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ANALYSIS OF THE EFFECTIVENESS OF THE COMBINED TREATMENT OF PATIENTS WITH LARYNGEAL PAPILLOMATOSIS

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Abstract

Laryngeal papillomatosis is one of the most common tumor diseases of the upper respiratory tract, the morphological substrate of which is papilloma, leading to chronic obstruction of the larynx and hoarseness, occurring in both children and adults. The main etiological factor of laryngeal papillomatosis is the human papillomavirus. There are data that about 10%-60% of the population is affected by latent human papillomavirus infection, as evidenced by the detection of human papillomavirus DNA in the externally unchanged mucosa in 78.5% of the examined. Papillomas make up 3.5% of all benign tumors of the ENT organs and from 20 to 45% of all benign tumors of this localization. Laryngeal papillomatosis is characterized by recurrent course and rapid growth, which requires multiple surgical interventions. In order to eliminate laryngeal stenosis for a long period, tracheotomy remained practically the only method of surgical treatment. Up to now, more than 50 different methods of treatment of laryngeal papillomatosis are known, but a radical etiopathogenetic agent has not been found yet. It should be noted that about 70% of patients have a frequent case of the disease. Therefore, the problem of treatment of respiratory papillomatosis is one of the most urgent in modern otorhinolaryngology and currently largely unsolvable.

Medical practice is based on three main directions and their different combinations: the improvement of surgical methods, the search for the new drugs (antiviral, immunotropic, etc.) and the development of vaccination methods. At present, there is no single international standard for the treatment of laryngeal papillomatosis, but it is known that it should be comprehensive.

The article presents a study of cold plasma surgery application and the use of a fundamentally new drug in otorhinolaryngology i.e. inductor of interferon synthesis.

Keywords: *laryngeal papillomatosis, human papillomavirus, cold plasma surgery, preventive treatment.*

Introduction

In modern otorhinolaryngology laryngeal papillomatosis remains one of the unresolved problems [*zur Hausen H, 1999*]. Up to now, quite a lot of treatment methods of this disease have been proposed, but the number of patients does not decrease, more and more common cases of papillomatosis and cases of tumor malignancy are detected. The detection of primary papillomatosis in adults is also increasing [*Sun A, 1993; Isakov V et al., 2007; Derkay C, Wiatrak B et al., 2008*].

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One of the main methods of treatment is surgical intervention [*Reidy P, Dedo H, 2004*]. The choice of papilloma removal technique depends on the surgeon's qualification and equipment of the clinic where the treatment is carried out [*Sidell D et al., 2014*]. Standard removal of papillomas with "cold" tools is carried out with the help of a set of micro instruments for laryngeal operations [*Lusar B et al.,1993*]. Often, using this technique, there is increased bleeding, capture of unaffected mucous membrane of the larynx, excessive traumatism of soft tissues. All this leads to a long rehabilitation period and contributes to the development of the scar process [*Rosen C, Bryson P,2003*]. Prevention of these consequences is possible with the help of point dosed effects on the affected tissue. This effect is possible by means of cold plasma surgery

i.e. coblation, which has drawn the attention of many experts around the world [*Rapp L, Chen J, 2008*]. The undoubted advantage of coblation is the targeted impact on the tissue in the absence of burn effects not only on the affected tissue, but also on the closest one, which is typical for laser and radio frequency equipment, traditionally used in many areas of surgery. It is the ability of the narrowly focused cloud of the heated plasma to 70° C that allows to act as a perfect and versatile surgical instrument [*Wiatrak B et al., 2004*]. All these properties lead to a reduction in the rehabilitation period after surgery, which consists in reducing the risk of scarring, the risk of bleeding and faster recovery of voice function. In addition, it is worth noting that the relative ease of use (after mastering the skills of the method) is also an important advantage for modern otorhinolaryngology [*Isakov V et al., 2007*].

Currently, the main task in the treatment is not only to choose the optimal surgical tactics of papilloma removal, but also prolongation of the interrelapse period [*Quiney R et al., 1989*].

Alpha interferon was first proposed as an adjuvant therapy for laryngeal papillomatosis. The exact mechanism of interferon action in laryngeal papillomatosis is unknown [*Li D et al., 2015*]. Probably, there is a mechanism similar to that in other viral infections - an increase in the production of endonucleases that inhibit the Assembly of virus proteins inside the cell, and protein kinases that change the structure of membranes of neighboring cells, which can prevent the introduction of viral particles in them [*Leventhal B, 1991; Isakov V et al., 2007*]. Interferon therapy allows achieving complete remission in 36.7% of cases (average duration of remission – 550 days), and 25% remain in remission after 4 years of therapy; partial remission was observed in 41.7% of cases (average duration – 400 days.) and 21.6% of the patients do not respond to interferon [*Leventhal B, 1991*].

