COMPARISON OF INDICATORS OF CELLULAR AND HUMORAL IMMUNITY IN ACQUIRED MYOPIA MILD AND HIGH DEGREE

POROVNANIE UKAZOVATEĽOV BUNKOVEJ A HUMORÁLNEJ IMUNITY U ZÍSKANEJ KRÁTKOZRAKOSTI MIERNEHO A VYSOKÉHO STUPŇA

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ABSTRACT

Introduction: Acquired myopia is one of the most fast-spreading visual impairment in the modern information society that affects primarily young population. Under adverse conditions myopia is prone to rapid progression and its high values significantly increases the risk of serious complications (visual acuity reduction, maculopathy, blindness). Despite the long study, the exact mechanisms of myopia development are still insufficiently studied, particularly its immunopathological aspect.

Objectives: The aim of the research was to analyze the characteristics of cellular and humoral immunity in conditions of non-pathological myopia acquired form mild and high degree. We studied the indicators of immunity system (the absolute number and relative content of leukocytes, lymphocytes, T-lymphocytes, T-helper/inducers, T-suppressors/cytotoxic, B-lymphocytes, IPI index, the concentration of IgM, IgG, IgA) in people aged 18 – 35 years with myopic refraction (n = 60, the experimental group) and people with normal vision (n = 60, the control group).

Methods: An immunological study was conducted with all volunteers after signing the Written Informative Consent. The examination included a Complete Blood Count and immunological study (flow cytometric immunoassay, ELISA) which were provided with standard diagnostic procedures carried out in accordance with the appropriate methodology and manufacturer's instructions.

Results: According to our results, the decrease in the level of leukocytes, lymphocytes, T-lymphocytes and T-helpers/inductors, and an increase in the level of T-suppressors/cytotoxic, B-lymphocytes, serum IgA and IgM (especially) were found in all myopes compared with control group. Consequently, we revealed the dysfunctions in immune homeostasis on the myopia background: the reducing immunoregulatory index, the depressing T-cellular and significant activation of humoral immunity. This negative tendency force with increasing myopia level, which reflects the involvement of immune system in mechanisms of its formation.

Key words: Myopia. Immunity. T-lymphocytes. B-lymphocytes. Immunoglobulins.

ABSTRAKT

Úvod: Získaná krátkozrakosť (myopia) je najrýchlejšie sa šíriacou formou zrakového postihnutia v modernej spoločnosti, ktorá postihuje predovšetkým mladých ľudí. Za nepriaznivých podmienok je krátkozrakosť náchylná k rýchlej progresii a jej vysoké hodnoty výrazne zvyšujú riziko závažných oftalmologických komplikácií (napr. znížená zraková ostrosť, makulopatia, slepota). Napriek dlhodobému štúdiu tohto problému nie sú stále pochopené presné mechanizmy vývoja myopie, najmä imunopatologický aspekt jej patogenézy.

Ciele: Cieľom štúdie bolo analyzovať vlastnosti bunkovej a humorálnej imunity u ľudí s nepatologicky získanou krátkozrakosťou mierneho a vysokého stupňa. Študovali sme parametre imunitného systému (absolútny počet a relatívny obsah leukocytov, lymfocytov, T-lymfocytov, T-pomocníkov / induktorov, cytotoxických T-supresorov, B-lymfocytov, koncentrácie sérových IgM, IgG, IgA) u ľudí vo veku 18 – 35 rokov s myopickou refrakciou (n = 60, experimentálna skupina) a ľudia s normálnym zrakom (n = 60, kontrolná skupina).

Metódy: Po podpísaní informačného súhlasu s účasťou sa so všetkými účastníkmi uskutočnila imunologická štúdia. Vyšetrenie zahŕňalo kompletný krvný obraz a imunologické štúdie (enzýmovo viazaný imunosorbentný test prietokovou cytometriou, ELISA). Použili sme štandardné diagnostické postupy, vykonávané v súlade s metodikou a pokynmi výrobcu.

Výsledky: Podľa našich výsledkov všetky myopy vykazovali pokles hladiny leukocytov, lymfocytov, T-lymfocytov a T-pomocných látok / induktorov, ako aj zvýšenie hladiny T-supresorov / cytotoxických, B-lymfocytov, sérových IgA a najmä IgM (v porovnaní s kontrolná skupina). Odhalili sme narušenie imunitnej homeostázy na pozadí získanej krátkozrakosti: nedostatok spojenia T-buniek so systémovou imunitou, zníženie imunoregulačného indexu a významná aktivácia humorálnej imunity. Zároveň sme zaznamenali, že so zvyšovaním hodnôt krátkozrakosti sa zvyšujú negatívne tendencie imunologických zmien, čo odráža vplyv imunitného systému na mechanizmy tvorby krátkozrakosti.

