Fluorescence diagnostics and photodynamic therapy of squamous cell carcinoma of the lateral surface of the tongue using the photosensitizer chlorin e6 by spectroscopic video fluorescence methods

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Abstract. This paper presents the results of using intraoperative fluorescence diagnostics (FD) with the endoscopic video system to increase the efficiency of photodynamic therapy (PDT). The molecular form of chlorin e6 was used as a photosensitizer (PS). All patients received an intravenous administration drug based on chlorin e6 at the concentration of 1 mg/kg. Diagnostics and therapy were carried out in 4 patients diagnosed by malignant neoplasms of the lateral surface of the tongue. Determination of the boundaries by changing the signal of the fluorescence intensity of the tumor was carried out before PDT and after PDT. The efficiency of PDT was assessed by the fluorescent signal of the tumor decreasing when compared with non-pathological normal tissue. The FD method allows to determine accurately the actual size of the tumor and its borders. All patients underwent PDT influenced by the therapeutic laser with a generation wavelength of 660 nm. It is shown that the using of fluorescence diagnostics improves the quality of the photodynamic therapy, since it is possible to assess the photobleaching of the drug during the operation.

1. Introduction
Up to half a million cases of head and neck cancer are diagnosed in the world every year [1]. In 2020, 12402 new cases of oral cancer and 5737 deaths from this cause were registered in Russia [2]. The anatomical structure of the mucous membranes of the head and neck requires extensive medical intervention [3]. Standard methods for diagnosing oncological diseases include ultrasound and X-ray scan, computed and magnetic resonance imaging, histodiagnosis [4]. All of these methods have some
disadvantages, such as low resolution, long study time, nephrotoxicity of contrast agents and high diagnostic costs. However, cancer detection at an early stage or determination of the exact boundaries of a tumor could not give a high accuracy result, since the above methods are a priority in working with bulk tumors. In addition, computed tomography images give to patients a dose of ionizing radiation, which limits the number of such images for a patient within the framework of radiation safety standards (for example, one image of the spine can fill the allowed safe dose for a patient). The described disadvantages lead to the searching for new physical diagnostic methods.

One of the promising methods for the diagnosis of malignant neoplasms in the early stages is fluorescence diagnostics (FD) [3]. FD is based on the interaction of laser radiation with PS, which selectively accumulates in tumor cells after intravenous administration. This diagnostic technique is safe and non-invasive, in addition, there is no dose load on the patient, since the laser radiation is not ionizing [5].

The standard treatments of head and neck tumors are surgical resection, chemotherapy, and radiation therapy. These methods have limitations in their application, high risks of incomplete tumor resection, which leads to relapses and the formation of new tumors and significantly reduce the quality of patient’s life. It is worth noting that secondary tumors are often more aggressive and less responsive to treatment. The main disadvantage of conventional treatments of head and neck cancers is the loss of the ability to vital skills such as chewing, swallowing, and the ability to speak. Photodynamic therapy is a promising technique for the treatment of head and neck tumors due to the possibility of selective destruction of cancer cells [6, 7].

FD and PDT are based on photochemical reactions on the interaction of PS, light of a certain excitation wavelength, and oxygen. The interaction mechanism is presented in the Yablonsky diagram Figure 1 [8].

![Yablonsky diagram](image)

This diagram shows the energy levels and transitions of two molecules: PS and triplet oxygen. The PS molecule absorbs by laser light and moves from the ground state to the excited state, then the PS molecule nonradiatively goes into the triplet state. After that two transitions are possible: the PS molecule returns to the initial state with the emission of a photon, or the energy of the triplet state is transferred to the oxygen molecule, transforming it into the singlet state [9, 10]. Otherwise, the oxygen molecule should be located close enough to the PS molecule (about 20 μm) for energy transfer according to the Franck-Condon principle [10, 11]. PS fluorescence phenomenon is observed due to exposing of low-intensity radiation, however a therapeutic effect appears by radiation with high light power [9, 12, 13, 14].
On the Yablonsky diagram, the red arrow indicates the absorption of a photon by the PS molecule, the blue dashed arrows correspond to nonradiative transitions, the yellow arrow corresponds to the emission of a fluorescent photon, the black wavy arrow indicates the transfer of energy from the triplet level of the PS molecule to the triplet level of the oxygen molecule, the black straight arrow corresponds to the excitation of the oxygen molecule from triplet to singlet level.

In this work, the effectiveness of FD and PDT with a drug based on Chlorin e6 was studied.

2. Research methodology

2.1 Video system design
The fluorescent intraoperative video system, manufactured by “BIOSPEC” LLC, consists of a white light source, a laser radiation source with a generation wavelength at 635 nm, two cameras for recording a color and a fluorescent image, Y-shaped light guide and an endoscope from “BIOSPEC” LLC (Figure 2).

