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STUDY OF THE COMPOSITION OF IMMUNOCOMPETENT CELLS AND HUMORAL FACTORS OF IMMUNITY AT NASOPHARYNGEAL CANCER IN CHILDREN ON THE BACKGROUND OF ANTIVIRAL THERAPY

***Karimova N. M., Polatova D. Sh., Rakhatullava D. T., Hayitov F. E., Nuriddinov K. R., Boboev M. M., Hayitova A. T. and Abdukhakimova M. I.**

Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan. Tashkent, Uzbekistan.

***Corresponding Author: Prof. Karimova N. M.**
Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan. Tashkent, Uzbekistan.

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SUMMARY

The aim of the study was to study the cellular and humoral parameters of immunity in children and adolescents after traditional therapy in combination with antiviral therapy. For this aim, 82 patients with nasopharyngeal tumors were examined, which were examined by various methods: clinical, laboratory, instrumental, immunological and virological methods. And also after examination and diagnosis, traditional therapy and antiviral therapy were prescribed. It was revealed that antiviral therapy, presented by acyclovir, favorably affects the state of cellular and humoral immunity, in connection with a significant suppression of viral load. Moreover, an improvement in the general condition is clinically noted, and in the future lengthening of the disease remission.

KEYWORDS: Nasopharyngeal cancer in children and adolescents, Epstein Barr virus, cellular and humoral immunity, antiviral therapy.

Topicality of the theme. Nasopharyngeal neoplasms are not often found in clinical practice, since the nasopharynx is less frequently affected than other parts of the pharynx.[1,4,6] A feature of childhood tumors is their predominant occurrence on the basis of developmental abnormalities or due to the presence in the child's body of undifferentiated embryonic primordia, which retained a great ability to grow. In the first case, angiomas, lymphangiomas, dermoids and other teratoid tumors can develop, in the second - ganglioneuromas, neuroblastomas, etc. with severe signs of true tumor.[2,5,8,9]

In malignant tumors, in most cases, complex therapy is carried out, the effectiveness of which depends on the prevalence of the tumor process, the histological structure and the individual nature of growth.[3,5,7,9] However, 5-year survival rates for malignant tumors of the nasopharynx vary greatly from different authors from 15 to 70%, which is due to their late detection and a tendency to dissemination and recurrence. Much attention has been paid in recent years to the relationship between nasopharyngeal cancer and the herpes-like Epstein-Barr virus (EBV). Therefore, the study of the mechanism of the occurrence of EBV - associated neoplasms, including nasopharyngeal cancer, is an important urgent problem in modern oncology.[8,9,12,14] Thus, the severity and variety of clinical manifestations, the lack of common ideas about the mechanism of occurrence and development, insufficient informational content of diagnostic tests for nasopharyngeal tumors in children and adolescents, determine the importance and relevance of this problem for oncology.

The aim of the study was to study the cellular and humoral parameters of immunity in children and adolescents after traditional therapy in combination with antiviral therapy.

MATERIAL AND RESEARCH METHODS

Our study is based on clinical observations of 82 patients with nasopharyngeal tumors in children and adolescents who were examined by various methods: clinical, laboratory, instrumental, immunological and virological methods. And also after examination and diagnosis, traditional therapy and antiviral therapy were prescribed. Children were in clinical conditions. Boys made up 75.0% of the examined, girls - 25.0%. The average age of patients was 14.5 ± 2.4 years. The most common nasopharyngeal tumor was found in children from 11 to 14 years old, which amounted to 38.8%, and in adolescence - 43.9%.

From the anamnesis it is known that in 80% of cases by nasopharyngeal tumors, the duration of the disease was

more than 10 months, in 21% of cases - up to 6 months, and in 5.2% of cases - up to 3 months.

The examinations were carried out in the following sequence: at the first stage, all patients underwent a finger examination, direct nasopharyngoscopy - a certain procedure for examining the nasopharynx with a mirror and a light source, a thorough medical history, examination and palpation of the neck, laryngoscopy, at the second stage to determine the volume, stage of the tumor and the choice of treatment method was performed fibrorinolaryngoscopy with biopsy, CT, MRI, ultrasound, general clinical examination, if necessary, consultation with an ophthalmologist, neurologist, neurosurgeon.