Antiviral drugs, such as acyclovir, cidofovir, indole-3-carbonyl, retinoids and many others were also used. Among the variety of drugs, methods and technical equipment, otorhinolaryngologists often meet with too high rate of papillomatosis recurrence [*Best S et al., 2012*]. Therefore, this problem requires the accumulation of the results of a number of studies of various techniques, drug regimens, which can lead to the definition of unanimous tactics of conducting such patients [*Demyrta Y, Yavuzer R, 2001; Derkay C, Wiatrak B et al., 2008*].

Modern immunology and virology use Allokin alpha (produced by FSUE "State.OCB research Institute" FMBA of RUSSIA, Saint-Petersburg; FGU "MD, PhD Rosmedtechnologies", APMB, Moscow) for the treatment of HPV-associated diseases as anti-relapse treatment. This is an original selective stimulator of natural immune factors, a new generation antiviral drug, the first drug that belongs to the group of drugs "prohibiting the shutdown of cytokines" [*Barnes L, 2001*]. The drug contains alloferon (derived from the immune system of insects), which is an Oligopeptide consisting of 13 amino acids (Histidin-glycyl-valyl-seryl-glycyl-histidyl-glycyl-glutaminyl-histidyl-glycyl-valyl-histidyl-glycin) [*Kashima H et al.,1993*]. This component is an effective inducer of synthesis of endogenous interferon and activator system of natural killer cells, able to stimulate the recognition and lysis of defective cells cytotoxic lymphocytes [*Lusar B et al., 1997*]. Alloferon acts as a cofactor activation of the immune response, for the formation of which requires the participation of the main signal –a viral antigen, which allows localizing the action of the drug in the focus of inflammation, avoiding excessive reactions of the immune system beyond its limits. Thanks to all these advantages, Allokin alpha can become a method of selecting new antiviral drugs. In gynecological practice, the inductor of endogenous interferon synthesis was previously used in the treatment of papillomavirus genital diseases. According to the results, the drug elimination of HPV reached 90% of cases. However, in papillomatosis of ENT organs, the inductor of interferon synthesis is used for the first time, which determined the purpose of our study [*Leventhal B, 1991*].

The aim of the study is to compare the effectiveness of treatment of two groups of adult laryngeal papillomatosis patients.

Material and methods

From 2013 to 2015 conducted the open randomized controlled clinical research on the basis of GBUZ MO MONIKI. M. F. Vladimirsky and First MSMU after I. M. Sechenov (Moscow). It included 50 patients (31 men and 19 women) aged

18 to 65 years with laryngeal papillomatosis. The results of earlier histological studies confirmed the diagnosis in all cases. The mean age of patients was 37.2±1.3 years. All patients were divided into two equal groups by blind sampling. The study group included 25 patients who had received drug treatment with the inducer of the synthesis of interferons (drug Allokin-alpha) scheme according to the Protocol in combination with endolaryngeal microsurgery with the application of cold plasma. Each patient received only 6 injections of the drug in 2 days subcutaneously at a dose of 1 *mg*: 2 injections before surgery, the 3rd injection on the day of surgery, and 3 more injections in the postoperative period. In the control group (n=25) patients were operated by the method of endolaryngeal microsurgery with the application of cold plasma. Drug therapy in the second group of patients was not conducted. Patients in both groups underwent ENT examination videopornolargogratis, type-specific HPV-DNA and quantitative study of the virus by method of polymerase chain reaction with the definition of viral load of HPV genotypes in saliva at baseline to beginning of treatment, after three months, six months and one year after the end of treatment. Also, with each surgical intervention, histological examination of the removed tissue, cytological examination, and study by pappilloma to determine HPV types 6 and 11 to confirm the infection of the patient with a lack of these indicators in saliva were conducted.

The studied groups according to nosological forms, age composition and sex are almost identical. Patients of the first and second groups were comparable in terms of duration of the disease, severity of the process and the nature of the laryngeal pathology. Concomitant somatic diseases in the studied groups of patients were also identical and did not have a significant impact on the course of the main disease.

