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CHANGES IN THE LEVEL OF 25-HYDROXYVITAMIN D IN ORAL FLUID IN CHILDREN WITH PROGRESSIVE MYOPIA

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Summary

Aim. Assess the level of 25-hydroxyvitamin D in oral fluid in children with progressive myopia.
Materials and methods. We examined 34 children (68 eyes) with mild myopia and 18 conditionally healthy children (36 eyes) without ophthalmological pathology. The children were divided into 2 groups: Group I (main) – 34 children (68 eyes) with mild myopia, in which subgroup Ia – 16 children (32 eyes) – with a progressive course of myopia and subgroup Ib – 18 children (36 eyes) – with a stable course of myopia. The control group consisted of 18 conditionally healthy children (36 eyes) without ophthalmological pathology. A standard ophthalmological examination was carried out: visometry, autorefractokeratometry before and after cycloplegia, biomicroscopy, ophthalmoscopy, determination of the axial length of the eye. The level of 25-hydroxyvitamin D in oral fluid was determined by the immunoenzymatic method.
Results. In children with a progressive course of myopia, the indicator of 25-hydroxyvitamin D is 1,2 times lower than in children with a stable course of myopia and 2,4 times lower than in children of the control group ($p<0,05$). Correlation analysis showed a significant inverse relationship between the axial length of the eye and the level of 25-hydroxyvitamin D ($r= –0,50$, $p<0,05$) and between the progressive course of myopia and the level of 25-hydroxyvitamin D ($r= –0,69$, $p<0,05$). According to the ROC analysis the optimal value of the cut-off threshold for the indicator of 25-hydroxyvitamin D in oral fluid in children was ≤ 20,154 ng/ml. (sensitivity is 87,9 %, specificity is 94,7 %), ($p <0,001$).
Conclusions. In children with a progressive course of myopia, the level of 25-hydroxyvitamin D is 2,4 times lower than the level of conditionally healthy children. A decrease in the level of 25 hydroxyvitamin D in the oral fluid is an additional risk factor of the progressive course of myopia in children.

Keywords: **Myopia, children, oral fluid, vitamin D**

INTRODUCTION

The high frequency of myopia in the population, its tendency to progress in childhood make this disease an important object of research in practical ophthalmology [1, 2]. A feature of myopic refractogenesis is the progressive course of this refraction anomaly and a significant percentage of subsequent ophthalmic complications [3]. Despite a sufficient number of publications, the pathogenesis of myopia is a complex and multifactorial process and continues to be the subject of research among clinicians. A variety of factors such as environmental exposure, lifestyle, distance learning, imbalance of micro and macro elements, genetic factors and co-morbidities play a significant role in the development of this disease [2, 3, 4]. The role of vitamin status in the aspect of myopic refractogenesis remains an actual and debatable issue. Literary data indicate the effect of 25-hydroxyvitamin D deficiency on the occurrence and course of myopia [5, 6, 7]. There are studies indicating a possible relationship between the degree of progression of myopia and the level of 25-hydroxyvitamin D in the blood serum [8, 9, 10, 11]. In addition to blood serum, any available biological substrate or liquid can be used as an alternative object as a material for studying the metabolism of various substances in the body, including vitamins. One of these biological substrates is oral fluid, the biochemical composition of which is multifactorial, containing proteins, lipids, carbohydrates, immune factors, vitamins and other components [12]. In the field of various medical specialties, the study of vitamin status in oral fluid, especially in childhood, is becoming increasingly widespread due to the non-invasiveness and availability of obtaining biological

material [13]. No publications were found in the literature regarding the content of 25-hydroxyvitamin D in the oral fluid of children with myopia, its influence on the course of myopic refractogenesis, which became the rationale for this scientific study.

AIM

Assess the level of 25-hydroxyvitamin D in oral fluid in children with progressive myopia.