The history of the disease was studied in detail in all patients in order to identify the most common diagnostic and medical errors of doctors before admission to the clinic. To determine the histological affiliation of the tumor process in the nasopharynx, the stage, the degree of its spread to neighboring structures, and decisions on the choice of treatment tactics, both traditional and modern diagnostic methods were used. At the same time, ultrasound diagnostics were standard, in order to determine the prevalence of the tumor, the limits of the nasopharynx, the detection of locoregional metastases, the degree of invasion into neighboring structures, computed tomography and magnetic resonance imaging were performed. A valuable and important method for the diagnosis of nasopharyngeal tumors is endoscopic examination (fibrorinolaryngoscopy). Thus, epifaringoscopy was performed in 84% of cases on an OLYMPUS ENF TIPE 1T10 apparatus. With this study, you can not only examine the tumor in detail, but also perform a biopsy of the suspicious tissue site (take a piece of the tumor for examination). Fibroepypharyngoscopy was performed from two opposite sides: through the nasal cavity - anterior epipharyngoscopy, and through the oropharynx - posterior epipharyngoscopy. This examination method has always been accompanied by taking material for histological examination. For the study, pieces of tissue were taken from visible areas of pathological tissue. Also, a biopsy was taken at first glance of intact tissues near the tumor, in which malignant cells were detected by histological examination. On the basis of clinical and instrumental studies, a histological type of tumor was established in all patients and a final diagnosis was made.

Immunological studies were carried out at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan. The study of the cellular link of adaptive immunity includes the study of the expression of CD4 + on T-helper / inducers, CD8 + on T-cytotoxic lymphocytes, CD16 + on natural killer cells, CD20 + on mature B-lymphocytes, as well as activation markers CD38 + on T- and B-lymphocytes and CD95 + on T-lymphocytes by flow cytofluorescence.[2,8] The study of circulating immune complexes was carried out using the Nihol test systems on a Stat Fax 2008 analyzer. The control group is represented by the normative values of 29 children of the same age and gender.

After being diagnosed, the children underwent traditional therapy, which is represented by polychemotherapy without surgery. All children were examined for infection with the Epstein Barr virus (EBV) by PCR analysis to detect viral load and resolve the issue of antiviral therapy (AVT). In this regard, a group of children with EBV infection was isolated and they were prescribed acyclovir in tablets as an antiviral drug at a dose of 20 mg / kg body weight (but not more than 800 mg) 4 times / day with a course of treatment of 21 days.

The development of the obtained data was carried out on a personal computer using standard ("MS Excel-2015", "Statistica 2017") and specially developed software tools that ensure the effective application of mathematical logic and statistical analysis methods.

The results of the study. In the modern sense, the study of the state of the immune system is a set of laboratory parameters characterizing the quantitative and functional activity of the cells of the immune system at a given time. Evaluation of the immune status is carried out using an immunological laboratory examination - an immunogram. A blood immunogram does not selectively reflect the state of a pathologically altered organ or system, but it allows you to evaluate the immune system as a whole. The determination of the cellular composition of blood lymphocytes is called immunophenotyping of lymphocytes, which is the main component in assessing the immune status. Immunophenotyping is a characteristic of cells obtained using monoclonal antibodies, which makes it possible to judge their type and functional state by the presence of a particular set of cell markers. Immunophenotyping of lymphocytes consists in the detection of differentiation markers, or CD antigens, on their surface. CD antigens are antigens on the surface of cells, markers that distinguish some types of cells from others. The differentiations of these antigens are studied and standardized, they are assigned certain numbers. CDs can be recognized using appropriate monoclonal antibodies. Using fluorescently labeled monoclonal antibodies that bind to specific CDs, it is possible to calculate the content of lymphocytes belonging to different function or stage of development of lymphocyte subpopulations using flow cytometry.[8,12] Therefore, an immunogram is a study of the main indicators of the human immune system. Typically, the basic parameters of a person's immune defense are determined: the number and functional ability of white blood cells, their percentage; cellular immunity - the total number of T-lymphocytes and their population. First of all, these are the so-called immunodeficiencies, in which one or several parts of the immune system are affected.[5,9]