Kľúčové slová: Krátkozrakosť. Imunita. T-lymfocyty. B-lymfocyty. Imunoglobulíny.

INTRODUCTION

Generally known, the prevalence of myopia, which is one of the important causes of reduced vision, has been increasing worldwide [1] especially among young people [2]. It's believed that most people have an induced form of myopia that appears throughout life, usually in school, and stabilizes during the third decade without pathological changes in the retina. High myopia increases the risk of maculopathy, glaucoma and other myopia-associated complications, which can lead to the loss of central visual function or even blindness [3-5]. It becomes a serious problem, not only because of rising

morbidity, but also because of the decreasing age of myopia onset [6]. It was shown that children with first myopic diagnosis at the age of 6-8 years ("early myopia") are at a greater risk of high myopia later in childhood [7].

Myopia is considered a complex of pathological conditions, generated by multiple etiologic factors but the leading mechanism of pathogenesis is still not defined. The myopia development can be influenced by lifestyle, environmental factors, academic load [6, 8-10], biomechanical weakening of the sclera, oxidative stress, excessive accommodation load [11, 4, 12]. The acquired form of myopia is considered as a functional adaptation of the visual sensory system, which develops as a result of prolonged work at close distances, and the eye acts as a part of the nervous system brought to the periphery [13, 14].

According to the Theory of immune-regulation of body functions [15], there is a functional unity of the nervous and immune systems and the condition of immune system may significantly impact general health. In this aspect, question of the state of immune system in people with myopia is very relevant. An analysis of literary sources shows that the immune status of myopes has been studied insufficient. It was found that the immunological factor plays an important role in enhancing refraction [16]. It was also revealed that under conditions of myopia a secondary immunodeficiency was formed, the signs of which is the relative suppression of T-cell immunity, a decrease in the functions of anti-infection protection, an imbalance of blood immunoglobulins [17-21]. However, these studies have a one-sided character and are devoted either to the myopia study at certain age period (mainly school), or to its certain level, or to its specific clinical form (congenital, progressive). Data of immune system's state on the background of acquired myopia of different levels are not fully presented in the literature. Thus, our research purpose was to determine the characteristics of cellular and humoral immunity in people aged 18–35 years with acquired myopia depending on its degree (mild and high).

MATERIALS AND METHODS

The research was performed according to the World Medical Association's Declaration of Helsinki and Council of Europe Protocol of the Convention on Human Rights and Biomedicine, approved by the Ethics Committee of Sumy State Pedagogical University named after A.S. Makarenko. Written informed consent was obtained from all the participants. This work was carried out within the framework of the scientific research topic of the Department of Human and Animal Biology, Sumy State Pedagogical University named after A.S. Makarenko.

Study groups

We analyzed data of 120 volunteers 18 – 35 years old, which were divided on the control group (healthy people without chronic and acute diseases with normal vision, n = 60) and the experimental group (people with an acquired myopia mild and high degree, n = 60). The diagnosis of "acquired form of myopia" and its degree were based on the doctor's conclusion after conducting standard eye examinations (measurement of visual acuity, refractometry, ophthalmoscopy, tonometry, skioscopy). Qualified staff at medical center "MedSoyuz" (Sumy, Ukraine) took the blood samples for immulogical study.

Assessment of cellular and humoral immunity

Blood sampling was performed in the morning before eating in compliance with the medical norms. Complete Blood Count (CBC) was measured in blood collected in EDTA samples (20 µl) using BC-5500 Hematology Analyzer (Mindray (UK) Ltd., China). Immunophenotyping of leukocytes was performed by flow cytometric immunoassay using whole blood and the sets of monoclonal antibodies AQUIOS Tetra Tests on a flow cytometer AQUIOS CL (both from Beckman Coulter, USA) according to the user's manual. The AQUIOS monoclonal antibody reagents (Tetra-1 Panel and Tetra-2+ Panel) are each a combination of murine monoclonal antibodies respectively, conjugated to the specified fluorochrome. Specific staining of leukocytes was accomplished by incubating whole blood with the monoclonal antibody reagent. The cyanide-free lytic reagent lysed red blood cells in preparation for white blood cell measurement in the flow cell. The total number and relative content of cell populations $CD3^+$ (T-lymphocytes), $CD4^+$ (T-helper/inducers), $CD8^+$ (T-suppressors/cytotoxic), $CD22^+$ (B-lymphocytes) and $CD4^+$:$CD8^+$ ratio (IPI index) were obtained using a panel of monoclonal antibodies with appropriate reagents.

