*ejpmr, 2018,5(9), 26-30*

**SJIF Impact Factor 4.897**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# STUDY OF THE STATE OF THE IMMUNORAACTIVITY OF THE ORGANISM OF CHILDREN WITH A NASOPHARYNGEAL CANCER

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**Article Received on 22/06/2018** **Article Revised on 12/07/2018** **Article Accepted on 01/08/2018**

## ABSTRACT

The purpose of this work was to study the state of immunoreactivity in children with nasopharyngeal cancer. Proceeding from the received results it is visible, that at a cancer of a nasopharynx at children the expressed unbalance cellular and humoral parts of immune system is observed. Imbalance in the cellular immunity unit was expressed in the suppression of the immunoregulatory index due to a decrease in the number of T-helpers / inducers and an increase in T-cytotoxic lymphocytes. Circulating immune complexes of large and small values were increased. Consequently, the role of immunological factors in the formation and flow of nasopharyngeal cancer in children is indicated by various changes in the state of immunoreactivity. Obviously, the T-cell response is much weaker and is directed against a smaller number of tumor epitopes, which suggests that clonal depletion of T-lymphocytes is possible against the background of activation of some humoral immunity factors.

**KEYWORDS:** Nasopharyngeal cancer in children, adaptive immune response, lymphocytes, cellular immunity factors, immunoglobulins.

## INTRODUCTION

It is known that imbalance in the immunity system is considered as an important mechanism for the formation, development and progression of many immunopathological processes, including malignant ones.[5,7] In this regard, 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 a very important problem due to the small number of studies in this field. The study of the main immunity parameters responsible for the antitumoral immune response remains one of the urgent problems of oncology, especially in childhood and adolescence, which is also caused by unsatisfactory results of therapy for nasopharyngeal cancer.[6,9] There are data that malignant nasopharyngeal tumors in children constitute 1-3% of all malignant tumors of childhood and 10-12% of head and neck tumors.[8,10] It is also known that an important role in the formation and development of this pathology is played by the Epstein-Barr virus.[5] Neoplasms of the nasopharynx occupy the second place in the frequency of lesion among tumors of the upper respiratory tract, accounting for about 25% of all tumoral diseases of the ENT organs, meeting from 0.4 to 3% among all localizations.[9,10] According to the world literature, the incidence of nasopharyngeal cancer (NC) is 0.1-3% among all malignant tumors, up to 2% among head and neck cancers and up to 50% among malignant lesions of the pharynx.[12]

Malignant tumors in children are few in the general structure of neoplasms. According to the International Agency for Research on Cancer (IARC), the incidence of malignant tumors in children is approximately 2-3% to 7-8% of all cancers.[6,12] It is noted that boys suffer from this pathology 2 times more often than girls. Great scientific and practical interest in the study of nasopharyngeal cancer in childhood causes the presence of an aggressive course of the disease with the involvement of lymph nodes, lungs, liver and bone. At 70-75% of patients at the time of diagnosis, there are already metastases in the cervical lymph nodes. At the same time, 95% of patients enter the clinic with a common tumor process in stages III-IV of the disease.[7,12] It should be noted that nasopharyngeal cancer is most often detected in children aged 10-15 years.[10,12] However, despite the significant deepening in the last decade of ideas about the etiology and immunopathogenesis of malignant processes, especially in childhood, many questions concerning mechanisms of development of the pathological process and its progress remain open. In some children, the swelling of the nasopharynx can proceed under the guise of an acute respiratory disease. In other cases, against the background of apparent well-being, there is difficulty in

nasal breathing, discharge from the nose, and nasal voices. The leading symptom of nasopharyngeal cancer is a disorder of nasal breathing.[14]

The aim of this work was to study the state of cellular and humoral factors of the adaptive immune response in nasopharyngeal cancer in childhood.