The functioning of the cellular link of adaptive immunity is provided by T-lymphocytes. It is known that T-lymphocytes recognize not “alien”, but altered “one's own” (immunogenic peptide in combination with HLA II molecules). T-helpers / inducers or CD3 + CD4 + T cells are lymphocytes that play a regulatory role in the immune response, literally "helping" the activation, reproduction and differentiation of other types of cells. According to the literature, it is known that the triggering and regulation of the effectiveness of the immune response is determined by the specific T-lymphocyte antigen, which allows the total number of T-lymphocytes to be identified.[9,11,14]

Depending on the clinical, virological and immunological results of the diagnosis and planned therapy, the children were divided into the following groups: group 1 consisted of children and adolescents with nasopharyngeal cancer with EBV infection who were prescribed only PCT as standard antitumor therapy; Group 2 consisted of children and adolescents with nasopharyngeal cancer with EBV infection who were prescribed PCT and antiviral therapy (AVT).

The results are presented in table 1, which shows the immunological results of children and adolescents with nasopharyngeal cancer with EBV infection with various treatment approaches.

The immunocompetent cells of adaptive immunity have been studied. The analysis showed that the content of the main markers of T-lymphocytes, CD3 + T-lymphocytes were significantly suppressed after PCT in children and adolescents. The results are presented in table 1.

The content of CD4 + T-lymphocytes was also significantly suppressed after PCT by almost 2 times. As you know, T-helpers are the main regulatory cells of the immune system. Moreover, in the 2nd group of patients where antiviral therapy with acyclovir was performed, a significant increase in T-helpers / inductors was observed. The CD4 + T-cell response is known to be an important cellular defense mechanism of a macroorganism, since CD4 + T-helpers stimulate antibody production by B-lymphocytes and activate CD8 + T-lymphocytes.[5,8] According to the literature, it is known that chemotherapy significantly suppresses cellular immunity and, against this background, increases the activity of viral infections.[4,9,10,11] And at the same time, the appointment of antiviral therapy in our case significantly reduced the viral load of EBV after the use of PCT.

Cytotoxic T-lymphocytes, or T-killers - immunocytes endowed with direct effector (damaging) functions. Often T-killers are called CD3 + CD8 + T-lymphocytes, because the CD8 coreceptor serves as a marker of this subpopulation. These cells recognize class I HLA complexes — a peptide on the surface of infected, mutant, or tumor cells.[9,11] With specific target recognition, T-killers secrete lymphotoxin (TNF-β), perforin and granzymes, which ensure apoptotic or necrotic death of compromised cells.[3,6] Defects of T-lymphocytes predispose to recurrent viral and fungal infections.[4,9] Analysis of the number of CD8 + T-lymphocytes in the peripheral blood showed that in groups of children after PCT there is a significant increase in T-cytotoxic lymphocytes. Moreover, in the group using antiviral therapy, this marker was slightly reduced. Although, no significant changes between groups of children were revealed, which is presented in the table. This range of values is acceptable, because T-cytotoxic lymphocytes are highly adaptive cells that play an important role in the pathogenesis of many pathological conditions. Their biological function is the removal of mutant and altered cells from the body.[6,12]

Table: Immunological parameters in cancer of the nasopharynx on the background of anticancer and antiviral therapy.