MATERIALS AND METHODS

We examined 34 children (68 eyes) with mild myopia and 18 conditionally healthy children (36 eyes) without ophthalmological pathology. The research was started after obtaining informed consent from the parents (representatives) of the children to participate in the clinical examination. The children were divided into 2 groups: Group I (main) – 34 children (68 eyes) with mild myopia, in which subgroup Ia – 16 children (32 eyes) – with a progressive course of myopia and subgroup Ib – 18 children (36 eyes) – with a stable course of myopia. The control group consisted of 18 conditionally healthy children (36 eyes) without ophthalmological pathology. The average age of patients in the observation groups did not differ significantly and ranged from 11 to 16 years. Visual acuity with correction in the control group was 0.9-1.0. The groups were representative in terms of age and sex.

Standard ophthalmological examination included: visometry, autorefractokeratometry before and after cycloplegia (autorefractokeratometer URK-700), biomicroscopy, ophthalmoscopy, determination of the axial length of the eye on an optical biometer (IOL Master 700 Carl Zeiss, Germany). On the basis of the University Clinic of the Zaporizhzhya State Medical and Pharmaceutical University, an enzyme-linked immunosorbent assay for the level of 25-hydroxyvitamin D in oral fluid was performed. Oral fluid was collected without stimulation by spitting into sterile test tubes, followed by centrifugation for 15 minutes at 10,000 revolutions per minute. The supernatant part of the oral fluid was poured into plastic tubes and stored at –30 ºC. The research was carried out on the immunoenzyme complex ImmunoChem-2100 (USA) using the commercial kit of reagents «25-HYDROXYVITAMIN D [25(OH)D] ELISA KIT» (cat. no. CAN-VD-510) of the company «Diagnostics Biochem Canada» (Canada) according to the manufacturer's instructions. Statistical processing of the obtained results was carried out on a personal computer in the program «STATISTICA 13 En» (StatSoft, license No. JRR709H998119TE-A). Statistical data are presented in the form of median and interquartile range of Me (Q25; Q75). Comparison of the data obtained in the groups was carried out using the non-parametric Kruskel-Wallis rank test. The relationship between the studied parameters was studied using the Spearman rank correlation coefficient (r). To assess the relative quality of the obtained data, ROC analysis was used, namely, the value of the area under the ROC curve (Area Under the Curve, AUC) with an assessment of sensitivity and specificity. The result was considered statistically significant at $p<0.05$.

RESULTS

No reliably significant differences in clinical refraction indicators were present in patients of both observation groups. The average values of clinical refraction were: -2,00 [-2,75; -1,50] dptr in patients of subgroup Ia and -2,00 [-2,00; -1,50] dptr in patients of subgroup Ib ($p>0,05$). Data on the axial length of the eye in both subgroups also had no sufficiently significant differences and amounted to: 24,63 [24,25; 25,13] mm, 24,41 [23,75; 25,14] mm, respectively, ($p>0,05$). The study of the level of 25-hydroxyvitamin D revealed its decrease in patients of the I group with myopia by an average of 2,2 times compared to the data of children in the control group: 18,02 [17,59; 19,77] ng/ml and 39,80 [38,98; 40,68] ng/ml, respectively ($p<0,05$). However, when examining the results of the study in detail, we drew attention to the heterogeneity of the obtained data within the group of patients with myopia. In subgroup Ia with progressive course of myopia, the indicator of 25-hydroxyvitamin D was 16,39 [13,63; 17,64] ng/ml, which is on average 1,2 times lower than in children of subgroup Ib (with a stable course of myopia): 19,68 [18,19; 20,19] ng/ml ($p<0,05$); and also, on average, 2,4 times lower than in children of the control group ($p<0,05$) (fig. 1).

In the future, we were interested in how the level of 25-hydroxyvitamin D affects the progression of the myopic process. For this, a correlation analysis was performed with the calculation of Spearman's rank correlation coefficient between the level of 25-hydroxyvitamin D and the axial length of the eye, as well as between 25-hydroxyvitamin D and the progression of myopia. (fig. 2, fig. 3).