## MATERIAL AND METHODS

The survey included 36 children and adolescents with nasopharyngeal tumors who were on treatment at the Russian Cancer Research Center from 2015-2018. Among the examined boys were (27) 75.0%, and girls - (9) 25.0%. The average age of children and adolescents was 14.9 ± 2.4 years. In children aged 11 to 15 years, the nasopharyngeal tumor was found in 38.8% of cases, and was most often detected in adolescence - in 43.9% of cases. From the history of 80% of children the duration of the disease was more than 10 months, 14% - up to 6 months and 8% - up to 3 months. All the children were examined comprehensively. Finger examination was performed, followed by direct nasopharyngoscopy, careful collection of anamnesis, examination and palpation of the lymph nodes of the neck, laryngoscopy, then performed instrumental studies - fibrolaryngarinography with biopsy, CT, MRI, ultrasound, general clinical examination, consultation of the oculist, neurologist, neurosurgeon. All patients underwent cytological and histological methods of investigation.

Immunological studies were carried out in all the examined children with the study of the state of the cell link of adaptive immunity, which includes the study of CD4 + expression on T helper / inducers, CD8 + on T-cytotoxic lymphocytes, CD16 + on natural killers, CD20 + on mature B lymphocytes, and activation markers CD38 + on T- and B-lymphocytes and CD95 + on T-lymphocytes by indirect rosette method according to the methodical recommendations of M.V. Zalyalieva.[2] The control group consisted of 29 healthy children of the same age and sex. The humoral link of immunity was evaluated using the Vector-Best test systems, (Russia), using the enzyme immunoassay (ELISA). The following parameters were evaluated: IgG, IgA, IgM, CIC3%, and CIC4% (circulating immune complexes). In the statistical analysis of the data presented in the study, the results of the study were entered into databases prepared in the Microsoft Excel XP program. Numerical (continuous) values were presented as mean arithmetic mean values and mean error (M±m). A comparison of the quantitative traits was carried out with the help of the Student's test, for continuous variables - the paired Student test. As a boundary comparative criterion for the statistical significance of reliability, $p < 0.05$ was assumed.

## RESULTS AND DISCUSSION

As is known, in recent years more and more data have accumulated on the importance of immunological mechanisms in the development of a particular pathology, including in malignant processes, especially in childhood. We assessed the state of cellular parameters of adaptive immunity in children and adolescents with patients with nasopharyngeal cancer who were on inpatient treatment in the children's oncology unit of the RSSPMCO&R MHRUz. Blood for immunological studies was taken after the diagnosis and before the beginning of antitumor therapy. According to the results obtained, the content of leukocytes was slightly increased in comparison with the values of the control group, there was no significant difference ($p > 0.05$). The study of the relative content of lymphocytes showed the presence of a reliable suppression of the total number of lymphocytes in the group of children with nasopharyngeal cancer when compared with the control values ($p < 0.05$). The phenotypic markers of T-lymphocytes include CD3 +, CD4 +, CD8 + receptors. It is shown that the initiation and regulation of the effectiveness of the immune response is largely determined by the specific antigen of T lymphocytes. Responsible for this function are antigen-recognizing receptors - TCR. It is known that the degree of surface expression of CD3 + receptors on the T-lymphocyte membrane reflects its transmissive function and allows the total number of T-lymphocytes to be identified.[2,6] Analysis of the immunophenotype of T-lymphocytes in children with nasopharyngeal cancer showed the presence of a significant suppression of CD3 + expression on T-lymphocytes and its absolute value in comparison with the values of the control group ($p < 0.05$). Obviously, a decrease in the total pool of T-lymphocytes (CD3 +) is mainly due to suppression of the number of T-lymphocytes expressing the CD4 + marker.