The main group of patients consisted of 25 patients, including 17 men and 8 women. The age of the patients was 18 to 60 years. In 3 patients, the duration of the disease was about 20 years (all patients had been suffering from the disease since childhood), while for a specified period of time they underwent about 10 operations. In 10 patients, the anamnesis of the disease ranged from 7 to 9 years (7 patients had had the disease since early childhood, the onset of the disease manifested at the age of 12-14 in 3 patients), they underwent 3 to 7 operations. In 8 patients, the duration of 3 to 5 years (the disease was first found in adulthood in 3 patients, 5 patients had had it since childhood), the number of operations is not more than 3. In 4 patients, the duration of the disease is about a year (all papillomatosis of adult age), within 12 months, 1 operation was carried out. According to the prevalence of the process: in 3 patients papillomas were determined only in the region of the epiglottis; in 5 patients papillomas were found in the area of the anterior commissural; papillomatous growths were visualized in the area of vocal folds in the anterior divisions with the transition to the anterior commissural in 11 patients; 6 patients had common papillomatosis.

In the control group of patients there were 25 patients, including 14 men and 11 women. The age of patients was 24 to 69 years. In 5 patients, the duration of the disease was about 20 years (since childhood), while for a specified period of time they underwent about 10 operations. In 9 patients, anamnesis of the disease was about 7 to 9 years, (in 8 children's type, in 2 adults) they underwent from 3 to 7 operations. In 6 patients the duration was from 3 to 5 years (in 3 patients the disease was diagnosed in childhood and in 3 patients had it at the adult age), the number of operations is not more than 3. In 5 patients, the duration of the disease was about a year (adult type), within 12 months 1 operation was performed. According to the prevalence of the process: 7 patients had papillomas in the anterior commissural, in 8 patients papillomatous growths were visualized in the area of vocal folds in the anterior divisions with the transition to the anterior commissural; 10 patients had common papillomatosis.

Complaints of patients of both groups were identical: hoarseness, recurrent cough, dyspnea at physical activity. The last complaint was regarded as a manifestation of papillomatosis of the larynx, as the concomitant diseases of broncho-pulmonary system, cardiovascular disease and high body mass index is not fixed

As already mentioned, all patients underwent surgical treatment-endolaryngeal microsurgical removal of papillomatosis foci with the use of cold plasma – coblation.

Radiofrequency cold ablation (coblation) involves the transmission of alternating electric current of a certain radiofrequency through the electrolyte salt solution in a small volume. This leads to the formation of a plasma field of sodium ions, which can destroy the intercellular connections, which leads to vaporization of tissue at a relatively low temperature of 60-65° C. In addition, the formation of aerosol particles due to the low temperature is excluded. The experience of its application in the treatment of recurrent laryngeal papillomatosis includes several descriptions of a series of cases in which there were significantly longer inter-relapse periods (compared to CO2 laser treatment), no scarring, better voice preservation in the near and distant postoperative period [*Leventhal B, 1991; Isakov V et al., 2007*].

With the help of this method, depending on the situation, the doctor can coagulate, dissect or destroy an array of pathologically altered tissues, without having a negative impact on nearby anatomical structures. The depth of penetration is only hundredths of a millimeter, which means that the surrounding layers are not affected. A very small thickness of the plasma layer allows to carefully dose the effect and carefully calculate the volume of removed and dissected tissue. In addition, the flexibility of the working electrode and the implementation of visual control through the operating microscope over the manipulations significantly increase their accuracy and efficiency.

We used the setup "Coblator II" produced by "ArthroCare" (USA) and the electrode "PROciseLW" (Registered in the Russian Federation, included into the state register of products of medical purpose and medical equipment registration certificate of FS No 2005/966).

All surgical interventions were performed as standard, there was low bleeding in the intraoperative period and a short period of postoperative recovery period. There were no differences and peculiarities in the operation between groups of patients.

Processing of all collected data was performed using the program SPSS (Statistical Package for the Social Sciences) 2010. Comparison of the effectiveness of treatment of the main and control groups was performed for two performance criteria: 1) Comparison of the frequency of relapses and the duration of the inter-relapse period in both groups; 2) Comparison of HPV in saliva after 12 months of therapy between groups with baseline values.

Results

In the course of surgical treatment of patients of both groups under the control of the microscope was carefully removed education of the larynx. All surgical interventions were performed as standard, there was low bleeding in the intraoperative period and a short recovery period. There were no differences and peculiarities in the operation between groups of patients. No one patient had postoperative complications, including bleeding, neither in primary nor in repeated operations.