The serum concentration of IgG, IgA, IgM were measured by enzyme-linked immunosorbent assay (ELISA) using a polystyrene 96-well plates MICROLON® (Greiner Bio-One, Germany) and the reagent kit "Immunoskrin-G,M,A-ELISA-Best" (Vector-Best, Russia). Monoclonal antibodies to human Ig labeled with horseradish peroxidase (Diaprof-Med, Ukraine) were used for ELISA. To visualize the reaction results in ELISA plates we used soluble chromogen 3, 3', 5, 5' - tetramethylbenzidine (Diaproph-Med, Ukraine) in 0.1 M citrate-phosphate buffer pH 4.5. ELISA results were recorded spectrophotometrically on a PR2100 reader (Sanofi Diagnostics Pasteur, Inc., France) at 450 nm. ELISA was performed according to the methodology [22] and manufacturer's instructions.

Statistical procedures

The data are expressed as arithmetic mean and standard error of the mean (M±m). Results were analyzed by using Mann-Whitney U-test (Microsoft Office Excel 2010, Statistica 8.0); differences with $p<0.05$ were considered significant. Correlation analysis was performed using the Pearson correlation coefficient.

RESULTS

The results of immunological study (Tab. 1) showed that in people with myopia mild and high degree most indicators of cellular and humoral immunity were significantly different from the control data.

Study results of CBC-test showed that in all myopic groups amount of leukocytes, lymphocytes (due to the reduction of segmented neutrophils) were lower than in healthy people with normal vision.

Analysis of $CD3^+$-lymphocytes content showed significant decrease of absolute and relative values of these cells in comparison to healthy people. We also observed a significant decrease of subpopulation of T-helpers/inductors ($CD4^+$) in all myopes. At the same time, the number of T-suppressors/cytotoxic ($CD8^+$) was increased compared to control data. Comparison of the immunoregulatory index showed reduced IPI of people with myopia compared to IPI of healthy people (2 times for mild myopia, 1.5 times for high myopia; $p<0.05$). In addition, leuko-T-cell index of persons with myopia was increased in comparison with control group.

The absolute and relative number of lymphocytes with membrane marker $CD22^+$ in all myopic

Table 1 The indices of cellular and humoral immunity in healthy people and people with acquired myopia.

Indicator		Healthy people (control group) (n=60), M±m	People with myopia (experimental group) (n=60), M±m	
			mild degree (n=30)	high degree (n=30)
Leukocytes	$^*10^9$/L	6.80 ± 0.12	6.03 ± 0.16	5.89 ± 0.20
Lymphocytes	$^*10^9$/L	2.20 ± 0.06	1.83 ± 0.15*	1.78 ± 0.14*
	%	32.87 ± 0.07	30.29 ± 0.12	32.67 ± 0.14
T-lymphocytes ($CD3^+$)	$^*10^9$/L	1.81 ± 0.04	1.20 ± 0.13*	1.29 ± 0.10*
	%	82.27 ± 0.36	66.70 ± 0.37*	70.67 ± 0.32*
T-helpers/inductor ($CD4^+$)	$^*10^9$/L	0.86 ± 0.04	0.67 ± 0.11*	0.61 ± 0.06*
	%	47.51 ± 0.14	38.00 ± 0.30*	36.33 ± 0.27*
T-suppressors/ cytotoxic ($CD8^+$)	$^*10^9$/L	0.32 ± 0.02	0.41 ± 0.06	0.33 ± 0.06
	%	17.68 ± 0.07	25.00 ± 0.42*	21.22 ± 0.40*
IPI ($CD4^+/CD8^+$)	cu	2.69 ± 0.11	1.46 ± 0.10*	1.75 ± 0.12*
Leuko-T-cellular index	cu	3.76 ± 0.06	5.28 ± 0.09*	4.85 ± 0.08
B-lymphocytes ($CD22^+$)	$^*10^9$/L	0.34 ± 0.04	0.40 ± 0.06	0.42 ± 0.05
	%	17.5 ± 0.38	22.14 ± 0.37*	25.78 ± 0.29*
IgG	g/L	15.10 ± 0.34	13.41 ± 0.28	16.32 ± 0.40
IgM	g/L	1.44 ± 0.16	6.41 ± 0.30*	7.78 ± 0.36*
IgA	g/L	1.85 ± 0.23	3.29 ± 0.15*	3.83 ± 0.21*
Immunoglobulin-producing ability of B-lymphocytes	g/L	18.39 ± 0.20	23.11 ± 0.24	27.93 ± 0.32*
Leuko-B-cellular index	cu	20.9 ± 0.16	18.62 ± 0.33	14.71 ± 0.32*

