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THE STUDY OF PATHOPHYSIOLOGICAL LINK OF AUTOIMMUNIZATION IN PATIENTS WHO USE SOFT CONTACT LENSES*

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In this work, the state of the immune system was studied in the patients using soft contact lenses for various variants of the normal microflora eyes. Analyzing the absolute number of lymphocytes in the peripheral blood, we found that these indices are within the limits of normal physiological fluctuations and do not have a significant difference between the groups. The same tendency was observed in the study of the content of common T-lymphocytes, and their number in all groups is within the limits of normal physiological parameters. The inhibition of the IL-2 expression activator confirms our assumption of latent suppression of the T-immune system, especially in patients who have a smoking habit. It has been found that in patients who use soft contact lenses and have a habit of smoking there is a decrease in the level of B-lymphocytes. However, despite the normal immunological parameters of B-lymphocytes, in the 1st group as well as in the 2nd group there is a hidden inhibition of the T-immune system.

Keywords: immune system, soft contact lenses, normal microflora

У роботі вивчали стан імунної системи у пацієнтів, що використовують м'які контактні лінзи при різних варіантах стану нормальної мікрофлори ока. Аналізуючи абсолютну кількість лімфоцитів у периферичній крові, ми виявили, що ці показники знаходяться в межах нормальних фізіологічних коливань і не мають суттєвої різниці між групами. Така ж тенденція спостерігалася при вивченні вмісту загальних Т-лімфоцитів, а їх кількість у всіх групах перебуває в межах нормальних фізіологічних параметрів. Інгибування активатора експресії ІЛ-2 підтверджує наше припущення про приховане пригнічення Т-імунної системи, особливо у пацієнтів, які палять. Було виявлено, що у пацієнтів які використовують м'які контактні лінзи та мають звичку паління має місце зниження рівня В-лімфоцитів. Однак незважаючи на нормальні імунологічні показники В-лімфоцитів, в I групі також як і в II групі спостерігається приховане пригнічення Т-системи імунітету.

Ключові слова: імунна система, контактні лінзи, нормальна мікрофлора

Wearing contact lenses increases the risk of eye infections, corneal damage, and changes the natural microflora of the eye. In any case, the results of wearing contact lenses can potentially be either dangerous or catastrophic. In order to understand the risks, it is useful to understand how the eye works and how contact lenses help correct vision. If a patient does not care about the eyes and lenses, one can increase the risk of complete loss of vision.

Contact lenses are literally in "contact" with the eye. These lenses are prescription based, and they are intended for correction of visual errors – in essence, they resemble miniature glasses. They change the place where the light focuses on the retina, which improves vision.

Soft lenses move along with the eye, floating above the surface of the cornea. The concept of contact lenses was demonstrated in 1508 by Leonardo da Vinci. The first lenses were made of glass in 1887.

For many years, they have evolved to plastic, soft lenses, disposable lenses, air-permeable versions; recently, silicone hydrogel has appeared, manufactured on an individual basis.

Air-permeable lenses are made of hard plastic, they have less water, they are less flexible than soft lenses, but are better resistant to bacteria. These lenses keep shaping when you blink and support diopters better than soft lenses.

In a 2016 newsletter from ABC, Dr. William Faulkner of the Eye Institute in Cincinnati warns that 20.000 people annually contract *Pseudomonas aeruginosa*. Accord-

ing to the Center for the control and prevention of diseases, 50% of people do not remove contact lenses overnight.

Unfortunately, this practice leads to an increased risk of eye infections, which can lead to further infection and loss of vision. If not treated, one can lose one's sight.

Faulkner believes that people who sleep in lenses (even those called "overnight"), and who have a habit of smoking, are 10 times more likely to contract eye infections.

Almost every fifth of eye patients has complications due to the use of contact lenses, which include corneal injuries, corneal transplantation, and loss of vision.

However, in the literature we did not find data on the study of the immune system in patients who use soft contact lenses.

The aim of the research is to investigate the state of the immune system in patients who use soft contact lenses in various variants of normal microflora of the eye.

Materials and methods

The study included 72 patients who have used soft contact lenses for at least one year. All patients were divided into two groups: the 1st group included 32 patients who had a habit of smoking and/or sleeping in lenses; the 2nd group – 40 patients, non-smokers, using soft contact lenses. The study group included patients aged 21 to 60 years old, with an average age of 34±6.2 years.

Only patients who have used soft contact lenses for at least 1 year and had no history of infectious eye diseases during the last 6 months have been included in this

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study. Non-specific protective factors were investigated in all patients, the leading link of which are phagocytic cells, involved in the elimination of foreign antigens due to their intracellular enzyme systems. The patients were evaluated for the total number of leukocytes, relative and absolute number of lymphocytes, phenotypes of lymphocytes at presence of CD antigens on their surface (CD3, CD4, CD8, CD16, CD22, CD25, CD95); the levels of immunoglobulins of classes A, M, G, E, circulating immune complexes (CIC), hemolytic activity of the complement (CH 50), phagocytosis parameters of neutrophil granulocytes with latex, lysosomal cationic proteins (LCP) and NST spontaneous and stimulated tests were studied.

