UDC: 616.327.2-006.6-08-059.849

N.M. KARIMOVA[1], *D.Sh. POLATOVA*[1], *D.T. RAKHMATULLAEV*[1], *K.R. NURIDLINOV*[1], *M.M. BOBOEV*[1], *A.T. KHAITOVA*[1], *M.I. ABDUKHAKIMOVA*[1]

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

Clinical and immunological aspects of nasopharyngeal cavity malignant processes in children and adolescents

Relevance: *Nasopharyngeal cancer is relatively rare in children. Therefore, its treatment results in children are understudied in the literature.*

The purpose of this study *was to analyze the clinical and immunological data of children with nasopharyngeal cancer to determine cellular immunity factors of this disease in children and adolescents.*

Results: *The detected characteristic changes in the cellular component of adaptive immunity included the suppression of CD3+T-lymphocytes, CD4+T-helpers / inducers, the immunoregulatory index, and the increased number of CD8+T-cytotoxic lymphocytes, CD16 + NKC-cells, as well as the expression of CD38+ and CD95+ on lymphocytes.*

Conclusions: *The research in this area shall be continued to accumulate knowledge about the immunopathogenesis of tumor processes in the nasopharynx. The revealed changes in the cellular component of the immunity necessitate further research to address the issues of immunodiagnostics and immunotherapy.*

Keywords: *nasopharyngeal tumors in children, immunocompetent cells, cellular immune response, T and B lymphocytes, malignant process.*

Introduction: Malignant nasopharyngeal tumors in children comprise from 1 to 3% of the total number of malignant neoplasms and from 10 to 12% of head and neck tumors in children [1-4]. This pathology is found everywhere, but the greatest prevalence is observed in residents of Southeast Asia, where it accounts for about 10-20% of all malignant tumors in children. The anatomical and topographic features of the nasopharynx determine the options for the clinical course of the disease, such as the spread of the tumor to nearby structures with the development of the corresponding symptoms. The pronounced biological activity of low-grade nasopharyngeal cancer in children explains the aggressiveness of the course with the development of both regional and distant metastases. Metastatic lesion of regional lymph nodes with undifferentiated nasopharyngeal cancer is observed in more than 90% of cases with a predominant localization of the tumor in the upper parts of the neck. Distant metastases can develop in the lungs, bones, soft tissues, liver and other organs [1, 4-6]. The WHO international histological classification defines three possible variants of nasopharyngeal cancer: keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma, and undifferentiated [2, 7]. The term "lymphoepithelial cancer" is often used for the last two options. Lymphoepithelial cancer occupies a major place among malignant epithelial tumors of the nasopharynx and occurs in 97% of cases [2, 3, 8, 9].

The diagnosis of nasopharyngeal tumors in the early stages of development is very difficult. The nasopharynx is considered a "blind" area, extremely difficult for primary diagnosis, viewing, and manipulation. Besides, inflammatory processes in the nasopharynx (adenoiditis, rhinopharyngitis) that are often found in children have similar symptoms with malignant neoplasms of the nasopharynx. Also, a large number of lymphadenopathies of various etiologies with localization on the neck leads to even greater difficulties in diagnosis. A large percentage of children entering specialized treatment at advanced stages of the disease, stage III-IV (about 70-80%), is associated with diagnostic difficulties [1, 6, 10].

Chemoradiation therapy is the main treatment for malignant nasopharyngeal tumors in children. According to published data for 2000-2010, the survival rate of children with nasopharyngeal cancer does not exceed 30% [3, 4]. However, due to the relatively low frequency of occurrence of this pathology in the territory of the Russian Federation, the schemes and results of treatment of this pathology are not adequately covered in modern Russian literature. As is known, standard treatment regimens (chemotherapy) are mainly used, without the appointment of etiopathogenetic therapy.

Imbalance in the immune system is considered as an important mechanism for the development and progression of many immunopathological processes, including malignant ones [1, 6, 7, 11]. Therefore, the study and evaluation of the main parameters of the immune response against the background of the development and course of malignant processes, including nasopharyngeal cancer in childhood, is an extremely important problem due

to the lack of full knowledge in the etiology and immunopathogenesis of the development of the disease and standard approaches to immunodiagnostics and treatment. The study of the main parameters of immunity responsible for the antitumor immune response remains one of the urgent tasks of oncology, especially in childhood and adolescence, due to the unsatisfactory results of the treatment of nasopharyngeal cancer [6, 10].

