

THE ROLE OF MOLECULAR BIOLOGICAL AND IMMUNOLOGICAL MARKERS IN THE DIAGNOSIS AND TREATMENT OF PATIENTS WITH OROPHARYNGEAL CANCER

Ermатов Nizom Djumakulovich¹, Ganiev Abduvaz Abdulhatovich², Nabieva Umida Pulatjanovna³, Samigova Nargiz Raimovna⁴, Khalmatova Matlyuba Artykovna⁵, Alimukhamedov Dilshod Shavkatovich⁶

ABSTRACT

It has been established that small-sized circulating immune complexes have great pathological potential, while a high level of small circulating immune complexes shows an active fight of immunity against virus antigens, on the one hand, and on the other hand, is associated with a violation of the mechanisms of virus elimination, which plays a significant role in the formation and maintaining the malignant process. All this contributes to the violation of immune regulation, triggering the mechanisms of carcinogenesis with the development of the disease. Thus, the combined determination of the level of VEGF, the CD95+ apoptosis marker, and the IRI immunoregulation index in complex clinical cases improves the diagnosis of OPR and allows timely prediction of a more severe course of oropharyngeal cancer to avoid unnecessary biopsies or sectoral resections.

Keywords: oncology, dentistry, cancer of the oral cavity and oropharynx, diagnostics, Epstein-Barr virus, circulating immune complexes, prognosis.

INTRODUCTION

Malignant neoplasms (MNs) are one of the most common causes of morbidity and mortality. Approximately 12 million new cases of cancer are diagnosed each year and more than 8 million deaths. Oral cancer is the sixth most common cancer in the world [8, 11, 12].

“Malignant neoplasm (MN) of the head and neck” is a group of neoplasms with a different histostructure, the localization of which is the mucous membranes of the lips, oral cavity, pharynx, larynx, cervical esophagus, nasal cavity, and paranasal sinuses, salivary glands. This group of tumors does not include tumors of the central nervous system (CNS), eyes, lymphatic system, nervous and endocrine, although they can be localized in this anatomical region.

Currently, an important role in the occurrence of OPR is played by the presence of previous precancerous OPD diseases, the possibility of their development against the background of virus carriage (Epstein-Barr virus [EBV]). The presence of precancerous OPD diseases, as noted by recent sources of literature, is associated with the occurrence of inflammatory processes in the body caused by Viruses [EBV]. The detection and activation of these viruses in the body are associated with altered behavioral responses and a "riotous"

lifestyle, which has recently been observed among young people around the world (non-traditional sexual orientation, homosexuality, alcoholism, drug addiction, etc.) [2, 5, 13].

It is known that even though all of them are potentially pathogenic, but has evolved with other species and their ancestors for more than a million years, they have achieved a balance in the "virus-host" position, the balance of which is critically dependent on the control of the host's immune system. Most representatives of herpesviruses are widespread in the population and often remain "silent" or are characterized by a slight manifestation when they first enter the child's body and then persist throughout life in a state of asymptomatic latent infection [1, 3, 9, 14, 16].

The Epstein-Barr virus (EBV) has unique abilities not only to induce cytolysis or persist chronically in cells, like all herpesviruses but is also able to transform B-lymphocytes, endothelial and epithelial cells of the host. In this connection, EBV is a latent, immunotropic virus that forms a deep immunodeficiency, against which a malignant proliferative process develops [4, 6, 7, 9, 10, 15].

All this contributes to the violation of immune regulation, triggering the mechanisms of carcinogenesis with the development of the disease. Works devoted to the study of

epidemiological features in OPD, changes arising from the immune system (IS) due to the presence of EBV, as well as to optimize surgical methods of treatment that improve the survival of patients over the past decades have not been carried out in the republic, which determines the relevance and relevance of this scientific research. research.

MATERIALS AND METHODS

The results of a survey of 65 patients with oropharyngeal cancer were included in the study. Patients underwent serological, immunological, and molecular genetic studies to clarify the pathogenetic mechanisms of virological and immunological factors in the development and course of oropharyngeal cancer variants. In the Tashkent regional branch of the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (TRB RSSPMCOR) from 2008 to 2017 (over the past 10 years), 1021 patients with oropharyngeal cancer (OPC) underwent

combined and complex treatment. According to the morphological study, all patients were diagnosed with OPR. Of these, the study included 427 patients who underwent combined and complex treatment. The study included 33 patients operated on at the Rostov Cancer Research Institute (RCRI). The patients were divided into 2 groups: the main group (n=149), who underwent standard treatment, including mandibulotomy with urostomy, and the control group (n=278), who did not undergo this type of operation.