CIC was determined in serum of blood by spectrophotometric method for Dijon RN. The method is based on the ability of polyethylene glycol (PEG 6000) at low concentrations to precipitate immune complexes in serum. The concentration of the protein in the precipitate was determined by the level of the CIC. Measurements were made using a spectrophotometer at a wavelength of 450 nm; the levels of the CIC were expressed in units of optical density. Determination of complement hemolytic activity was performed by the method of 50% hemolysis in the modification of Reznikova L. C. The principle is based on the phenomenon of erythrocyte lysis in the presence of antierythrocytic antibodies and complement. To evaluate the complement activity, the hemolytic system was introduced into the serum. The detection of lysosomal cationic proteins (LCP) was performed by the cytochemical method according to MG Shubichik. Microscopy of the smears was performed under a microscope with an increase of x

90 in the immersion system, 100 neutrophils were counted, and% of NST-positive cells containing the dyformasan was determined. In the spontaneous NST test, the norm is 10%, in the stimulated – from 40%. In addition, the neutrophil activity index (IAN) was calculated according to the Astaldy-Berg formula.

The microbiological study was performed on all patients using soft contact lenses. Identification of bacteria was carried out according to generally accepted criteria. The culture of bacteria was grown on a liquid nutrient medium for 1-2 days at 37 °C to a concentration of 2 billion cells / ml.

All stages of the study were performed on the clinical basis of the medical diagnostic center "ASK-Health".

The research was conducted in compliance with all ethical and legal norms and protocols, in accordance with the legislation of Ukraine.

The results of the study were processed using the EXCEL and STATISTICA 6 applications.

Results and discussion

The lack of data on the state of immunity of patients using soft contact lenses in the literature was the basis for its study, the study may contribute to the resolution of the pathophysiologic formation of immunological disorders and as a consequence of infectious complications. The analysis of the state of the immune system provided for the determination of the total number of all lymphocytes and their varieties in peripheral blood in absolute and relative values (Table 1).

Table 1
The state of cellular immunity in patients who use soft contact lenses

Patients' groups	Indicators of the state of the immunity system							
	The number of lymphocytes, %	The absolute number of lymphocytes x10 ⁹ /L	CD3,%	CD3, x10 ⁹ /L	CD4,%	CD4, x10 ⁹ /L	CD8,%	CD8, x10 ⁹ /L
Comparison group	18-38	0.72-3.42	67-76	1.1-1.7	38-46	0.7-1.1	31-40	0.5-0.9
Ist group (smokers)	53.4± 2.45*	2.45± 0.24	75.7± 1.6	1.8± 0.25	38.1± 1.5	1.2± 0.20	30.9± 1.1	0.56± 0.04
IInd group	43.6± 3.35	2.31± 0.15	75.1± 1.25	1.73± 0.13	46.8± 1.3	1.0± 0.11	31.2± 2.1	0.81± 0.10

Notes: P<0.05 – differences in values when compared between groups.

The study found that the relative number of lymphocytes varies widely and tends to increase, on average, (44.73±5.1)%. However, patients with a smoking habit of the 1st group have a significantly higher incidence (p≤0.05) the reaction voltage from the lymphoid system, while the number of lymphocytes in such persons was (53.4±2.45)%. High indicators of the relative number of lymphocytes are also observed in the second group – (43.6±3.35)%. The increased relative numbers of lymphocytes in both groups of study, in our opinion, show a different pathophysiologic mechanism of the reaction of the lymphoid system. In group II, lymphocytes can reflect the expressed reactivity of the immune system on the pathological process, and in group I – indicates the presence of chronic inflammatory reaction in the body due to smoking.

Analyzing the absolute number of lymphocytes in the peripheral blood, we found that these indices are within the limits of normal physiological fluctuations and do not have a significant difference between the groups. The same tendency was observed in the study of the content of common T-lymphocytes, and their number in all

groups is within the limits of normal physiological parameters. There was a stable uniform content of total T-lymphocytes at the upper limit of the norm – (from 75.7±1.6% to 75.1±1.25%).