As is known, CD4 + T-cell response to viral proteins is an important mechanism for protecting the macroorganism, since CD4 + T-helpers stimulate the production of antibodies by B lymphocytes and activate CD8 + T lymphocytes specific for virus-infected cells.[5,9,10,12] Thus, in children with nasopharyngeal cancer, a significant inhibition of CD4 + expression on T-lymphocytes was observed in comparison with the values of the control group ($p < 0.05$). It is noted that both the relative and absolute content of CD4 + T-helper / inducers in the group of children with nasopharyngeal cancer was significantly suppressed ($p < 0.05$). Thus, in children with nasopharyngeal cancer the number of CD4 + T-helper / inducers was 26.9 ± 2.1%, while in the control group - 34.6 ± 1.28%. It is established that CD4 + T-helpers / inducers are divided into two types of helper lymphocytes in functional respect: the so-called T helper type 1 (TX1) and the second type (TX2). It was shown that TX1 produced cytokines of the cellular immune response, and TX2 - a humoral immune response.[12]

It is known that cytotoxic CD8 + T-lymphocytes play an important role in the pathogenesis of tumor diseases.[8] The function of these cells is the recognition of antigens

on the cell surface in complex with molecules of MHC class 1. Since they are present on almost all nuclear cells of the body, any cell carrying MHC class 1 molecules in combination with an antigenic peptide can activate a clone of cytotoxic T lymphocytes. The biological role of this activation is the removal of mutant or virus-infected cells.[10,12] CD8 + T-cytochrome lymphocytes play a major role in the pathogenesis of viral and neoplastic diseases, which on the one hand are capable of causing the death of infected cells expressing the corresponding peptides presented by MHC class I molecules, and on the other hand, the ability to secrete antiviral and antitumor factors, such as proinflammatory cytokines - IFN-α, TNF-α and many others).[7,10] Analysis of CD8 + T-cytotoxic lymphocytes in children with nasopharyngeal cancer showed a significant increase in the relative content of CD8 + T-cytotoxic lymphocytes when compared with the control group (26.6 ± 1.2% in patients, in the control group - 18 , 3 ± 0.58%, $p < 0.05$). According to the literature, it is known that the Epstein-Barr virus can play an important role in the etiopathogenesis of nasopharyngeal cancer, which can be detected by specific cytotoxic T-lymphocytes, which have a damaging effect.[9] However, it is shown that the virus is able to persist even in the presence of CD8 + T-cytotoxic cells, which becomes the main mechanism of disease progression.[14]

The immunoregulatory index (IRI), which is the ratio of the number of CD4 + T-helper / inducers to the number of CD8 + T-lymphocytes, is of significant importance in tumor processes. In the norm of IRI, in practically healthy children, an average of 1.4 ± 0.03. Obviously, suppression of CD4 + T-helper / inducers against the background of an increase in the number of CD8 + T-lymphocytes leads to a decrease in IRI in the group of children with nasopharyngeal cancer, which was 1.02 ± 0.03 ($p < 0.05$). Consequently, the reduction of IRI is an important criterion for the depth of the T-cell immunodeficiency state in this pathology.

Natural killer cells (NKC) are the third population of lymphocytes providing maintenance of genetic homeostasis, which phenotypically and functionally differ significantly from T and B lymphocytes. The EBC are classified as the main effectors of natural or innate immunity, which are capable of lysing target cells or carrying out antibody-dependent cellular cytotoxicity. ECC are involved both in antiviral, antibacterial, antiprotozoal protection, and in antitumor immunity. It is their inherent performance of the functions of the first line of defense before the emergence of immune T-lymphocytes and specific antibodies.[7,12]

We studied the subpopulation of NKC on the membrane immunophenotype - CD16 +. A significant increase in the relative number of CD16 + NKC in the main group of children was revealed compared with the control group. So, in the group of sick children the number of CD16 + NKC was 23.2 ± 1.1%, while in the control group this figure was 18.2 ± 0.8%.