It is believed that childhood tumors arise mainly due to developmental abnormalities or the presence in the child's body of undifferentiated embryonic primordia retaining a great ability to grow. However, we cannot deny the infectious theory of the etiopathogenesis of nasopharyngeal cancer in children, the more so since there is evidence of the role of the Epstein-Barr virus in the formation and development of this pathology [4, 8]. Neuroblastoma, lymphosarcoma, and undifferentiated cancer of the nasopharynx (lymphatic epithelioma) are often found among malignant tumors of the nasopharynx [9].

The aggressive course of the disease with damage to the lymph nodes, lungs, liver, and bones is of great scientific and practical interest in the study of nasopharyngeal cancer in children. 70-75% of patients already have metastases in the cervical lymph nodes at the time of diagnosis. It is worth noting that nasopharyngeal cancer is more common in children aged 10-15 years and is more often in boys [2, 7].

Based on the above, the purpose of this study was to analyze the main clinical and immunological factors in nasopharyngeal cancer in children and adolescents.

Material and Methods: The main group consisted of 32 children and adolescents with nasopharyngeal tumors who were diagnosed and then combined treatment at the National Medical Research Center for Oncology from 2016-2018. Among them, boys were 23 (72%), and girls - 9 (28%). The average age was 14.6±2.5 years. A nasopharyngeal tumor was recorded in children aged 11 to 14 years (38% of cases) and aged 14 to 18 years (62% of cases). The duration of the disease before diagnosis, according to the anamnesis, averaged 4.2±2.8 months. All children were examined comprehensively in the hospital: direct nasopharyngoscopy, a thorough medical history, examination and palpation of the lymph nodes of the neck, laryngoscopy, fibrorinolaryngoscopy with biopsy, CT, MRI, ultrasound, general clinical examination. All patients underwent cytological and histological examinations and were diagnosed with stage III or IV nasopharyngeal cancer. The study of the cellular link of adaptive immunity included 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 the method of indirect rosette formation according to the methodological recommendations of M.V. Zalyalieva [2].

The control group included 29 children of the same age and gender with normative values of interest.

An assessment was made of the cellular immunity in children and adolescents with nasopharyngeal cancer, which were in stationary conditions. Blood for immunological studies was taken after diagnosis and before the start of anticancer chemotherapy.

When carrying out a statistical analysis of the data presented in work, the results of the study were entered into databases prepared in the Microsoft Excel XP program. Numerical (continuous) values were presented as arithmetic means and mean errors (M±m). A comparison of quantitative characteristics was carried out using Student's criterion and for continuous variables using the paired Student's criterion. $P < 0.05$ was taken as a boundary comparative criterion of statistical significance.

Results and Discussion: In recent years, more and more data have been accumulating on the importance of immunological mechanisms in the development of a particular pathology, in particular with nasopharyngeal cancer.

According to morphological variants, the tumors were divided as follows: 70.6% of cases - undifferentiated cancer, 23.5% - non-keratinizing squamous cell carcinoma, 5.9% - highly differentiated transitional cell cancer.

According to clinical signs, a typical symptom complex for this pathology was noted: nasal congestion, a conglomerate of lymph nodes in the neck. In patients with a verified stage IV of the disease, the following was determined: in one patient, a locally distributed process with germination in the cranial cavity and damage to the temporal lobe of the brain, and in one patient, damage to the lymph nodes of the supraclavicular region and mediastinum with metastatic brain damage.

On average, the leukocytes quantity in peripheral blood was slightly higher than the value of the control group, but no significant difference was found. Reliable suppression of the total number of lymphocytes in the group of children with nasopharyngeal cancer was revealed compared with the standard values ($p<0.05$). It is known that the degree of surface expression of CD3+ receptors on the T-lymphocyte membrane reflects its transmissible function and allows the total number of T-lymphocytes to be identified [3, 10]. Thus, an analysis of the immunophenotype of T-lymphocytes in children with nasopharyngeal cancer revealed a significant decrease in the expression of CD3+ on T-lymphocytes (relative number) and the absolute value in comparison with the values of the control group ($p<0.05$). The decrease in the total pool of T-lymphocytes (CD3+) could be mainly due to the suppression of the number of T-lymphocytes expressing the CD4 + marker [2, 4]. A significant decrease in the expression of CD4+ on T-lymphocytes compared with the values of the control group ($p<0.05$) was recorded in children with nasopharyngeal cancer. It is noted that both the relative and absolute contents of CD4+ T-helpers/in-

ducers in the group of children with nasopharyngeal cancer were significantly suppressed (p <0.05). So, the number of CD4+ T-helpers/inducers averaged 25.9±2.4% in children with nasopharyngeal cancer, while in the control group, this indicator was 34.6±1.3%.

