Article

Rapid diagnosis of laryngeal cancer using Raman fluorescence spectroscopy

Alina Timurzieva [1], Vladislav Kotov [2*], Valentin Popadyuk [3], Igor Ganshin [4]

[1] Department of otorhinolaryngology, RUDN University, 117198, Russia; alinko9977z@mail.ru
[2] Department of plastic surgery, RUDN University, 117198, Russia; fnkc.vladislav@gmail.com
[3] Department of otorhinolaryngology, RUDN University, 117198, Russia; lorval04@mail.ru
[4] Department plastic surgery, RUDN University, 117198, Russia; gibdoc@yandex.ru
* Correspondence: fnkc.vladislav@gmail.com; Tel.: +7 921 127 96 77

Abstract: Aims: to provide a scientific and practical justification for the use of Raman fluorescence spectroscopy in the early express-diagnostics of laryngeal cancer. Patients and methods. A total of 83 patients with a diagnosis of laryngeal cancer were examined. The spectra of the intact mucous membrane of the larynx, the anatomical structures of the larynx in cancer and precancer were analyzed. The individual features of the bands of Raman scattering of light and fluorescent signals in intact tissues, in case of dysplasia, and squamous cell carcinoma of the larynx were evaluated. To compare the data of Raman fluorescence spectroscopy, the histological method was used in this study. The study evaluated the sensitivity and specificity of the method. To take the spectra, a laser setup was used using both fluorescent and Raman components, with a wavelength of 532 nm. Measurements were taken immediately after removal of the laryngeal tissue (1-5 seconds) involved in the malignant process. Results. In this study, using Raman fluorescence spectroscopy, individual spectral characteristics of the tissues of the larynx were obtained in intact tissues and in laryngeal cancer, as well as in dysplasia (precancer). In this study, in the above groups there are differences both in fluorescence signals and in Raman scattering bands. This information can be useful in the early diagnosis of laryngeal cancer for the clinicians. Conclusions. In the future, Raman fluorescent medical technologies can be used for the purpose of early express diagnostics of tumor diseases of the larynx. Clinical Medicine and Public Health and Healthcare in general need the development of technologies of this kind in order to improve the quality and efficiency of diagnosing and treating diseases at the stage of their initiation.

Keywords: Raman-fluorescence medical technologies, laryngeal cancer, squamous cell carcinoma of the larynx, early diagnosis of cancer, tumor diseases of the ENT-organs..

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1. Introduction

More than 600,000 of new of malignancies cases are registered in Russia annually, and there are approximately 300 thousand deaths from this nosology every year [1]. Laryngeal cancer is one of the most common types of squamous cell carcinoma of the head and neck [2-6]. According to statistics, 13,000 of new cases are expected annually in the USA with a 70% survival rate [7]. Laryngeal cancer ranks 20th in the structure of the most common types of malignant neoplasms, more than 150,000 of new cases are detected every year in the world [8]. Moreover, this type of cancer occurs in one third of cases of malignant neoplasm of head and neck with squamous cell transformation [9]. Optimal treatment of this pathology requires an interdisciplinary approach with the participation of different medical specialists [10].

Determination of tumor diseases biomarkers is one of the most important tasks in clinical practice [11-12]. Late diagnosis and a significant decrease in the life quality of patients with cancer of different localization serve as an additional motivation for the development of early methods for diagnosing cancer. Early express diagnostics of tumor diseases of the ENT organs is no exception [8,13,14]. In particular, the search for markers of the oncological process in laryngeal cancer