Correlation analysis showed a significant inverse relationship between the axial length of the eye and the level of 25-hydroxyvitamin D ($r= –0,50$, $p<0,05$). Also, a statistically significant inverse relationship was found between the progressive course of myopia and the level of 25-hydroxyvitamin D ($r= –0,69$, $p<0,05$).

We were interested in determining exactly what level of 25-hydroxyvitamin D might be a marker of myopia progression. According to the ROC analysis, a quantitative characteristic of the sensitivity and specificity of 25-hydroxyvitamin D was obtained in the subgroup of children with a progressive course of myopia (Ia), which had statistically significant differences from the indicators of children of the subgroup Ib with a stable course of myopia (fig. 4). The optimal value of the cut-off threshold, which provides the maximum values of sensitivity and specificity, for the indicator of 25-hydroxyvitamin D in oral fluid in children is ≤ 20,154 ng/ml. When choosing this threshold, the sensitivity is 87,9 %, the specificity is 94,7 %. The area under the ROC curve AUC is 0,95±0,03 (CI 0,89-0,98) ($p <0,001$).

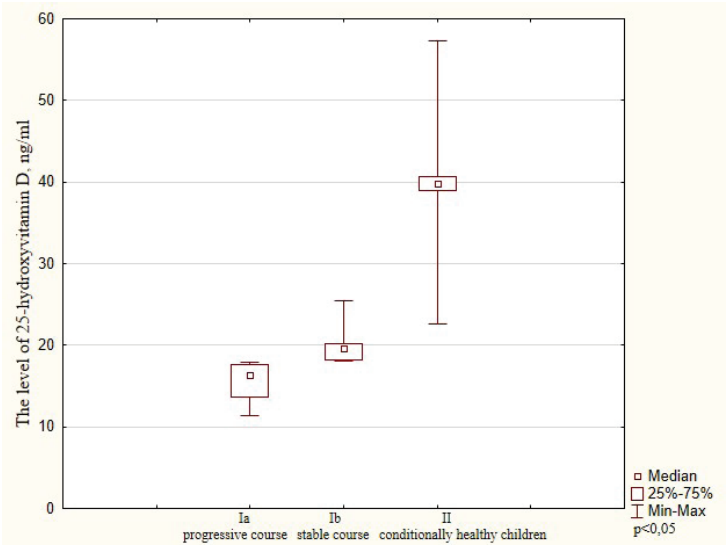

Figure 1. The level of 25-hydroxyvitamin D in the oral fluid of children with progressive and stable myopia and conditionally healthy children.

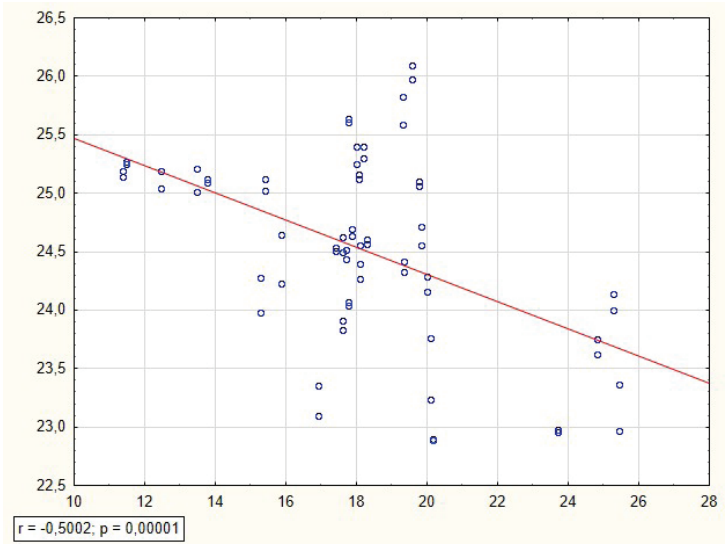

Figure 2. The relationship between the axial length of the eye and the level of 25-hydroxyvitamin D in children with myopia.