After surgery, all patients disappeared complaints of cough and shortness of breath. With regard to the restoration of voice function, its improvement was noted in patients with a history of multiple surgical interventions that do not have scar processes in the voice Department of the larynx.

In the main group of patients during the observation period repeated surgical removal of papillomas was required 8 patients out of 25, 6 of them were operated once, and 2 patients underwent the operation twice. In one of these two patients, repeated surgery was performed 3 months later, then 9 months from the beginning of observation. The second patient was operated on within 6 months and 11 months from the beginning of the study. These patients suffered from papillomatosis for about 20 years and had previously undergone more than 10 operations.

The remaining 6 patients of the main group, who needed repeated surgery, were operated once: 4 of them in terms of 6 to 8.5 months, 2 patients in the period of 11 months from the beginning of the study.

As for the prevalence of the process, in 5 patients who once needed surgery, papillomas were initially located in the area of vocal folds with the transition to the anterior commissural, and in case of relapse - only in the anterior commissural region. Another patient with initially common papillomatosis was re-operated after 8 months. In this patient, papillomas were determined only in the area of the left vocal fold (no more than the anterior 1/3 of the length) and in the area of the anterior commissural.

In 2 patients, who underwent surgery twice in 12 months, initially and with repeated relapses, a com-

mon localization was determined (more than 2/3 of the vocal folds and the area of the anterior commissary).

All 8 patients who required repeated surgery suffered from papillomatosis from childhood.

Based on the obtained data, out of 17 patients who did not require surgery, 5 had an anamnesis of the disease from 7 to 9 years and they underwent from 3 to 7 operations; 8 patients had a duration of 3 to 5 years and the number of operations did not exceed 3. In 4 patients, the duration of the disease is about a year, during which time 1 operation was transferred.

Analyzing histological and cytological findings, it is worth noting that in all patients who did not require repeated intervention, juvenile papillomas with abundant vascular network and signs of keratosis were determined. During the initial examination of the removed tissue, 4 of 8 re-operated patients had adult papillomas of pink type of dense elastic consistency, and 4 of them had juvenile papillomas. No early signs of tumor malignancy were detected in any patient. Subsequent histological and cytological results had no significant differences.

From the above it can be concluded that the inclusion in the combined treatment of laryngeal papillomatosis inducer synthesis of endogenous interferon is possible both in childhood papillomatosis and adult type. However, with previous multiple surgical interventions, this method of treatment is less effective.

In the Control group of patients during the observation period, repeated surgical removal of papillomas was required in 19 patients. Of these, 7 were operated three times and 4 twice. Moreover, in re-operated patients there were no changes in the prevalence and localization of recurrent foci of neoplasms, ie papillomas were located in the same area of the larynx, where the primary examination.

Repeated removal of tumors in 3 patients of the control group (in 2 with common papillomatosis, operated three times and in 1 with localization in the anterior commissural, operated twice) determined denser and lighter overgrowth with the presence of fibrosis. However, according to the findings of the histological study, they revealed juvenile-type papillomas.

The average period of remission in the control group was about 3.5±0.5 months, and in the main group it was about 9±0.5 months.

The difference between the curves of the inter-relapse period was estimated by log-rank test. Differences between groups are statistically significant ($p<0.001$). The midpoint of the inter-relapse period in the main group was not achieved. The control group median inter-relapse period was 5 months.

According to the results of the study of HPV 6 and 11 types in saliva in patients of the main group, the following results were noted: in 13 of 25 patients at the baseline level before treatment, both types of HPV were determined, and by 12 months of observation during treatment – only in 5 patients. In 3 patients before treatment revealed only HPV type 11, after 12 months of observation, this type of virus in saliva was determined in all these patients. In 1 patient, both types of HPV infection were detected before treatment, and after a year of type 6 HPV in saliva was not determined, but HPV type 11 was left. Isolated HPV type 6 before use of the drug was found in 1 patient, and 12 months after treatment of such patients was not. In 8 of 25 patients of the main group before the use of alpha Allokin in saliva, no type of HPV was detected, in a year the number of such patients increased to 16.

HPV saliva test in the control group of patients showed that all initial data of qualitative determination of the virus remained unchanged, i.e. there were no significant dynamic differences in viral load on any control period of observation.