Cytotoxic CD8+ T-lymphocytes play a known important role in the pathogenesis of tumor diseases [2, 4, 5, 10]. CD8+ T-cytotoxic lymphocytes play a major role in the pathogenesis of viral and tumor diseases. On the one hand, they can cause the death of infected cells expressing the corresponding peptides represented by MHC 1 class molecules, and on the other hand, they can secrete antiviral and antitumor factors, such as anti-inflammatory cytokines - IFN-α, TNF-α and many others [10, 12]. Analysis of the content of CD8+ on T-cytotoxic lymphocytes in children with nasopharyngeal cancer revealed a significant increase in the relative content of CD8+T-cytotoxic lymphocytes when compared with the control group (27.9±1.2% vs. 18.6±0.59%, p<0.05).

Immunoregulatory index (IRI), which shows the ratio of the number of CD4+ T-helpers/inductors to the number of CD8+ T-lymphocytes, is of significant importance in tumor processes. In practically healthy children, IRI averages 1.4±0.03. The suppression of CD4+T-helpers/inducers with an increase in the number of CD8+ T-lymphocytes leads to a decrease in IRI in the group of children with nasopharyngeal cancer. So, IRI was 1.12±0.05 (p<0.05) in children of the main group. The decrease in IRI is an important criterion of the state of the T-cell immunodeficiency in this pathology.

Further, natural killer cells (NK cells) were studied, which are the third population of lymphocytes that support the maintenance of genetic homeostasis and which are phenotypically and functionally significantly different from T and B lymphocytes. NK cells belong to the category of the main effectors of natural or innate immunity, which can lyse target cells or carry out antibody-dependent cellular cytotoxicity. NK cells are involved in both antiviral, antibacterial, antiprotozoal protection, and antitumor immunity. They perform the functions of the first line of defense before immune T-lymphocytes and specific antibodies arise [6, 7]. Analysis of the NK cells subpopulation by membrane immunophenotype - CD16 revealed a significant increase in the relative number of CD16+ NK cells in the main group of children with nasopharyngeal cancer in comparison to the control group: 22.8±1.22% vs 18.6±0.9%.

B-lymphocytes, along with T-lymphocytes, are the main effectors of immunity. The function of B-lymphocytes in the fight against infection in the body is the production of antibodies. A change in the expression of surface receptors of B-lymphocytes indicates their active participation in the antiviral response [4, 9]. The content of B-lymphocytes by the expression of CD20+ receptors involved in the activation of B-lymphocytes was studied, on the basis of which it was found that there was a significant increase in the main group of children in comparison with the values of the control group (22.9±1.2% vs 18.4±0.58%, p <0.05). The revealed increased expression of CD20+ B-lymphocytes is an interesting and important topic for continuing research in this area.

The data on the activation markers of peripheral blood lymphocytes in the literature is ambiguous and insufficient, which may be due to insufficient knowledge of immunopathogenesis data for various variants of the course of nasopharyngeal cancer, especially when infected with herpes infections in a highly endemic region. In this regard, it was interesting to analyze the expression of activation markers of lymphocytes in nasopharyngeal cancer in children. The study of activation markers of lymphocytes has studied just recently, and we could find a limited number of works devoted to the functional activity of activation markers of lymphocytes, in particular with nasopharyngeal cancer in children and adolescents. The available literature data shows that the study of activation markers of lymphocytes has important scientific and practical value, especially for tumors and infectious diseases. An analysis of activation markers of lymphocytes allows one to study the processes of activation, proliferation, differentiation, and apoptosis of immunocompetent cells and characterizes cell cycles associated with these processes [10, 12]. CD38+ is an activation marker represented by a transmembrane glycoprotein, which is considered as a multifunctional protein. In turn, it is an ectoenzyme that catalyzes the synthesis and hydrolysis of CDP-ribose. The enzymatic functions of CD38+ provide its main immunoregulatory role; this is the binding of various agents to this receptor, which enhances the synthesis of cytokines, activation of kinases, and protein phosphorylation [7]. In theory, CD38+ is a precursor to plasma cells. It is expressed on immature T-lymphocytes and B-lymphocytes, activated T-lymphocytes, and plasma cells. An analysis of the expression of CD38+ on lymphocytes revealed a significant increase in this marker in the main group of children compared with the control group (26.8±1.4% vs. 22.6±0.8%, p<0.05). Therefore, an increase in the expression of CD38+ activation markers indicates the activation of cellular immunity with the presence of immature forms of lymphocytes. Of course, research in this area must be continued.

There is information in the literature on the role of APO-1/Fas (CD95+) receptors in the process of apoptosis, and its degree is a reflection of the level of lymphocyte apoptosis [5, 11]. An increase in the expression of CD95+ receptors on lymphocytes indicates an excessive and ineffective process of stimulating blood lymphocytes, which indicates an apoptotic pathway for the death of lymphocytes [2, 4]. Increased expression of CD95+ on peripheral blood lymphocytes was detected in patients with nasopharyngeal cancer (26.2±1.5% vs. 21.2±0.45% normal). Increased apoptosis of lymphocytes may explain the presence of T-cell immunode-

ficiency. Apparently, excessive apoptosis in the tumor process, in combination with deep T-cell immunodeficiency contributes to the progression of the disease.