deserves close attention [15]. Among other things, in laryngeal cancer strict adherence to the boundaries of tumor tissue resection during surgery is very important in order to avoid the recurrence of the pathological process, so it is necessary to develop an accurate and rapid intraoperative determination of the boundaries of prevalence malignancy in laryngeal cancer [16,17]. Recent scientific articles describe the use of Raman scattering in combination with deep irradiation and related hardware and software systems in relation to the identification of laryngeal squamous cell carcinoma [18]. The question of increasing the sensitivity and specificity of methods for early indication of the pathological process in laryngeal cancer remains in demand due to the increasing frequency of cases with the relapse of the disease [19]. Despite the use in practice such methods as histological [20], immunohistochemical [14], and polymerase chain reaction [21], scientists around the world are faced with the important issue of accelerating the process of identifying the oncological process - the implementation of its immediate determination in real time. Patients with this pathology visit doctor at an advanced stage, when treatment may be either completely ineffective or insufficiently effective. Accordingly, doctors faced a difficult task - to conduct high-quality, highly sensitive and specific, fast, non-invasive diagnosis of cancer at an early stage, even before clinical manifestations. Accordingly, doctors are faced with a difficult task - to conduct a high-quality, highly sensitive, highly specific, fast, non-invasive diagnosis of cancer at early stage, even before clinical manifestations. Histological examination, despite its accuracy, very often takes a lot of time, and also, often, is carried out at an advanced stage of larynx cancer, given the peculiarities of routing and patient referral, which is a separate challenge. The search for modern methods for the early detection of oncological diseases, including laryngeal cancer, using highly sensitive, highly specific, accurate, fast, non-invasive diagnostic methods is one of the most important tasks of public health and healthcare [22-24]. Raman fluorescent medical technologies have proven themselves as such diagnostic methods [25-30]. Currently, Raman scattering is used in the diagnosis of tumor diseases of the ENT organs, including laryngeal cancer [31-32].

Today there are certain difficulties for using in clinical practice these methods, such as the high cost of equipment, the lack of doctors who can do this technology and, accordingly, training in this area; insufficient development of algorithms for diagnosing tumor and inflammatory diseases of the ENT organs, including cancer of the larynx, as one of the most common types of oncological pathology in the practice of otorhinolaryngologist and oncologist. In connection with the above, the purpose of the study was set.

The aim of the study: to provide a scientific and practical justification for the use of Raman fluorescence spectroscopy in the early express-diagnostics of laryngeal cancer.

2. Patients and Methods

A total of 83 patients with a diagnosis of laryngeal cancer were examined. The spectra of the intact mucous membrane of the larynx, the anatomical structures of the larynx in cancer and precancer were analyzed. The individual features of the bands of Raman scattering of light and fluorescent signals in intact tissues, in case of dysplasia, and squamous cell carcinoma of the larynx were evaluated. To compare the data of Raman fluorescence spectroscopy, the histological method was used in this study. The study evaluated the sensitivity and specificity of the method. To take the spectra, a laser setup was used using both fluorescent and Raman components, with a wavelength of 532 nm.

Measurements were taken immediately after removal of the laryngeal tissue (1-5 seconds) involved in the malignant process.

In this study we used the WHO classification accepted in Paris in 2005, according to which 3 stages of laryngeal intraepithelial neoplasia of squamous epithelium (LIN) are distinguished: LIN 1, LIN 2, LIN 3: mild dysplasia, moderate dysplasia, severe dysplasia, and cancer in situ.

3. Results.

The main distinguishing feature of the intact tissue surrounding the tissue of the larynx involved in the tumor process in squamous cell carcinoma, as shown in Figure 1, is that in this case there are no peaks at wavenumbers of 1100 cm -1 and 1500-1650 cm -1, which registered at all stages of the course of squamous cell carcinoma of the larynx, including at the stage of LIN 3-dysplasia, grade 3.

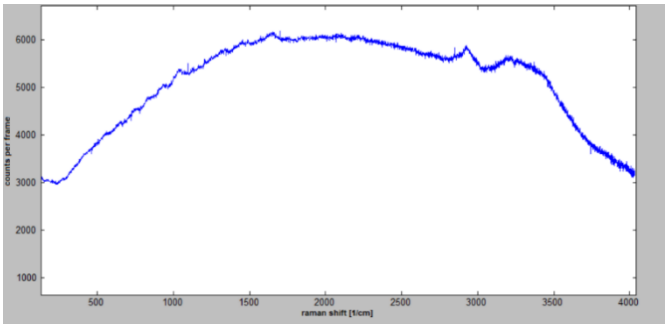

Figure 1. Spectrum of intact laryngeal tissue not involved in the tumor process, surrounding larynx squamous cell carcinoma. On the abscissa axis - the magnitude of the wave numbers in reciprocal centimeters (cm -1), along the ordinate axis - the intensity of fluorescence (in relative units - OE).