It is shown that along with T-lymphocytes, B-lymphocytes are the main effectors of immunity. The function of B-lymphocytes in the struggle of the organism with infection consists in the production of antibodies. It was shown that changes in the expression of surface B-lymphocyte receptors indicate their active participation in the antiviral response.[5] The change in the expression of surface B-lymphocyte receptors indicates their active participation in the antitumor and antiviral immune response.[4,7,13,15] An auxiliary component of B-lymphocytes is a complex of co-receptor molecules, including CD19 +, CD20 +, CD21 +, CD72 +.[8]

We studied the content of B-lymphocytes from the expression of CD20 + receptors involved in the activation of B-lymphocytes. A study of CD20 + B-lymphocyte counts revealed the presence of a significant increase in the main group of children in comparison with the values of the control group ($p < 0.05$). Thus, the relative number of CD20 + B-lymphocytes in children with nasopharyngeal cancer was 23.0 ± 1.4%, and in the control group - 18.4 ± 0.58%. The increased expression of CD20 + on B-lymphocytes is more typical for latent viral infections and proliferative processes. In this case, such a picture can be associated with the antitumor strategy of immunity and the possible presence of viral aggression. However, the protective efficacy of B-lymphocytes is limited in such conditions.[12]

Data on activation markers of peripheral blood lymphocytes in the literature are ambiguous and not sufficient. In this regard, we have analyzed the expression of activation lymphocyte markers in nasopharyngeal cancer in children, which is of great scientific and practical importance, especially for tumoral and infectious diseases. The analysis of activation markers of lymphocytes allows studying the processes of activation, proliferation, differentiation and apoptosis of immunocompetent cells.[4,9]

It is known that CD38 + - the activation marker, represented by the transmembrane glycoprotein, is considered as a multifunctional protein. In turn, it is an ectoinzyme, which catalyzes the synthesis and hydrolysis of CADF-ribose. The enzymatic functions of CD38 + provide its main immunoregulatory role, it is the binding of various agents to this receptor, which enhances cytokine synthesis, kinase activation and protein phosphorylation[8], CD38 + is a precursor of plasma cells. It is expressed on immature T- and B-lymphocytes, activated T-lymphocytes, plasmocytes. An analysis of the expression of CD38 + on lymphocytes revealed a significant increase in this marker in the children of the main group compared with the control group ($p < 0.05$). Thus, expression of CD38 + in the main group was 26.8 ± 1.6%, and in the control group - 22.0 ± 0.9% ($p < 0.05$).

Consequently, the growth of expression of CD38 + activation markers indicates the presence of inadequate activation of cellular immunity.

According to the literature, there is information about the role of APO-1 / Fas (CD95 +) receptors in the process of apoptosis, and its expression is a reflection of the level of apoptosis of lymphocytes.[14,15] The growth of expression of CD95 + receptors on lymphocytes indicates an excessive and ineffective process of stimulation of blood lymphocytes, which indicates an apoptotic pathway of lymphocyte death. The membrane molecule Fas (CD95 +) is a specialized signal receptor for the induction of apoptosis and belongs to the receptor family for TNF-α and nerve growth factor.[2,5] The natural ligand for the Fas receptor is the FasL ligand (TNF-α homolog), which is expressed on a part of the cells under the influence of activation, in this case on lymphocytes. Binding of CD95 + to the Fas ligand induces apoptosis of cells expressing CD95. Thus, an increased expression of CD95 + on peripheral blood lymphocytes in patients with nasopharyngeal cancer was detected, which indicates an increase in apoptosis of lymphocytes, which explains the presence of T-cell immunodeficiency. In the main group of children and adolescents with nasopharyngeal cancer, expression of CD95 + was 26.7 ± 1.2%, and in normal cases - 20.6 ± 0.49%. Apparently, excessive apoptosis in the tumor process in combination with deep T-cell immunodeficiency contributes to the progression of the disease. Analysis of the results shows that in the main group of children with nasopharyngeal cancer there are certain changes in the cellular link of adaptive immunity, which are manifested by suppression of CD3 + T-lymphocytes, CD4 + T-helpers / inducers, immunoregulatory index, but an increase in the number of CD8 + T cytotoxic lymphocytes, CD16 + NKC cells. Analysis of activation lymphocyte markers also revealed an increase in the expression of CD38 + and CD95 +.

