SURFACE PHENOTYPE OF BLOOD LYMPHOCYTES IN CHILDREN WITH MEDIUM AXIAL MYOPIA IN THE PRESENCE OR ABSENCE OF SECONDARY IMMUNODEFICIENCY

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Investigating the role of secondary immunodeficiency in the development of myopia in children is a promising research area. We studied the surface phenotype of blood lymphocytes in healthy children and children with medium axial myopia in the presence or absence of secondary immunodeficiency clinical manifestations. The mean age of study participants was 16 ± 0.25 years. The control group and each of the two experimental subgroups included 8 children. Using indirect immunofluorescence, the expression of CD3, CD4, CD8, CD16, CD56, CD20, CD72, CD38, CD25, CD71, HLA-DR, CD95, CD54, mIgM, mIgG, ICAM-1 antigens was studied. For children with myopia and secondary immunodeficiency, only one statistically significant ($p < 0.05$) difference from the control group was detected, namely, a reduced expression of CD4 antigen. For children with myopia and without secondary immunodeficiency, a statistically significant ($p < 0.05$) increase in CD20 antigen expression and a reduced ICAM-1 antigen expression were observed.

Keywords: nearsightedness, myopia, medium axial myopia, secondary immunodeficiency, lymphocytes, lymphocyte surface phenotype, antigens

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ПОВЕРХНОСТНЫЙ ФЕНОТИП ЛИМФОЦИТОВ КРОВИ У ДЕТЕЙ С ОСЕВОЙ МИОПИЕЙ СРЕДНЕЙ СТЕПЕНИ ПРИ НАЛИЧИИ И ОТСУТСТВИИ ВТОРИЧНОГО ИММУНОДЕФИЦИТА

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Изучение влияния вторичного иммунодефицита на развитие близорукости у детей — перспективное направление исследований. Нами был изучен поверхностный фенотип лимфоцитов крови у здоровых детей и детей с осевой миопией средней степени при наличии и отсутствии клинических признаков вторичного иммунодефицита. Средний возраст участников исследования составил 16 ± 0,25 года. В контрольную группу и в каждую из двух опытных подгрупп включили по 8 детей. Изучали экспрессию антигенов CD3, CD4, CD8, CD16, CD56, CD20, CD72, CD38, CD25, CD71, HLA-DR, CD95, mIgM, mIgG, ICAM-1 методом непрямой иммунофлюоресценции. Для детей с близорукостью и вторичным иммунодефицитом выявили лишь одно достоверное ($p < 0,05$) отличие от показателей контрольной группы — сниженную экспрессию антигена CD4. Для детей с близорукостью и без вторичного иммунодефицита отметили достоверное ($p < 0,05$) усиление экспрессии антигена CD20 и снижение экспрессии антигена ICAM-1.

Ключевые слова: близорукость, миопия, осевая миопия средней степени, вторичный иммунодефицит, лимфоциты, поверхностный фенотип лимфоцитов, антигены

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Myopia is not only the most common type of refractive error, but is also ranked first in the general structure of ocular pathology [1]. The prevalence of myopia is growing. According to available data, myopia of varying degrees affected approximately 1.6 billion people worldwide in 2000. This figure is expected to in crease to 2.5 billion by 2020 [2]. It is important to note that prevalence of the disease among children [3, 4] is growing, and progression of the disease is often observed in school children [5, 6].

Myopia is considered a polyetiological disease, but some of its causes are not yet fully studied. Studying the role of immune disorders in the development of myopia is a promising research area [5–9]. Children with myopia have clinical signs of secondary immunodeficiency more often than their peers with other types of clinical refractive errors [5, 7–9].

Our study aims at investigating the surface phenotype of blood lymphocytes in children with emmetropia and medium axial myopia, with or without clinical signs of secondary immunodeficiency.

METHODS

The study was conducted in 2013–2015 and featured 24 school children in Moscow aged between 10 and 18 years (mean age of 16 ± 0.25 years): 16 boys and 8 girls. The control group included 8 children (16 eyes) with emmetropia without chronic diseases but with incidence of acute respiratory infections (ARIs) for less than five times a year. The experimental group consisted of 16 children (32 eyes) and was divided into two subgroups. The first subgroup (Group I) included 8 children diagnosed with medium axial myopia and clinical signs of secondary immune deficiency (SID). These children were observed with increased incidence of ARIs (more than 7 times a year). The exclusion criterion was the presence of autoimmune diseases. The second subgroup (Group II) included 8 children with medium axial myopia, but without clinical signs of SID and with incidence of ARIs for less than five times a year and absence of ARIs for two months prior to the study. The diagnosis was verified at the Diagnostic and Consultative Unit, Children's Medical Centre of the Administrative Directorate of the President of the Russian Federation, Moscow, Russia. The presence or absence of SID was determined based on dispensary data.

All the children examined underwent standard ophthalmic examination, including visometry, (digital chart OAP-250, Carl Zeiss, Germany), autorefractometry (auto kerato-refractometer KR-8900, Topcon, Japan), biomicroscopy (SL 120 slit lamp, Carl Zeiss, Germany), identification of relative accommodation reserves by Avetisova method [10], and ophthalmoscopy and echo biometry (HiScan scanner, OPTICON, Italy).