A total of 24 patients were examined, 24*20=480 spectra. As we can see, there is a small peak at 2900 cm -1, after which an additional rise in fluorescence is recorded. In 11 patients with squamous carcinoma of the larynx (undifferentiated type, with an average degree of keratinization), 220 spectra were analyzed from the surface of the tissues of the larynx. In this case, Raman peaks were registered at 1250, 1400, 1900/cm. 2 rises in fluorescence were registered with maximum peaks at 11500 and 12100 relative units (RU) (maximum fluorescence intensity was visualized).

In 12 patients, 240 spectra were analyzed. As noted in Figure 2, 2 fluorescence signals are recorded with a maximum at 1200 cm -1 (8500 OU), 2100/cm (12000 OU). When this is visualized Raman peaks on the values of the wave numbers: 1200 cm -1, 2900 cm -1.

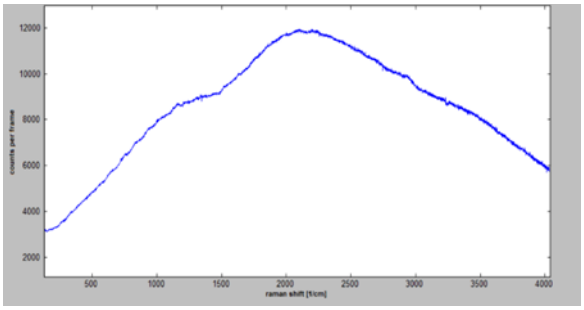

Figure 2. Spectrum of squamous cell carcinoma of the larynx, poorly differentiated, non-keratinizing type of tumor.

In 22 patients, 440 spectra were analyzed. According to Figure 3, the main peaks are recorded at wave numbers 1100 cm -1, 1500 cm -1, 2900 cm -1, additional peaks are recorded at 800, 900, 1000, 1250, 1400, 1600, 1700, 2700 cm -1. The first maximum is at 13200 OE, the second maximum is at 13000 OE, the third maximum (main peak) is at 9000 OE.

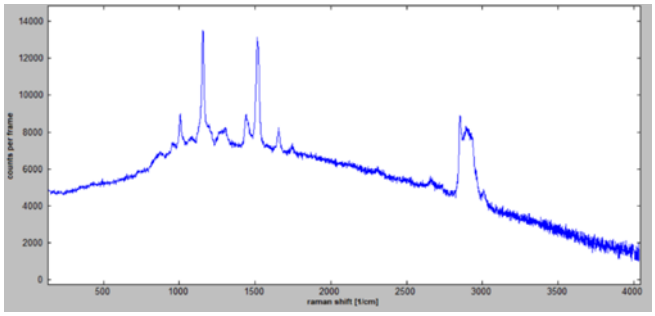

Figure 3. Spectrum of squamous cell carcinoma of the larynx with metastasis in regional lymph nodes.

Figure 4 shows that the intensity of the main peaks is lower, their configuration is less pronounced, with smoother transitions, however, these peaks stand out against the general background of the generalized fluorescence signal. There are peaks at 1100, 1500, 2900 cm -1, with a maximum fluorescence intensity at 6500, 6900 and 4800 cm -1. In 14 patients, 280 spectra were recorded.

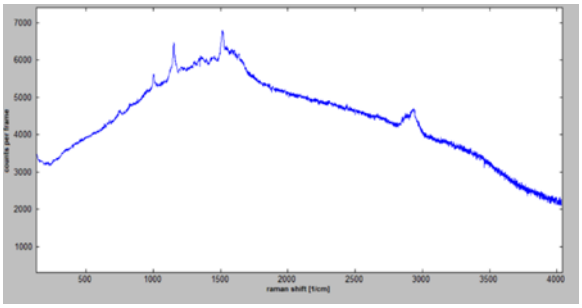

Figure 4 Spectrum of tumor tissue during the formation of squamous cell carcinoma of the larynx (LIN 3 with the transition to cancer in situ), a highly differentiated type of tumor tissue.

As noted in the course of the study, the more highly differentiated type of squamous cell carcinoma, the more the spectrum correlates with the spectrum of intact laryngeal tissue, and vice

versa, with a low-differentiated type, a smoother spectral curve is noted, a more powerful fluorescence signal.