Legend: * – statistically significant differences myopic group relative to control data (Mann-Whitney U-test, $p<0.05$); $^*10^9$/L – the absolute cell number; % – the relative cell count; CU - the standardized unit of measurement.

groups exceeded control data, which led to activation of their immunoglobulins secretion. According to obtained results, the overall immunoglobulin-producing activity of B-lymphocytes was higher in myopes as compared to the control group. We observed the increase in concentrations of immunoglobulins A and M classes in myopes blood ($p<0.05$). The decrease of concentration of IgG was observed under mild myopia, while an increase was detected under high myopia. Simultaneously with an increase of B cells content, leuko-B-cell index of persons with myopia was reduced in comparison with control data.

DISCUSSION

Immune disorders are primarily indicated by fluctuations (the decrease or increase) in the content of leukocytes and lymphocytes. In our research, it was detected that under myopia the amount of leukocytes and lymphocytes was decreased. This trend has been described in some other research studies [16, 20, 21]. We reveal new data that these changes intensify with increasing myopia values. It is generally known that leukopenia and lymphopenia are the markers of acquired immunodeficiency and neutropenia can determine higher infection risk [18, 19]. This may cause the weakened anti-infective immunity on the background of acquired myopia: it has been shown that children with myopia more often suffer from infectious and viral diseases than their healthy peers or children with other visual impairment [23, 24].

Our further analysis, which included the assessment of T-cell-mediated immunity, showed that the $CD3^+$ and $CD4^+$ values, the IPI index were decreased and the amount of $CD8^+$ subpopulation was increased in myopic groups compared with control data. These results confirm the findings of several other authors who studied the characteristics of immunity in conditions of myopia. In most cases, a decrease in the level of T-lymphocytes (in particular of T-helpers subpopulation) has been proven [16, 20, 21]. Regarding $CD8^+$ cells, their values in people with myopia varies: There were described cases of their content increasing [20, 24] and decreasing [19]. Supposedly, different age of participants or other cohort features are the explanation of this fact. In conclusion, our results confirm the formation of the signs of T-cell immunodeficiency in people with myopia, caused by suppression of the T-helper population.

The defect of a particular function of T-lymphocytes, as a rule, leads to a dysregulation of humoral response. Our results revealed statistically significant increase in $CD22^+$ amount and concentration of IgA and IgM in all myopes groups. At the same time, we disclosed that the IgM content in myopic groups exceeded the upper limit of normal values. The increased IgM content is a symptom of dysregulation in the T-helper cells' functions responsible for transmitting co-stimulation signals to B-cells for switching IgM synthesis of another class of immunoglobulin synthesis. Since IgG is a T-dependent immunoglobulin, regulates the production of specific antibodies by feedback, and is responsible for the secondary immune response, the decrease in its level is the indirect evidence of disorder in T-cell immunity. Other authors obtained similar results [20, 24], but in our study we found a decrease in the concentration of IgG in people with mild myopia, and its increase in the group with a high myopia. The elevated value of IgA, which was showed in our study, is probably a reflection not only of immune dysregulations, but also of a chronic inflammatory process, that is present in people with myopia [23]. The increasing level and frequency of dysimmunoglobulinemiya depending on the myopia degree are shown in our other work [25]. According to some sources, increasing B-cell content is an adaptive response of the immune system, which is aimed at compensating for the defect in T-cell link [26].

When investigating relationships in humoral and cellular immunity of people with mild and high myopia, we observed a significant correlation between these cohorts (Fig. 1).

However, in some cases these relationships were different in mild and high myopia. These changes are the result of adaptive rearrangements of populations of immune cells that occur to maintain internal homeostasis. Thus, it can be assumed that in the range of certain values of myopia (its degree), a special functional state of the immune system was formed.