RESULTS AND DISCUSSION

The main criteria for inclusion in the study were clinically and morphologically established widespread OPR. Patients with early cancer - oropharyngeal zone (OPZ) - 233 (22.8%) patients were not included in the study. The distribution of patients by sex was as follows: in the main group - men 60.4% (90), women - 39.6% (59); in the control - 58.6% (163); 41.4% (115), respectively (Table 1).

Table 1 Distribution of patients by gender in the control and main groups of oropharyngeal cancer

№	Gender	Primary		Control		Total
		abs	%	abs	%	
1.	Men	90	60.4%	163	58.6%	253
2.	Women	59	39.6%	115	41.4%	174
Total		149	100%	278	100%	427

The distribution of patients by sex and age intervals showed that among patients men are predominant: 253 (59.3%) compared with women 174 (40.7%), the ratio is 1.45:1. The average age of the patients was 59.0 ± 1.4 years for men and 59.6 ± 2.08 years for women; the age range was from 19 to 85 years. It was established that common OPR occurred at any age, however, two-thirds of patients with stage IV OPR were patients in the age range of 50-70 years.

All patients underwent immunological studies in peripheral blood serum at the initial admission after the diagnosis was established before treatment to identify the values of the most vulnerable immunological markers that play an important role in the diagnosis, dynamics of treatment, and prognosis of oncological diseases.

Determination of circulating immune complexes of various sizes (CIC) by ELISA analysis on the Stat-Fax analyzer (USA) was carried out in the laboratory of immune cytokines of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan. CEC sizes are determined using different concentrations of PEG. Low concentrations of PEG precipitate large CECs with a predominance of antigen, while high concentrations precipitate small CECs with a predominant content of antibodies.

To study the humoral link of immunity in patients with oropharyngeal cancer, we studied circulating immune complexes (CIC). One of the main characteristics of the CEC is its size. In the patients examined by us, an increase in the values of the CIC and large values (3%) and small values (4%) was

revealed. Moreover, the CEC values of 3% and 4% were higher than the control group by more than 2-3 times. Considering that small circulating immune complexes have great pathological potential, it is important to identify this indicator. And it should be taken into account that a high level of small circulating immune complexes shows an active fight of immunity against virus antigens, on the one hand, and on the other hand, it is associated with a violation of the mechanisms of virus elimination, which plays a significant role in the formation and maintenance of the malignant process. Violation of the elimination of viruses, in this case, depends on a change in the function of the cells of the monocyte-macrophage system - cells whose main function is the absorption and disintegration of immune complexes.

Large CECs (3%), having the ability to bind complement, but due to their large size and insolubility, are rapidly phagocytosed and have low pathogenicity, as a result of which they are not pathogenetically unfavorable in the prognosis and course of oropharyngeal cancer. Analysis of the obtained results showed that in oropharyngeal cancer against the background of a long-term chronic course of EBV infection, dysregulation of the cell link is observed due to a decrease in the expression of T-helpers/inducers and an increase in the expression of T-cytotoxic lymphocytes against the background of activation of the humoral immunity link. And the existing, insufficient T-cell immune response can be the result of both clonal depletion of T-lymphocytes-helpers, and be a consequence of a dysfunction of the immune system in the presentation of antigen to cells, as well as a decrease in the efficiency of T-cytotoxic lymphocytes.

For an in-depth study of certain stages of the pathogenesis of the development of neoplasms in the conditions of the tumor degeneration of cells, the study of the neoangiogenesis factor characterizing tumor growth is an important factor in terms of diagnosis in the study of malignant growth activity in oropharyngeal cancer.