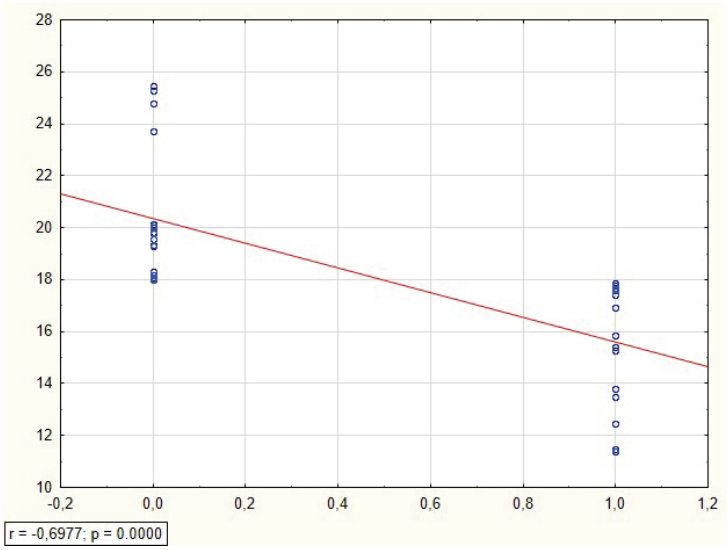

Figure 3. The relationship between the progressive course of myopia and the level of 25-hydroxyvitamin D in children with myopia.

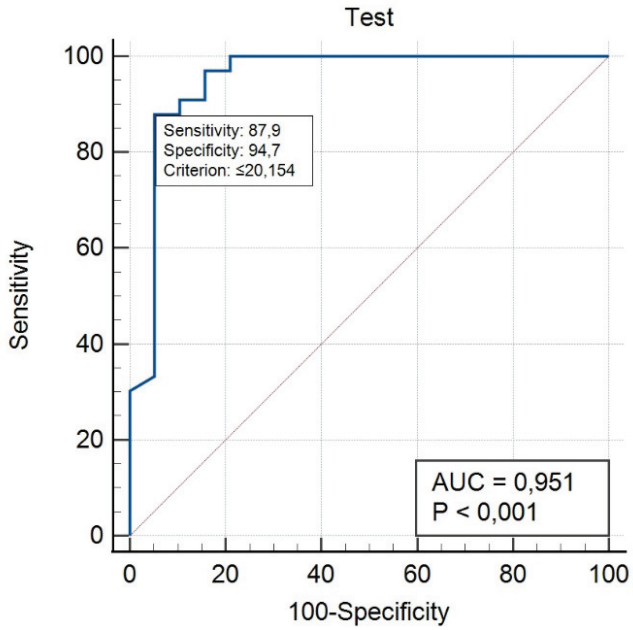

Figure 4. ROC-analysis of the diagnostic efficiency of the indicator of 25-hydroxyvitamin D in oral fluid in the diagnosis of the progressive course of myopia.

DISCUSSION

Despite a sufficient number of publications, the pathogenesis of myopia is a complex and multifactorial process and continues to be the subject of research among clinicians. The pathogenesis of progressive myopia is of particular interest among ophthalmologists around the world. The aetiology of myopia is complex, involving both genetic and environmental factors. The study of cause-and-effect relationships is important for planning appropriate therapeutic and preventive strategies in deciding the tactics of introducing children with myopia.

In publications of recent years, special attention is paid to the imbalance of 25-hydroxyvitamin D in the development of various ophthalmic diseases, including myopia [2, 7].

The widest range of questions regarding the assessment of the level of 25-hydroxyvitamin D in the body in myopia is based on publications related to blood plasma studies, such as both in adults and in children [2, 6, 7].