The decreased immunoreactivity of the T-cell link on the background of long chronic pathological processes is considered as a result of a disruption in the representation of the antigen of the APC, as well as a violation of the function of the T-cells themselves □1,3,5,8□.

According to the literature, it is known that IL-2 is a nonspecific growth factor of T cells, thus forming a functional activation of T-lymphocytes.[9,14] The main IL-2 producing cells are T-lymphocytes with the T-helper phenotype, which have functional signs of type 1 T helper. For IL-2, stimulation of proliferation of CD8 + T-lymphocytes and ECC is characteristic. It was shown that IL-2 acts only on cells carrying high-affinity receptors to IL-2. These receptors are absent on cells at rest and participate in specific responses in response to an antigenic stimulus.[8,10]

Expression of the activation marker CD25 + on lymphocytes in the main group of children was reduced in comparison with the value of the control group ($p < 0.05$). Thus, in the control group, CD25 + expression on lymphocytes averaged 22.4 ± 1.2%, and in the group of sick children - 18.6 ± 0.92%. Obviously, such suppression of CD25 + expression in the tumor process is associated with depletion of the T-cell pool of lymphocytes and, subsequently, a decreased production of IL-2, which is a growth factor of T lymphocytes.

Next, we studied the humoral factors of immunity in nasopharyngeal cancer in children, despite the fact that these factors are nonspecific, but reactive, which even more attracts the attention of researchers. It has been established that one of the most important biological functions of immunoglobulins is antigen binding and the formation of circulating immune complexes (CIC). An important characteristic of the CIC is their magnitude. Thus, in the main group of children there is an increase in the average values of the CIC 3% (large values) and 4% (small values). Moreover, all CIC values were significantly increased in the main group of children compared to the control group. Thus, in the main group of children with nasopharyngeal cancer, the CIC3% is increased 3.5 times, and the CIC4% is increased - 4.2 times with respect to the value of the control group. It is known that the increase in the CIC is most often observed in chronic infectious processes and is characterized by the fact that for example, the CIC3% are formed with an excess of antibodies, although they are able to bind complement but are large, insoluble, rapidly phagocytosed and have low pathogenicity. The greatest pathological potential is possessed by soluble immune complexes of small sizes (CIC4%), which also formed with an excess of antigen. Consequently, an increased number of CIC3% and CIC4% may be due not only to the activation of a depleted immune response to tumor antigens, but also to existing viral antigens, as well as suppression of their elimination mechanisms, i.e. phagocyte-monocyte system. The latter may be due to the weakening of the function of cells of the monocyte-macrophage system-cells that absorb and disintegrate immune complexes. Some authors cite data on the decrease in phagocytic activity of peripheral blood neutrophils.[5,8,10,15]

Consequently, in case of nasopharyngeal cancer, inadequate activation of the humoral link of immunity is observed in diseased children along with depression of immunoglobulins, ie depletion of humoral immunity. It is also possible to indirectly judge the weakness of the T-cell immune response, which is directed against a smaller number of epitopes, suggesting clonal depletion of T lymphocytes. In addition, it is possible to judge the inadequate activation and depletion of serum immunoglobulins.

Thus, based on the results obtained, it can be seen that in nasopharyngeal cancer, a marked imbalance in the cellular and humoral parts of the immune system is observed in children. Imbalance in the cellular immunity

unit was expressed in the suppression of the immunoregulatory index due to a decrease in the number of T-helpers / inducers and an increase in T-cytotoxic lymphocytes. Circulating immune complexes of large and small values were increased. Consequently, various changes in the state of immunoreactivity of patients play an important role in the formation and progression of nasopharyngeal cancer.

## CONCLUSION

1. Inadequate activation of the humoral link of immunity In nasopharyngeal cancer is observed in diseased children along with depression of immunoglobulins, that is, depletion of humoral immunity.
2. Misbalance in the cellular immunity unit was expressed in the suppression of the immunoregulatory index due to a decrease in the number of T-helpers / inducers and an increase in T-cytotoxic lymphocytes.

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