Lymphocytes were separated from peripheral blood using a one-step Boyum density gradient technique [11]. The expression of CD3, CD4, CD8, CD16, CD56, CD20, CD72, CD38, CD25, CD71, HLA-DR, CD95, CD54, mIgM, mIgG, ICAM-1 antigens was studied using monoclonal antibodies ICO and LT by indirect immunofluorescence under the Luman I-3 microscope (LOMO, Russia).

The results were statistically processed using software package Statistica. The statistical significance was estimated using Student's test.

RESULTS

The research results are presented in the table. Group I children (with SID signs) showed reduced expression of CD3, CD4 and CD8 antigens in comparison with the control group. However, the difference was significant only for lymphocytes with surface phenotype $CD4^+$ ($p < 0.05$). In Group II children (with no SID signs), on the contrary, the number of lymphocytes expressing CD3, CD4 and CD8 antigens was higher than that of the control group.

But for all of them, the difference was insignificant. Differences in expression of CD16 and CD56 antigens identified for both groups in comparison with the control group was also not statistically confirmed.

The content of lymphocytes with surface phenotype CD20+ in Group I children was 14.54 ± 2.36 %, which is lower than similar indicator in the group of healthy children (19.87 ± 2.15 %). However, the difference was insignificant. A significant increase in the expression of this antigen in Group II children was observed. The identified differences on lymphocytes with

Lymphocyte count with different surface phenotype in the peripheral blood of children in the experimental and control groups (% of the total lymphocyte count)

Surface markers	Group I	Group II	Control group
$CD3^+$	39.97 ± 2.01	58.47 ± 1.96	56.33 ± 3.35
$CD4^+$	29.81 ± 4.38*	44.73 ± 4.76	38.03 ± 0.87
$CD8^+$	22.66 ± 2.49	32.02 ± 2.63	26.48 ± 0.98
$CD16^+$	19.01 ± 3.12	25.74 ± 4.51	23.01 ± 3.07
$CD56^+$	18.28 ± 4.62	19.88 ± 3.52	18.06 ± 1.65
$CD20^+$	14.54 ± 2.36	29.21 ± 2.84*	19.87 ± 2.15
$CD72^+$	15.08 ± 1.63	24.805 ± 4.07	19.23 ± 2.27
$CD38^+$	16.17 ± 3.16	25.93 ± 5.41	22.89 ± 2.08
$CD25^+$	17.11 ± 2.13	19.52 ± 2.22	17.92 ± 4.23
$CD71^+$	18.73 ± 4.14	21.17 ± 2.99	17.8 ± 2.84
$HLA\text{-}DR^+$	22.27 ± 2.06	24.63 ± 3.88	21.45 ± 2.20
$CD95^+$	15.88 ± 2.68	20.97 ± 2.46	15.73 ± 1.87
IgM^+	10.69 ± 1.73	27.19 ± 5.79	15.84 ± 1.07
IgG^+	16.29 ± 3.67	20.49 ± 3.23	20.49 ± 3.23
$ICAM\text{-}1^+$	6.69 ± 0.70	22.905 ± 6.42*	11.93 ± 1.40

* — $p < 0.05$ compared with the control group.

surface phenotypes CD72, CD38, CD25, CD71, HLA-DR, CD95, mIgM and mIgC were not statistically confirmed.

A significant ($p < 0.05$) decrease in the level of expression of ICAM-1 antigen was observed in Group II: number of lymphocytes corresponding to the phenotype was 6.69 ± 0.70 % against 11.93 ± 1.40 % in the control group.

Thus, children with medium axial myopia and clinical signs of SID showed reduced expression of CD4 antigen when compared with healthy children. In children with the same diagnosis, but with no clinical signs of SID, there was a significant increase in the expression of CD20 antigen and reduced expression of ICAM-1 antigen.

DISCUSSION

Despite the fact that most of the differences revealed were statistically insignificant, it is undeniable that children with medium axial myopia and clinical signs of SID tend to have decreased immunity. Examining a larger number of patients is most probably required. However, there is reduced number of major subpopulations of T-lymphocytes and NK-cells in sickly children compared with healthy children.

Increased expression of adhesion molecules in children with myopia can be associated with the action of peroxy compounds on lymphocytes [12, 13]. It is known that transretinal — the product of isomerization of cis-retinal in the light-dependent visual cycle process – activates lipid peroxidation [14]. Also, myopia is associated with the toxic effect of peroxide compounds on the sclera and increase in the longitudinal dimensions of the eyeball [15].

CONCLUSIONS

The trend towards inhibition of expression of the antigens characterizing lymphocyte subpopulations in myopia in children with clinical signs of SID shows that the immune system is involved in the pathological process, and is apparently not associated with myopia.

Children with medium axial myopia without clinical signs of SID exhibit elevated blood lymphocytes expressing ICAM-1 antigens in the leukocyte membrane. This may be associated with production of free radicals, which are generated during visual act by one of the active metabolites of vitamin A.

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