DISCUSSION

The obtained results showed a greater effectiveness of treatment in assessing this criterion in the main group of patients ($p=0.4396$).

Thus, the results revealed that the combined treatment, including the surgical stage with the use of cold-plasma surgery and anti-relapse treatment with the use of inductor synthesis of endogenous interferon (drug Allokin alpha) can reduce the number of repeated surgical interventions, increase the inter-relapse period and reduce the viral load of HPV 6 and 11 types in saliva.

On this basis, the proposed scheme of complex treatment with the use of this drug can be recommended for wide application in clinical practice for the treatment of patients over 18 years of recurrent respiratory papillomatosis, with the best result being assumed in patients who have undergone fewer surgical interventions in history.

REFERENCES

1. *Barnes L.* Diseases of the larynx, hypopharynx, and oesophagus. Textbook surgical pathology of the head and neck. L. Barnes. – New York/Basel: Marcel Dekker. 2001. 154-116.
2. *Best SR, Friedman AD, Landau-Zemer T, Barbu AM, Burns JA.* Safety and dosing of bevacizumab (avastin) for the treatment of recurrent respiratorypapillomatosis. Ann Otol Rhinol Laryngol. 2012; 121(9): 587-593.
3. *Demyrta Y, Yavuzer R.* Coblation in aesthetic facial rejuvenation. Aesthetic Plast Surg. 2001; 25(5): 372-377.
4. *Derkay CS, Wiatrak B.* Recurrent respiratory papillomatosis: a review. Laryngoscope. 2008; 118(7): 1236-1247.
5. *Kashima H, Mounts P, Leventhal B, Hruban RH.* Sites of predilection in recurrent respiratory papillomatosis, Ann Otol Rhinol Laryngol. 1993; 102, 580-583.
6. *Leventhal BG.* Long-term response of recurrent respiratory papillomatosis to treatment with lymphoblastoid interferon alfa-N1. Papilloma Study Group. N Engl J Med. 1991; 325(9): 613-617.
7. *Li D, Zhang Q, Ding M.* Application of coblation treatment via endoscopy in epiglottic benign tumors, Clin Otolaryngol. 2015; 29(7): 616-617.
8. *Lusar B, Gale N, Kambic V.* Human papillomaviruses infection and expression of p53 and c-erbB2 protein in laryngeal papillomas. Acta. Otolaryngol. Suppl. (Stockh). 1997; 527: 120-124.
9. *Pou AM, Rimell F, Jordan J.* Adult respiratory papillomatosis: human papillomavirus type and viral coinfections as predictors of prognosis. Ann Otol Rhinol Laryngol. 1995; 104: 758-762
10. *Quiney RE, Wells M, Lewris FA.* Laryngeal papillomatosis: correlation between severity of disease and presence of HPV6 and 11 detected by in situ DNA hybridization. J Clin Pathol. 1989; 42(7): 694-698.
11. *Rapp L, Chen J.* The papillomavirus E6 proteins. Biochim Biophys Acta. 2008; 1378(1): 1-19.
12. *Reidy PM, Dedo HH.* Integration of human papillomavirus type 11 in recurrent respiratory papilloma-associated cancer. Laryngoscope. 2004; 114(11): 1906-1909.
13. *Rosen CA, Bryson PC.* Indole-3-carbinol for recurrent respiratory papil Predictors of remission in juvenile-onset recurrent respiratory papillomatosis. Arch-Otolaryngol Head Neck Surg. 2003; 129: 12751278.
14. *Sidell DR, Nassar M, Cotton RT, Zeitels SM.* High-dose sublesional bevacizumab (avastin) for pediatric recurrent respiratorypapillomatosis. Ann Otol Rhinol Laryngol. 2014; 123: 214-222
15. *Sun AK.* Detection of human,papillomavirus DNA with biotinylated probes in laryngeal papillomas by in, situ hybridization. Chung Hua Erh Pi Yen Hou Ko Tsa Chili. 1993; 28(5): 297-299.
16. *Wiatrak BJ, Wiatrak DW, Broker TR, Lewis L.* Recurrent respiratory papillomatosis: a longitudinal study comparing severity associated with human papilloma viral types 6 and 11 and other risk factors in a large pediatric population. Laryngoscope. 2004; 114: 1-23.
17. *zur Hausen H.* Yohei Ito Memorial Lecture: Papillomaviruses in human cancers. Leukemia. 1999; 13(1): 1-5.