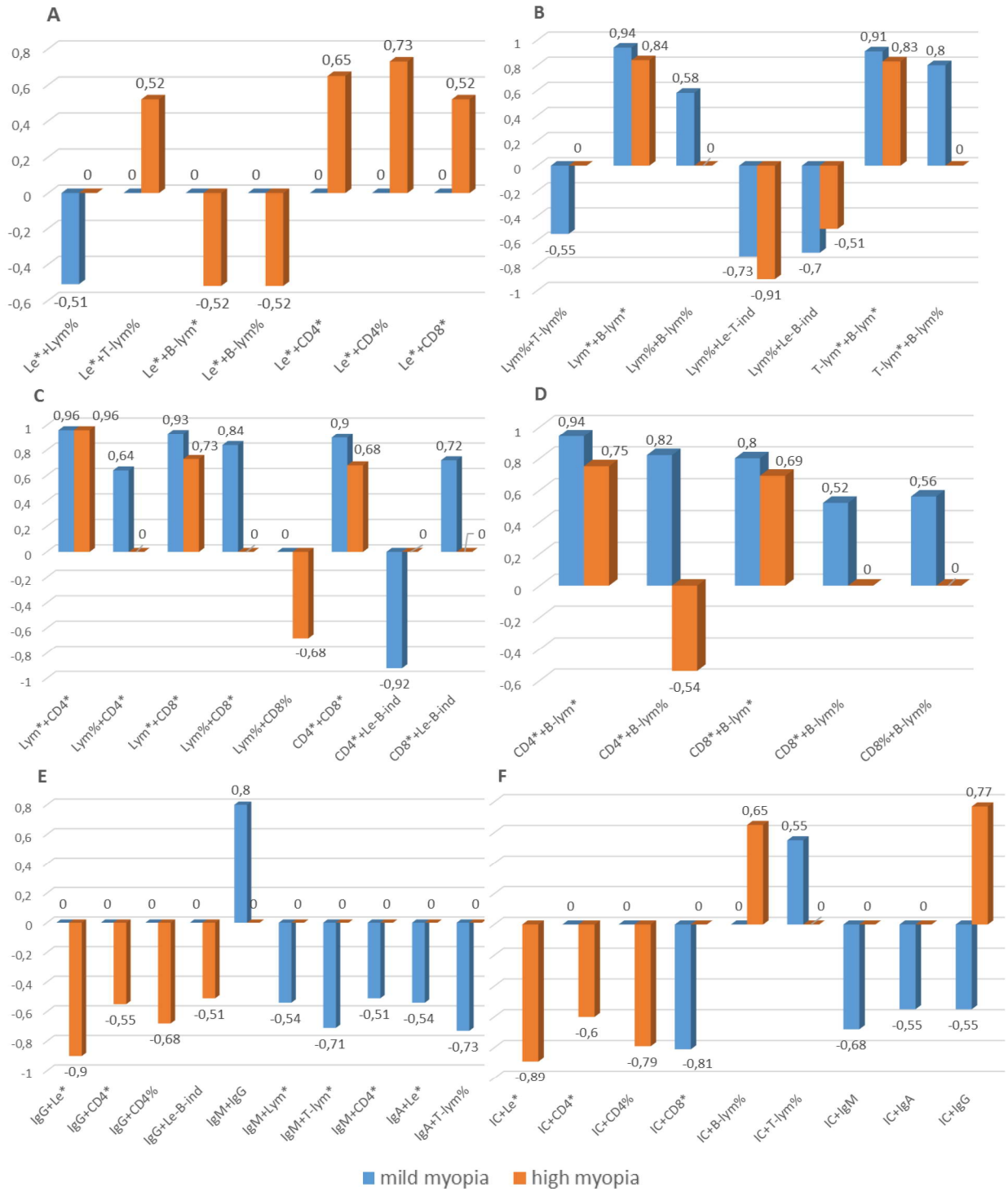

Figure 1 Correlation between the characteristics of CBC, cellular and humoral immunity in myopic groups (n=60). *Legend:* Le – leukocytes; Lym – lymphocytes; B-lym – B-lymphocytes; T-lym – T-lymphocytes; CD4 –T-helper/inducers; CD8 – T-suppressors/cytotoxic; Le-B-ind – Leuko-B-cellular index; IC - circulating immune complex; * - the absolute cell count; % - the relative cell count.

CONCLUSIONS

Our study revealed changes in the indices of cellular and humoral immunity in people with acquired myopia in comparison to healthy people with normal vision. We have established that under conditions of myopia of certain degree, a special functional state of immune system, which is expressed in adaptive reorganizations of immune cells populations, was observed. In general, a tendency to leukopenia, lymphopenia, T-cell depression (by reducing the number of T-lymphocytes and T-helpers/inductors) and simultaneous activation of humoral (elevated B-lymphocytes, immunoglobulins M and A) immunity in myopes mild and high degree were identified. It should be noted that this negative changes were aggravated with an increase in the level of myopia. In general, immune resistance of people with acquired myopia was lowered. The obtained results confirm the involvement of immune system in pathophysiological mechanisms of development of acquired myopia.

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