We have determined the level of VEGF in blood serum in newly diagnosed patients with oropharyngeal cancer (T3-4N0M0 stages), as well as in the control group (healthy individuals). The studies were carried out in dynamics - upon admission of patients and after 14 days after surgery (to exclude the release of VEGF due to the initiating effect of surgical intervention). Analysis of the results of the

conducted studies revealed that the level of VEGF in the blood serum of patients was increased relative to the control group. Thus, in the control group, the level of vascular endothelial growth factor was 35.4 ± 0.43 ng/ml, and in patients with initially diagnosed oropharyngeal cancer, this indicator was significantly higher ($P < 0.05$) and averaged 531.21 ± 58.3 ng/ml. It should also be noted that if we consider this indicator separately for groups of patients depending on the presence of metastases, then the level of VEGF in the group of patients with metastases (874.21 ± 67.3 ng/ml) was approximately 3 times higher compared to the data of patients without metastases (362.21 ± 53.1 ng/ml).

The level of VEGF after surgery on day 14 averaged 308.18 ± 43.1 ng/ml, which is still significantly higher than in the control group, but already almost 2 times lower than before surgery. Considering that all patients were under dispensary observation after treatment, we conditionally divided them into those who still had a high level of VEGF after treatment and those who had this level significantly lower. And as a result of retrospective observation, it was found that among patients who had a high level of VEGF after tumor removal, the number of relapses was 2.1 times more than in patients with a lower level of VEGF after tumor removal.

To identify pathogenetic relationships, we carried out a complete analysis of the correlation relationships between the level of VEGF with all studied virological, molecular genetic, and immunological parameters. We studied the relationship between changes in the levels of VEGF in the blood serum with the age of patients and revealed a direct correlation of the average level of significance between the level of VEGF in the blood serum and the age of patients ($r = 0.51$; $p = 0.041$). by TNM degrees ($r = 0.45$, $p = 0.018$). The presence or absence of concomitant diseases did not change this relationship. That is, the older the patient was, the higher the VEGF levels were determined in him.

Further correlation analysis revealed a significant direct correlation between the level of VEGF and the apoptosis marker CD95+ ($r = 0.75$; $p = 0.004$) and an inverse correlation with the IRI immunoregulation index ($r = -0.487$; $p = 0.021$). Next, we examined the occurrence of these indicators depending on the distribution by TNM stages. And found that in patients T4N0M0 stage of oropharyngeal cancer in 89%

of cases there was a high level (above 531.21 ± 58.3 ng/ml), increased expression (above $33.4 \pm 1.53\%$), and a decrease in the immunoregulatory index (below 1.0), or at least a combination of 2 of the above indicators. Whereas such values of these 3 interrelated indicators at the T3N0M0 stage were found only in 58% of all cases of the disease. Thus, the joint determination of the level of VEGF, the CD95+ apoptosis marker, and the IRI immunoregulation index makes it possible to predict a more severe course of oropharyngeal cancer.

As a result of comprehensive studies of serological and molecular genetic parameters, it was determined that patients with oropharyngeal cancer were characterized by more frequent (56%) detection of capsular antigen (VCA) and more often in combination with nuclear (NA) Epstein-Barr virus, which is an indicator chronic persistence of the virus, as well as the frequent detection of viral DNA in the saliva of these patients in the absence of replication in the blood plasma, which gives reason to use as predictive criteria for the possible development of oropharyngeal cancer. In oropharyngeal cancer, against the background of a long-term chronic course of EBV infection, dysregulation of the cell link is observed due to a decrease in the expression of T-helpers and an increase in the expression of T-cytotoxic lymphocytes against the background of activation of the humoral link of immunity.

Among patients who had a high level of VEGF after tumor removal, the number of relapses was 2.1 times more than in patients with a lower VEGF level after tumor removal. A significant direct correlation was found between the level of VEGF and the apoptosis marker CD95+ ($r=0.75$; $p=0.004$) and an inverse correlation with the immunoregulation index (IRI) ($r=-0.487$; $p=0.021$).

In patients with T4N0M0 stage of oropharyngeal cancer, in 89% of cases, there was a high level (above 531.21 ± 58.3 ng/ml), increased expression (above $33.4 \pm 1.53\%$), and a decrease in the immunoregulatory index (below 1.0), or at least a combination of 2 of the above. Whereas such values of these 3 interrelated indicators at the T3N0M0 stage were found in 58% of all cases of the disease. That is, the joint determination of the level of VEGF, the CD95+ apoptosis marker, and the IRI immunoregulation index makes it possible to predict a more severe course of oropharyngeal cancer.