Thus, Lingham G. and co-authors indicate the relationship between the time spent outdoors, the biomarker of which is the concentration of 25-hydroxyvitamin D in the blood serum of children and adolescents, and myopic refractogenesis [8]. Along with this study, there are data provided by Tideman J. W. and co-authors: studying the relationship between the growth of the axial length of the eye and the level of 25-hydroxyvitamin D in 6-year-old children with myopia, they claim that although a decrease in the concentration of 25-hydroxyvitamin D is associated with higher axial length of the eye and a high risk of developing myopia, but does not depend on the influence of the surrounding environment [10].

Tang S. M., Tiffany L. found a significant association between vitamin D in the blood and myopia for individuals aged older than 18 years [11].

The scientific work of Frolova T. and Bezdetko R., who have not revealed a correlation between the level of 25-hydroxyvitamin D and the degree of myopia, since its deficiency was determined in all children with progressive myopia, but assessing vitamin D deficiency and the gradient of myopia progression for 12 months, note a high inverse correlation ($r = -0,99$, $p<0,05$) between these parameters, is of interest [14].

A literature review of studies Jung B. J. and Jee D. in myopic adults over 20 years of age also indicated an inverse association between serum 25-hydroxyvitamin D and myopic progression, with an odds ratio of 0,75 (95 % Confidence Interval [CI]; 0,67-0,84, $P < 0,001$) [9].

Despite evidence that insufficient vitamin D negatively affects eye health, there is currently no research supporting an association between insufficient vitamins and myopia in children. That is why studies to identify ways to prevent or slow the progression of myopia in both developing and developed countries are urgently required, including any that implicate vitamins deficiency [5].

Oral fluid is a biological substrates, the biochemical composition of which is multifactorial, containing proteins, lipids, carbohydrates, immune factors, vitamins

and other components [12]. In our work, when studying the level of 25-hydroxyvitamin D in children with myopia, oral fluid was used as a biological substrate, because its study is a non-invasive and more convenient method of diagnosis, which is important in childhood. The data we obtained are consistent with the results of other scientists and allow us to draw conclusions about the existence of a close relationship between 25-hydroxyvitamin D and the course of myopia in children, which is confirmed by the above data of correlation analysis. In our study, correlation analysis showed a significant inverse relationship between the axial length of the eye and the level of 25-hydroxyvitamin D ($r = -0,50$, $p<0,05$). Also, a statistically significant inverse relationship was found between the progressive course of myopia and the level of 25-hydroxyvitamin D ($r = -0,69$, $p<0,05$).

The results of the diagnostic efficiency of the level of 25-hydroxyvitamin D in the oral fluid (≤ 20,154 ng/ml, sensitivity 87,9 %, specificity 94,7 %, $p <0,001$), determined in this study, can supplement the knowledge base regarding the features of myopic refractogenesis, be used as an additional biomarker of the progression of myopia in the practical work of an ophthalmologist and in the planning of treatment measures.

CONCLUSIONS

1. In children with myopia, a decrease in the level of 25-hydroxyvitamin D in the oral fluid was determined. So, in children with myopia, the level of 25-hydroxyvitamin D was on average of 2,2 times lower compared to conditionally healthy children ($p<0,05$). However, when examining the results of the study in detail, we drew attention to the heterogeneity of the obtained data within the group of patients with myopia. In children with a progressive course of myopia, the level of 25-hydroxyvitamin D is 2,4 times lower than the level of conditionally healthy children ($p<0,05$).

2. Correlation analysis showed a significant inverse relationship between the axial length of the eye and the level of 25-hydroxyvitamin D ($r = -0,50$, $p<0,05$). Also, a statistically significant inverse relationship was found between the progressive course of myopia and the level of 25-hydroxyvitamin D ($r = -0,69$, $p<0,05$).