Thus, the characteristic changes in the cellular link of adaptive immunity, which were manifested by the suppression of CD3+ T-lymphocytes, CD4+ T-helpers/inducers, immunoregulatory index, increase in the number of CD8+ T-cytotoxic lymphocytes, CD16+ NK cell, as well as increased expression CD38+ and CD95+ on lymphocytes are observed in nasopharyngeal cancer in children and adolescents. Research in this area is ongoing; it is necessary to accumulate knowledge in the field of immunopathogenesis of nasopharyngeal cancer. Given the identified changes in the state of cellular immunity, further studies are necessary to address the issues of immunodiagnostics and immunotherapy.

References:

1. Davydov M. I., Axel E. M. Statistika zlokachestvennykh novoobrazovaniy v Rossii i stranakh SNG v 2007 g. (Statistics of malignant neoplasms in Russia and the CIS countries in 2007) // Vestnik RONTS (Bulletin of the Russian Cancer Research Center). – 2009. – Vol. 20, № 3, Annex 1. – P.1–156 [in Russian];

2. Zalyaliyeva M.V. Sposoby opredeleniya subpopulyatsiy limfotsitov (Methods for determining subpopulations of lymphocytes). №1 DP. 20000774 D/P МКП 6601 № 33/48 26.02.2001 [in Russian];

3. Kamalov Z.S., Aripova T.U., Madzhidov A.V. Funktsional'naya aktivnost' EK i produktsiya IL-1 i IL-2 u zhiteley Uzbekistana (Functional activity of EC and production of IL-1 and IL-2 among Uzbekistan population) // Immunologiya (Immunology). – 2000. – №6. – P. 51–54 [in Russian];

4. Korolenkova L.I., Stepanova Ye.V. Molekulyarno-biologicheskiye markery proliferatsii i apoptoza, kak faktory progressii tservikal'nykh intraepitelial'nykh neoplaziy i raka sheyki matki (Molecular biological markers of proliferation and apoptosis as factors in the progression of cervical intraepithelial neoplasia and cervical cancer) // Rossiyskiy bioterapevticheskiy zhurnal (Russian Biotherapeutic Journal). – 2010. – Vol. 9, № 4. – P.11–16 [in Russian];

5. Khaitov R. M., Pinegin B.V. Otsenka immunnogo statusa cheloveka v norme i pri patologii (Assessment of the human immune status in normal and pathological conditions) // Immunologiya (Immunology). – 2001. – № 4. – P. 4–6 [in Russian];

6. Romagnani S. Regulation of the T cell response // Clin. Exp. Allergy. – 2006. – Vol. 36. – P. 1357–1366;

7. Pinegin B.V., Khaitov R.M. Immunodiagnostika zabolevaniy, svyazannykh s narusheniyem immuniteta (Immunodiagnosis of diseases associated with impaired immunity) // Gematologiya i transfuziologiya (Hematology and transfusiology). – 1997. – Vol. 42, №2. – P. 40–44 [in Russian];

8. Khaitov R.M., Pinegin B.V., Yarilin A.A. Rukovodstvo po klinicheskoy immunologii. Diagnostika zabolevaniy immunnoy sistemy: rukovodstvo dlya vrachey (Guidelines for Clinical Immunology. Diagnostics of immune system disorders: Manual for Doctors). – M.: GEOTAR-Media, 2009. – 352 p. [in Russian];

9. Antony P., Restifo N. CD4+ CD25+ T Regulatory Cell, Immunotherapy of Cancer, and Interleukin-2 // J. Immunother. – 2005. – V.28. – P.120–128;

10. Ginadi L., Farahat N., Matutes E. et al. Differential expression of T-cell antigens in normal peripheral blood lymphocytes : a quantitative analysis by flow cytometry // J. Clin. Pathol. – 1996. – Vol. 49, № 1. – P. 539–544;

11. Khaitov R.M., Pinegin B.V. Vtorichnyye immunodefitsity: klinika, diagnostika, lecheniye (Secondary immunodeficiencies: clinic, diagnosis, treatment) // Immunologiya (Immunology) .– 1999. – №1. – P. 14–17 [in Russian];

12. Merser J.C., Ragin M.J., August A. Natural killer T-cells: rapid responders controlling immunity and disease // Int. J. Biochem. & Cell Biology. – 2005. – № 37. – P. 1337–1343.