![Figure 2. Schematic representation of a fluorescent video system.](image)

Images can be obtained in a familiar color via the white light source, which allows doctors to orientate themselves in the area of interest. A laser radiation source with a generation wavelength at 635 nm is required to excite the PS fluorescence during diagnosis. Two cameras - color and black and white - are needed to work in three different modes: color image, fluorescence fixation in black and white image, and overlay mode. When switching to overlay mode, the image of the fluorescent signal from the black and white camera is superimposed on the color image and colored in pseudo green. Then the system software calculates the average value of the radiation intensity in the cursor area: for a black-and-white camera, the intensity of the image pixels is taken, for a color camera, the intensity of the image pixels of the red component of the spectrum is taken. These intensity values are normalized to the parameters of the black-and-white and color cameras, respectively, after which the ratio of the first parameter to the second is calculated. The final value of the obtained fluorescence index is displayed on the computer screen, and different parts of the investigated area are colored green in accordance with the intensity of the PS fluorescence signal. The spatial resolution of the developed system is 0.05 mm [14]. This method makes it possible to assess the PS accumulation in different tumor tissues and accurately determine the boundaries of the tumor.

2.2 Chlorin e6 as a photosensitizer
We used the medicine based on Chlorin e6 as a photosensitizer with the commercial name “Photoditazin”, produced by “VETA-GRAND” LLC. The maximum accumulation of chlorin e6 in tumor tissue was recorded in 2 hours after intravenous administration [15]. Chlorin e6 has a tropism for
tumor cells, which allows PDT to be performed only on pathological tissues without damaging normal tissues [15]. In figure 3, the maximum absorption peak of chlorin e6 is observed at 660 nm wavelength.

![Absorption and emission spectra of chlorin e6](image)

**Figure 3.** Absorption and emission spectra of chlorin e6.

The generation wavelength of a therapeutic laser for PDT should be within this absorption peak. In this work, a therapeutic laser with a generation wavelength of 660 nm was used, which makes it possible to transfer the largest number of chlorin e6 molecules to an excited state and obtain the greatest therapeutic effect. The generation wavelength of the diagnostic laser at 635 nm is observed at the tail of the absorption peak and excites fluorescence, but the radiation power density is much lower than the radiation power density of the therapeutic laser, therefore the therapeutic effect was manifested only when the therapeutic laser was used.

### 2.3 Performing FD and PDT

Diagnostics and therapy were carried out at the moment of maximum accumulation of chlorin e6 in 2 hours after intravenous administration at a concentration of 1 mg/kg. Before PDT, each of the patients underwent fluorescence diagnostics to assess the accumulation of chlorine e6. The fluorescence index of the tumor was compared with the fluorescence index of healthy tissue, the index of which is 10 relative units. After determining the size and border of the tumor, the time of exposure to a therapeutic laser for PDT was calculated. After PDT, a control FD is performed to determine the effectiveness of photodynamic exposure. A new fluorescence index of the tumor is recorded and compared with the fluorescence index before PDT. For complete photobleaching of the PS, it is necessary to reduce the fluorescent signal of the tumor to the values of normal tissue.

The study of the effectiveness of PDT was carried out on 4 patients aged from 61 to 76 diagnosed with squamous cell keratinizing cancer of the lateral surface of the tongue. Each patient was injected intravenously with a preparation based on chlorin e6 at a concentration of 1 mg/kg. Fluorescence diagnostics and photodynamic therapy of tongue cancer were carried out. All patients received a radiation dose from 100 to 150 J/cm².

### 3. Results and Discussion

In one of the patients with squamous cell keratinizing cancer of the lateral surface of the tongue, fluorescence signal and tumor boundaries were quantitatively determined during fluorescence
Two tumor areas were detected, each of them was irradiated with 100 J/cm² dose and a power density of 1.02 W/cm². Each of the regions was first irradiated for 6 minutes, after FD was performed for photobleaching control. The PS fluorescence signal decreased from 22.5% to 45% for both zones, which turned out to be insufficient for a therapeutic effect; therefore, each zone was irradiated for another 4 minutes with a power density of 1 W/cm².

Figure 4. Images from the video system: (a), (b) is a tumor area before PDT, tumor fluorescence index 20-38; (c), (d) is a tumor area after PDT fluorescence index 9. Yellow arrows indicate the area of interest.

Figure 5. Images from the video system: (a) tumor area before PDT, tumor fluorescence index 22; (b) tumor area after PDT, fluorescence index 3. Yellow arrows indicate the area of interest.
In another case, in a patient with invasive squamous cell keratinizing cancer of the lateral surface of the tongue, during fluorescence diagnostics, the fluorescence index of the tumor was twice the fluorescence index of normal tissue.

The tumor was visually divided into several areas with a diameter of 1 cm each and were irradiated with a power density of 1.9 W/cm² for 2 minutes. As a result of PDT, the fluorescence index decreased to the values of normal tissue, which indicates a significant photobleaching of chlorin e6.

4. Conclusion
The results, which were obtained using a fluorescent intraoperative video system, show the selective accumulation of chlorin e6 in the tumor area and its burnout after photodynamic exposure. Assessment of PS accumulation before and after PDT significantly increases the effectiveness of PDT, reducing the risk of recurrence and the formation of secondary tumors. This technique allows, on the one hand, to correct the radiation dose in time and achieve the desired therapeutic effect, and on the other hand, to prevent the supply of excessive power density if the therapeutic effect is achieved.

The video system allows to assess of chlorin e6 photobleaching during therapy, which enables oncologists to make immediate flexible decisions for more effective treatment of malignant neoplasms. By photobleaching of chlorin e6 immediately after therapy, it was determined that the fluorescent signal decreased several times. Based on the results of operations, all 4 patients have no data of relapses.

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