3. According to the ROC analysis, a quantitative characteristic of the sensitivity and specificity of 25-hydroxyvitamin D was obtained in the subgroup of children with a progressive course of myopia (Ia), which had statistically significant differences from the indicators of children of the subgroup Ib with a stable course of myopia (fig. 4). The optimal value of the cut-off threshold, which provides the maximum values of sensitivity and specificity, for the indicator of 25-hydroxyvitamin D in oral fluid in children is ≤ 20,154 ng/ml. When choosing this threshold, the sensitivity is 87,9 %, the specificity is 94,7 %. The area under the ROC curve AUC is 0,95±0,03 (CI 0,89-0,98) ($p <0,001$).

4. A decrease in the level of 25 hydroxyvitamin D in the oral fluid is an additional risk factor of the progressive course of myopia in children.

Prospects for further research. The results of determined in this study, can supplement the knowledge base regarding the features of myopic refractogenesis, be used as an additional biomarker of the progression of myopia in the practical work of an ophthalmologist and in the planning of treatment measures.

COMPLIANCE WITH ETHICAL REQUIREMENTS

The authors adhered to the principles contained in the 1964 Declaration of Helsinki and their latest amendments. All parents (guardians) gave oral and written voluntary informed consent for examination, tests and data processing. The work with patients was prepared and carried out in accordance with the principles of ethics. The permission to conduct the study and the study protocol were approved by the bioethics committee of the institution.

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Резюме

ЗМІНИ РІВНЯ 25-ГІДРОКСИВІТАМІНУ Д У РОТОВІЙ РІДИНІ У ДІТЕЙ З ПРОГРЕСУЮЧОЮ МІОПІЄЮ

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Мета роботи. Оцінити рівень 25-гідроксивітаміну Д у ротовій рідині у дітей з прогресуючою міопією.
Матеріали та методи. Під обстеженням перебувало 34 дитини (68 очей) з міопією слабкого ступеню та 18 умовно-здорових дітей (36 очей) без офтальмологічної патології. Діти були розподілені на 2 групи: I група (основна) – 34 дитини (68 очей) з міопією слабкого ступеню, в якій було виділено підгрупу Ia – 16 дітей (32 ока) – з прогресуючим перебігом міопії та підгрупу Ib – 18 дітей (36 очей) – з стабільним перебігом міопії. Контрольну групу склали 18 умовно-здорових дітей (36 очей) без офтальмологічної патології. Проведено стандартне офтальмологічне обстеження: візометрію, авторефрактокератометрію до та після циклоплегії, біомікроскопію, офтальмоскопію, визначення аксіальної довжини ока. Імуноферментним методом визначено рівень 25-гідроксивітаміну Д у ротовій рідині.
Результати. У дітей з прогресуючим перебігом міопії показник 25-гідроксивітаміну Д в 1,2 рази нижче дітей з стабільним перебігом міопії та у 2,4 рази нижче дітей контрольної групи ($p<0,05$). Кореляційний аналіз показав достовірний зворотній зв'язок між аксіальною довжиною ока та рівнем 25-гідроксивітаміну Д ($r= –0,50$, $p<0,05$) і між прогресуючим перебігом міопії та рівнем 25-гідроксивітаміну Д ($r= –0,69$, $p<0,05$). За даними ROC-аналізу отримано оптимальне значення порогу відсікання для показника 25-гідроксивітаміну Д у ротовій рідині у дітей ≤ 20,154 нг/мл (чутливість становить 87,9 %, специфічність – 94,7 %), ($p <0,001$).
Висновки. У дітей з прогресуючим перебігом міопії рівень 25-гідроксивітаміну Д у 2,4 рази нижче від рівню умовно-здорових дітей. Зниження рівню 25 гідроксивітаміну Д у ротовій рідині є додатковим фактором ризику прогресуючого перебігу міопії у дітей.

Ключові слова: **міопія, діти, ротова рідина, вітамін Д**