CONCLUSION

1. Activation of the humoral link of the immune system was noted, which is manifested by an increase in circulating immune complexes, both large and small.

2. The joint determination of the level of VEGF, the CD95+ apoptosis marker, and the immunoregulation index IRI in difficult clinical cases improves the diagnosis of OPR and allows timely prediction of a more severe course of oropharyngeal cancer to avoid unnecessary biopsies or sectoral resections.

LIST OF REFERENCES

1. Azova M.M., Gigani O.B. The role of the Epstein-Barr virus in the occurrence and development of tumor diseases // *Natural History and Humanism*. - Tomsk, 2006. - T. 3. - No. 3. - p. 3.
2. Gazhva S.I., Stepanyan T.B., Goryacheva T.P. The prevalence of dental diseases of the oral mucosa and their diagnosis // *International Journal of Applied and Fundamental Research*. - Moscow, 2014. - No. 5. - S. 41-44.
3. Davydov M.I. *Oncology: textbook*. - Moscow: GEOTAR, Media 2020. - 920 p.
4. Dadamov A.D., Zhilonov A.A., Kayumova R.R. Sonoelastography in the early diagnosis of neoplasms of the maxillofacial region // *Dentistry*. - Tashkent, 2013. - No. 3-4. - S. 75-81.
5. Zarkumova A.E. The structure of the incidence of the oral mucosa // *Bulletin of KazNMU*. - Kazan, 2017. - No. 3. - S. 168-175.
6. O. I. Kit, E. M. Frantsyants, and I. V. Neskubina, Acoust. Study of the content of VEGF-A and TGF- β in biopsy specimens of squamous cell carcinoma of the tongue and mucous membrane of the floor of the mouth during polychemotherapy with monoclonal antibodies - cetuximab // *Research and practice in medicine*. - Moscow, 2019. - No. 6(3). - S. 20-28.
7. Korytova L.I., Sokurenko V.P., Maslennikova A.V. Current trends in the treatment of locally advanced cancer of the oropharynx and oral cavity. - St. Petersburg: Folio, 2011. - 110 p.
8. Rakhmankulova G.S., Tulebaev K.A., Zholdybaev S.S. Oncological alertness in diseases of the oral cavity (literature review) // *Bulletin of KazNMU*. - Kazan, 2017. - No. 1. - S. 168-172.

9. Tillyashaikhov M.N. Organization of the oncological service of Uzbekistan at the present stage and prospects for further development // *Clinical and experimental oncology*. - Tashkent, 2017. - No. 1. - S. 5-8.
10. Ugarov I.V., Arutyunov S.D. Molecular biological markers of diagnostics and monitoring of therapeutic response in patients with oral cancer // *Bulletin of KazNMU*. - Kazan, 2018. - No. 1. - S. 526-529.
11. Freidlin I.S., Kuznetsova S.A. Immune complexes and cytokines // *Medical Immunology*. - St. Petersburg, 1999. - V.1. - No. 1-2. - S. 27-36.
12. Shilova O.Yu. Association of laryngeal cancer with human papillomavirus and Epstein-Barr // *Siberian journal of oncology*. - Tomsk, 2007. - No. 2. - S. 126-127.
13. Brocklehurst P., Kujan O., O'Malley L.A., et al. Screening programs for the early detection and prevention of oral cancer // *Cochrane Database Syst Rev*. - 2013. - №11. - P. 149-156.
14. Crossman T., Warburton F., Richards M.A. (2016) Role of General Practice in the Diagnosis of Oral Cancer // *British Journal of Oral and Maxillofacial Surgery*. - 2016. - №54. - P.208-212.
15. Kimura H., Miyake K., Yamauchi Y., Nishiyama K., Iwata S., Iwatsuki K., Gotoh K., Kojima S., Ito Y., Nishiyama Y. Identification of Epstein-Barr virus (EBV)-infected lymphocyte subtypes by flow cytometric in situ hybridization in EBV-associated lymphoproliferative diseases // *J. Infect. Dis*. - 2009. - Vol. 200. - №7. - P. 1078-1087.
16. Oliveira L.R., Ribeiro-Silva A. Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma // *Int J. Oral Maxillofac Surg*. - 2011. - №40. - P.298-307.