, and response to light can help to diagnose neurological diseases and ocular disorders.[20] Many conditions affect pupil diameter. Pharmacological agents, arousal state, and head trauma may affect pupillary response in studies.[21] Measures of pupillary reactivity inversely correlated with intracranial pressure in acute brain injury.[22] Pupil diameter reaches a peak baseline level during adolescence and then gradually decreases with age.[23] While there was an inverse correlation between age and pupil size, there was no relation between pupil size and gender or refractive error.[24] However, the effect of $VB_{12}D$ on pupil width has not been examined so far.

Case studies reported that adults with $VB_{12}D$ developed optic neuropathy, resulting in progressive vision loss.[25] In a study in which patients with symmetric optic neuropathy were examined for 15 months, $VB_{12}D$ was identified as more common among the etiological conditions.[26] $VB_{12}D$ affects the peripheral nervous system and central nervous system; it may also lead to autonomic dysfunction.[27] In the study of Südiqui *et al*.,[28] autonomic dysfunction occurred in a patient with cobalamin deficiency, resulting in adynamic ileus. The clinical findings improved with parenteral cobalamin treatment. Orthostatic hypotension developed in vitamin B_{12}-deficient patients due to a sympathetic system activation defect.[29] Çelik *et al*.[30] found that subclinical atherosclerosis may develop in proportion to homocysteine elevation in adolescents with $VB_{12}D$ and this was associated with the cardiovascular autonomic imbalance occurring in vitamin B_{12}-deficient children. Hansen *et al*.[31] found that as the vitamin B_{12} level decreased, the risk of developing cardiovascular autonomic neuropathy increased in patients with type 2 diabetes mellitus. Studies supported the significance of vitamin B_{12} in the regulation of the autonomic nervous system.

The analysis of the pupillary light reflex is an indicator of the balance between the sympathetic constrictor and parasympathetic dilator systems in the autonomic nervous system.[32] Objective and reliable investigation methods are needed in the evaluation of pupil functions. Automated pupillometry devices today enable quantitative, non-invasive, objective, and repeatable measurement of pupil diameter.[33] In studies with automated pupillometers, pupil functions in many diseases have been explored. Erdem *et al*.[34] found that pupillary reactions occurred in both groups with and without retinopathy in patients with diabetes mellitus. Murase *et al*.[35] found that pupillary findings in diffuse axonal damage were a strong predictive indicator of the prognosis. Pupillometry studies have also been performed in obstructive sleep apnea, and diseases such as sepsis and autonomic dysfunction have been reported in such studies.[36,37]

Studies involving pupillometry have also been carried out in the pediatric age group. Patwari *et al*.[38] found in their study that the pupillary diameter was significantly different in pediatric patients with congenital central hypoventilation syndrome compared to the control group, and the fact that dysregulation occurred in the autonomic nervous system in this syndrome was evidenced with the help of pupillometry. Pena *et al*.[39] found that pupillometry was a more sensitive test than cardiovascular reflexes in the assessment of autonomic nerve dysfunction in adolescent patients with diabetes mellitus. In this study, pupil diameters were observed to be larger and there was insufficient constriction in the pupil in vitamin B_{12}-deficient children under mesopic and photopic conditions. This study shows that there is autonomic nervous system dysfunction and particularly the parasympathetic system is affected in the $VB_{12}D$ group. To the best of our knowledge, there is no other study examining pupillary functions in $VB_{12}D$ to which we can compare our findings. However, there were few studies investigating pupillary response in nutritional vitamin deficiencies. A few studies used pupillometric testing in patients with vitamin A deficiency in relation to nutritional status.[40,41] In the study of Healy *et al*.,[36] it was found that nutritional status affected visual functions and there was a correlation between the serum retinol level and pupillary threshold. In a study carried out on children from Zambia, regular use of provitamin A carotenoids strengthened pupillary functions in children with vitamin A deficiency.[37] Aytemir *et al*.[42] obtained that there was autonomic dysfunction in vitamin B_{12}-deficient patients with a decrease in parasympathetic nervous system effects. Our study was similar to Aytemir's study in this respect.

The paucity of similar studies investigating pupillary functions in children with $VB_{12}D$ in the literature is the strong point of our study. However, this study has some limitations. Children under the age of 6 were not included in the study because they cannot achieve success in automated pupillometry measurements. Other studies recommended testing homocysteine levels in people with $VB_{12}D$. In the studies, examining the homocysteine level in $VB_{12}D$ is a more sensitive laboratory method that supports the diagnosis.[43] Pupillometry measurement data under dynamic conditions were not obtained because these pediatric patients could not adapt to it.

While investigation of pupillary reflexes is used in the diagnosis of many neurological diseases, it should be ascertained that the level of vitamin B_{12} is normal. It may be necessary to measure vitamin B_{12} levels in pediatric patients with impaired pupil response. Today, research using automated pupillometry measurements on how pupil functions are affected by a variety of diseases appears in the literature frequently. Pupillometry can be used as a screening method to determine the effect of $VB_{12}D$ on pupil functions.

Conclusion

Pupillary responses can be affected in pediatric patients with $VB_{12}D$ and $VB_{12}D$ can lead to decreased parasympathetic activity. The pupillary diameters are significantly larger in mesopic and photopic conditions in children with $VB_{12}D$. We believe that further studies on how $VB_{12}D$ affects pupillary functions will shed light on this issue.

Declaration of patient consent

Written informed consent was obtained from the parents.

Author contributions

ZYÖ: Literature search, clinical studies, manuscript preparation, manuscript editing, manuscript review, guarantor. GYB: Literature search, clinical studies, experimental studies, manuscript preparation, manuscript review. KRZ: Experimental studies, data acquisition, data analysis, statistical analysis, manuscript review. FK: Data analysis, statistical analysis, manuscript editing, manuscript review.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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