Indicator	Normative values	The 1st group - after a course of chemotherapy	The 2nd group - after a course of PCT + AVT
CD3+, %	59,5±1,32	38,6±1,21*	49,94±1,5*^
CD4+, %	34,8±1,25	24,7±0,74*	28,74±1,66^
CD8+, %	18,6±0,65	25±2,24*	24±1,64*
CD16+, %	18,6±1,04	24,2±1,5*	19,2±0,81^
CD20+, %	19,8±0,7	16,4±1,02*	19,9±0,55^
CD38+, %	22,7±0,85	29,83±1,92*	21,8±1,6^
CD95+, %	22,5±1,28	28,69±2,24*	21,9±0,94^
IRI (CD4+/CD8+)	1,5±0,05	0,82±0,11*	1,3±0,21^
IgG, g/l	12,80±3,75	9,5±2,51	10,24±0,56
IgA, g/l	1,24±0,60	3,02±0,52*	2,08±0,52^
IgM, g/l	1,10±2,4	2,1±0,41	1,59±0,42
CIC (IgM, c.u.)	8,52±1,35	48,35±2,45*	27,40±1,35*^
CIC (IgG, c.u.)	14,58±1,20	39,6±2,99*	22,84±4,41*^

Note: * - significance of differences p <0.05 with control; ^ - significance of differences p <0.05 between the studied groups.

It has been established that the immunoregulatory index (IRI), which is the ratio of the values of CD4 + / CD8 +, is essential in the diagnosis of immunity. It is known from the literature that, in normal cases, IRI in healthy individuals is on average 1.52 ± 0.02.[5,14] According to our data, it is clear that the maximum value of IRI was observed in the group of children after the appointment of antiviral therapy. moreover, in the group of children after PCT without the use of acyclovir, IRI suppression is almost 2 times.

From the available literature data, it is clear that the study of activation markers of lymphocytes, especially in proliferative processes, is of great scientific and practical importance, because the analysis of activation markers of lymphocytes allows us to study the activation, proliferation, and apoptosis of immunocompetent cells and characterizes cell cycles associated with these processes.[7,13] Analysis of CD95 + on lymphocytes showed that in groups of children after PCT there was a significant increase in the activation of apoptosis, and in the group of children using acyclovir, there was a slight decrease in apoptosis and its approximation to the normal value. Again, such fluctuations are considered absolutely solvable, since the main function of these molecules is the activation on the one hand of proliferative processes of immunocompetent cells, and on the other hand, pronounced apoptosis of cells that have performed their direct function in the body. For example, it was found that an increase in the expression of CD95 + receptors on lymphocytes indicates an excessive and ineffective process of stimulating blood lymphocytes, which indicates a large-scale apoptotic pathway for the death of lymphocytes.[6] The same situation is observed in the expression of CD38 +. After traditional PCT, the expression of CD38 + increased significantly. And in the group of children using antiviral therapy, there is a significant suppression of the expression of this marker, which indicates a suppression of the viral load of EBV.

From humoral factors of immunity, we have analyzed circulating immune complexes (CIC) of large and small sizes. CIC3% of large values formed with an excess of antibodies, although they can bind complement, but are large, insoluble, quickly phagocytized and have low pathogenicity.[9] The greatest pathological potential is possessed by soluble immune complexes of small sizes, which were formed with an excess of antigen.[4,9] A high level of CIC can be caused not only by activation of the immune response, but also by suppression of the mechanisms of their elimination.[9] The latter, apparently, is associated with a weakening of the function of the cells of the monocyte-macrophage system - cells that absorb and disintegrate immune complexes. CIC3% quickly disintegrate in the body, so they have no pathological potential. The analysis showed that the average values of CIC3% and CIC4% were significantly increased. It was revealed that the range of individual values ranges from 4 to 38 for large CICs, and from 5 to 68 for small CICs. As shown, the values of circulating immune complexes were also reduced by almost 2 times in the group of children using antiviral therapy. Moreover, the content of the main immunoglobulins did not differ significantly in all the examined groups. It should be noted that immunoglobulin A was significantly increased in the group of children after PCT without the use of antiviral therapy.

Thus, we have shown that antiviral therapy, represented by acyclovir, favorably affects the state of cellular and humoral immunity, in connection with a significant suppression of viral load. Moreover, an improvement in the general condition is clinically noted, and in the future lengthening of the disease remission.

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