As a result of the study, the absolute number of CD-3 T lymphocytes in the 1st group was 1.82x10⁹/L, which is significantly higher (p≤0.05) than in the comparison group. The obtained results of the study suggest that the main population of common T-lymphocytes (CD3) is represented by T-helper. Indicators of the second group were significantly higher than normal, however, the difference between patients and both groups was not detected. The analysis of the relative level of T-helper cells – CD4 in the blood corresponds to the normal value in patients of group II and remains at the level of the upper limit of norm – (46.8±1.3)%. In patients of group I, the relative level of CD4 is at the lower limit of the norm and has no significant difference when compared with the control values. However, it should be noted that the absolute number of T-helper (CD4) in patients of group I deviates from the control values in both directions, as in the direction of decrease – 26.78% in comparison with

the norm, and in the direction of increasing their number – at 13.5% of cases. Such a situation may be due to the voltage of the T-system of immunity and the T-helper type 2 immune response at the onset of the disease, that is, in the process of prolonging the disease, the immune status of the organism is suppressed.

Relative content of T suppressors – CD8 did not have a significant difference between the groups.

The absolute number of T suppressors – CD8 in patients of Group I is at the lower limit of the norm – $0.56 \times 10^9/L$, while in the second group of patients the number of T suppressors – 1.2 times higher and reaches the upper limit of the norm – $0.81 \times 10^9/L$.

Consequently, despite the normal immunological performance in all groups, the latent immune suppression of the T-immune system is nevertheless traced in group I.

The abovementioned data finds its confirmation in the study of the dynamics of other indicators of immunity (Table 2). Absolute content of natural killers CD16 in patients of group I is significantly ($p \leq 0.05$) lower than in group II, although it corresponds to the norm – $0.31 \times 10^9/L$. That is, the content in the blood of natural killers, although consistent with the norm, similar to T-lymphocytes, in the 1st group, this limit is significantly lower.

Table 2
Some indicators of the state of immunity in patients who use soft contact lenses

Patients' groups	Indicators of the state of the immunity system								
	CD16, %	CD16, $\times 10^9/L$	CD20, %	CD20, $\times 10^9/L$	CD25, %	CD25, $\times 10^9/L$	CIC with 3.5 % PEG, un.	CD95, %	CD95, $\times 10^9/L$
Comparison group	10-19	0.2-0.4	11-16	0.2-0.4	10-18	0.54-1.16	10±2	5-20	0.1-0.5
Group I	18.26±1.68	0.31±0.14**	16.4±0.67	0.27±0.09**	20.7±0.16	0.32±0.05**	12.9±1.15	18.55±2.5*	0.33±0.01
Group II	17.4±0.08	0.45±0.01	14.0±0.01**	0.39±0.05	19.6±1.10	0.47±0.02	10.4±1.42**	13.6±0.14**	0.31±0.02

Notes: * – $p < 0.05$ – the differences are significant from the indicators of other age groups;

** – $p < 0.05$ – the differences are significant from the indicators of other age groups and comparison group.

When analyzing the absolute content of B-lymphocytes in the blood of patients in the studied groups, we found that their lowest rates were found in the group of smokers who use soft contact lenses.

Investigation of the content in the activator of expression of interleukin 2 (IL 2) – CD25 in absolute numbers has established a significant ($p < 0.05$) decrease in its content in both groups in comparison with the norm. Patients in group I with a habit of smoking, this figure was particularly low, but had a significant difference in comparison between the groups ($20.7 \pm 0.16\%$). Interleukin 2 is a mediator of inflammation and an activator of the synthesis of T-lymphocytes, on the one hand, and on the other – participates in the process of differentiation and maturation of T-cells. The inhibition of the IL-2 expression activator confirms our assumption of latent suppression of the T-immune system, especially in patients who have a smoking habit.

Except for the detected dynamics, the rates of circulating immune complexes in the studied patients, in which they exceeded the control values, and in smokers, this figure already ($12.9 \pm 1.15\%$).

In the study of apoptosis receptors – CD95, it was found that in absolute numbers in all groups their number was practically the same and did not have a significant difference.

The study of apoptosis receptors on CD95 lymphocytes did not reveal any disturbances in their composition. The number of lymphoid cells with apoptotic receptors, CD95 in both relative and absolute numbers, is within normal physiological oscillations, which suggests that apoptosis does not play a role in the violation of cellular immunity.

Analyzing the obtained data of the state of the T-system of immunity, it should be noted that when using soft contact lenses, there is dissociation of it in general, and in particular in its various links, as evidenced by the increase and decrease in the number of common T-lymphocytes, the activation of T-helper cells and de-

crease in the number of T-suppressors. The latter may be the pathophysiologic cause of autoimmunization of the patient's body and accumulation of the CIC.

Summarizing the analysis of the status of the B-immune system in patients under study, we can conclude that there is no significant change in this immune system. One can only note the increase in the absolute number of B-lymphocytes in smokers and the tendency to reduce the local immunity in this group.

Conclusions. Based on the obtained results, it can be concluded that in patients who use soft contact lenses and have a habit of smoking there is a decrease in the level of B-lymphocytes. However, despite the normal immunologic parameters of B-lymphocytes, in the 1st group as well as in the 2nd group there is a hidden inhibition of the T-immune system.

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