Also, important acquires the process of keratinization in squamous cell carcinoma of the larynx. In squamous cell carcinoma of the larynx without keratinization and with keratinization, spectral differences are noted, which in the future can be used to determine the type of tumor.

4 Discussion

The obtained results correlate with the world literature data. Thus, the use of transnasal Raman spectroscopy for differentiation of tumor and intact tissue during endoscopic examination in the "real time" mode (in the range of wave numbers 2800-3020 cm -1) in the region of the anatomical structures of the larynx is described.

This study showed that Raman spectroscopy has the potential for non-invasive diagnosis and real-time detection of laryngeal cancer at the molecular level [33]. Raman spectra in the range of 800-1800 cm -1 from various tissue sites, taken immediately after surgery for 5 seconds, can illustrate the changes occurring in the tissue with a diagnostic sensitivity of 88.0% and a specificity of 91.4% in relation to the identification of malignant neoplasms larynx [34].

Conducting fiber-optic Raman spectroscopy in the range of 800-1800 cm -1 for the diagnosis of cancer of the larynx in vivo in the course of an endoscopic study has demonstrated effectiveness in differentiating tissues of the larynx in cancer and normal. The Raman spectra of intact and tumor tissues of the larynx differ significantly, which may be due to different amounts and ratios of proteins, lipids, nucleic acids, and the content of bound water in the cells of the anatomical structures of the larynx [35].

The literature describes the state of the art in optical techniques, with particular attention paid to the combined intraoperative use of fluorescence imaging and Raman spectroscopy for targeted imaging during resection of tumor tissue within an intact area with well-defined margins [36].

When using Raman light scattering (wavelength 785 nm) and registration of excitation in the range of wave numbers 50-1500 cm -1, it seems possible to determine the types of biomolecules of each group of tissues under study (phospholipids, amides, tyrosine, phenylalanine, collagen, etc.) [37].

The technology of surface-enhanced Raman scattering of light has shown promise in diagnosis of cancer of various organ locations in vivo. Ordinary Raman signals are extremely weak and are easily distorted by fluorescence. The sensitivity and specificity of the combined method, according to some authors, were 99.2% and 98.4%, respectively (for the identification of intact and tumor tissues) [31].

Thus, clinical practice today requires the development of accurate methods for diagnosing cancer in situ and rapid identification during surgical intervention for the purpose of resection of a malignant neoplasm, which is the key to reducing cancer morbidity and mortality. To date, there are many diagnostic methods, however, none of them has a sufficient level of specificity, sensitivity, spatial and temporal resolution, speed and accuracy. In addition, most methods cannot provide information on the molecular composition of the tissue.

In this case, Raman fluorescence spectroscopy is of particular interest in the differentiation of resection margins of tumor tissue within intact tissue in "real time" mode [25]. The Raman

spectrum illustrates the internal "molecular imprint" of the tissue, and any biochemical change associated with an inflammatory or tumor state of the tissue is reflected in this "spectral imprint". Currently, Raman spectroscopy systems in combination with modern hardware and software systems and machine learning methods can serve as additional diagnostic and treatment tools in oncological practice [38-40]. Raman measurements in vivo have been made possible by recent technological advances in Raman endoscopy and signal amplification facilities [41].

As a result of the study, it was noted that the method of Raman fluorescence spectrometry in the future can be used to expressly identify the tumor and pre-tumor process in laryngeal cancer, in particular, in squamous cell carcinoma of the larynx at its various stages and with various types of tumors. Given that the tissue spectra in normal and laryngeal cancer differ, the clinician needs a database of spectra that allows identifying malignant growth even at the initiation of the precancerous process, since at this stage, patients, as a rule, do not go to the doctor yet, but can be examined as part of a medical examination.

5. Conclusion

Summarizing the above, it should be noted that in the development of a special algorithm for combined diagnostics, Raman fluorescence spectroscopy can be used as a screening technique for the purpose of early rapid identification of larynx cancer with subsequent application in clinical practice, which is the goal of further research.

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Conflicts of Interest: The authors declare no conflict of interest.

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