CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.2604489

Available online at: http://www.iajps.com *Research Article*

PROSPECTS OF USE OF FLUORESCENT MEDICAL TECHNOLOGIES IN DIAGNOSTICS OF INFLAMMATORY DISEASES IN OTORHINOLARYNGOLOGY

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Article Received: January 2019 **Accepted:** February 2019 **Published:** March 2019

Abstract:

To substantiate use of fluorescent medical technologies in diagnostics of inflammatory diseases in otorhinolaryngology a technology was applied which is based on phenomenon of autofluorescence of tissue und on effect of combinatory scattering of light (Raman effect) that allows to register individual molecular vibrations in crystal lattice of substances which are parts of the tissue being examined. In future this effect will allow to use the medical technology as a diagnostic one. During the research a probe radiation with wavelength of laser radiation 532 and 405 nm was used. Spectral characteristics of tissues were obtained by means of hardware-software complexes EnSpectr R532 and EnSpectr M405 for registration of indexes both in vivo and in vitro. In addition, the study presents data which have been obtained in respect of microflora of pathologic focus (palatine tonsils) in an inflammatory process in order to evaluate contribution of microbial factor to resulting indexes of spectral characteristics of a biologic object. Results of the study show a possibility to use fluorescent technologies not only for evaluation of metabolic, morphometric and functional indexes of tissue in vivo over time, but also for analysis of individual spectral data of microbial composition of a pathologic focus and for analysis of histologic features of tissue in vitro. Nowadays diagnostic potentials of optic technologies (including fluorescent ones) are actively studied. This study presents experience of use of the above mentioned technologies for substantiation of prospects of application and for determination of range of their clinic use in clinic ENT practice.

Key words: *fluorescence, sensitivity, specificity, noninvasive medical technology, quick diagnostics, Raman effect (effect of combinatory scattering of light), inflammatory diseases, otorhinolaryngology.*

Abbreviations: ENT - otorhinolaryngology, RFD - Raman fluorescent diagnostics, AI - aerobicity index, NIF - normalized index of fluorescence, CFU - colony-forming units, CT DF - chronic tonsillitis, decompensated form.

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Please cite this article in press A. B. Timurzieva et al., ***Prospects of Use of Fluorescent Medical Technologies in Diagnostics of Inflammatory Diseases in Otorhinolaryngology.,*** *Indo Am. J. P. Sci, 2019; 06(03).*

INTRODUCTION:

Today inflammatory diseases of ENT organs are among the most widespread diseases in general population [4]. In particular, one of the most important tasks is development of methods of diagnostics of chronic tonsillitis and other inflammatory diseases of ENT organs [3]. It is known that one of key factors in development of inflammatory diseases of ENT organs is microbial factor [5]. Furthermore, disturbance of symbiosis between macro- and microorganisms, acquisition of pathogenic properties by the latter ones [2] can lead to a disease too, despite absence of pathogenic flora. In addition to many various methods of diagnostics of pharyngeal diseases such as bacteriologic, bacterioscopic, cytological methods, PCR diagnostics, the fluorescent methods should be developed and studied because they are among the most sensitive, specific, low-invasive and quick ones [1, 6, 8, 9].

Laser fluorescent method with Raman component can be considered as one of such methods [7]. Raman effect is based on the fact that when a substance is irradiated by light with a certain wavelength, its inelastic scattering is detected on molecules; this scattering is accompanied by a considerable change of irradiation frequency [9].

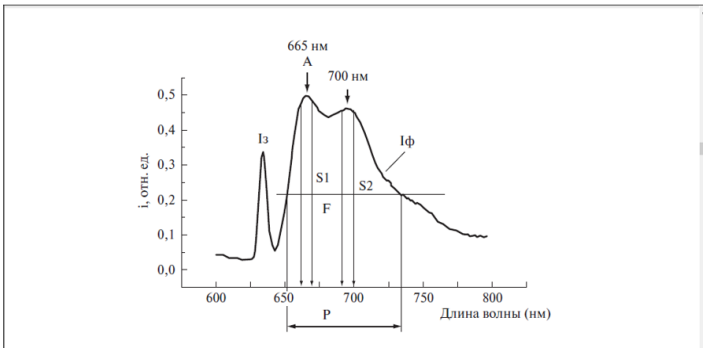
Goal of our study is to substantiate prospects of use of fluorescent methods in diagnostics of diseases of ENT organs.

The presented study has the following main tasks:
- spectral characteristics of tissue/pathologic substrate in inflammatory diseases of ENT organs must be registered and the received data have to be analyzed;
- sensitivity and specificity of the developed technique must be evaluated in an ENT clinic;
- results of study have to be compared with features of spectral data of a causative agent (microbial cultures in pure form) and possibility of use of the technique in its various variations must be substantiated.

MATERIALS AND METHODS:

Participants in the study were patients with chronic tonsillitis in decompensated (N=68) and compensated (N=72) form as well as patients with chronic rhinosinusitis (N=16) and polypous rhinosinusitis (N=12), chronic purulent otitis media (N=14) and healthy volunteers (N=69).

For measurements we used hardware-software complexes EnSpectr R532 and EnSpectr M405 (certificate No. RZN 2015/2419 dated May 18, 2015). The hardware-software complex (HSC) includes a green solid-state laser and a blue one Nd:YAG (2w) with wavelengths 532 nm and 405 nm, CCD matrix, spectrometer with diffraction grating 1200 grooves/mm with spectral resolution 4 cm^{-1}, it also includes an optical system for focusing and filtering of signal, controller and computer with software for recording of spectra. Laser power is 10 mW, recording of spectra was performed during exposure time 1 sec. Registration of spectral characteristics of tissue by means of HSC EnSpectr in inflammatory diseases of ENT organs was performed by contact, stable method. To secure accuracy we took measurements in several points on surface of the biologic object, which was examined - from 4 to 10 points. Sensitivity and specificity of the method was calculated on the basis of results of histologic examination of tissue and amounted to more than 95%. Picture 1 below shows and explains the indexes which we used in our work:

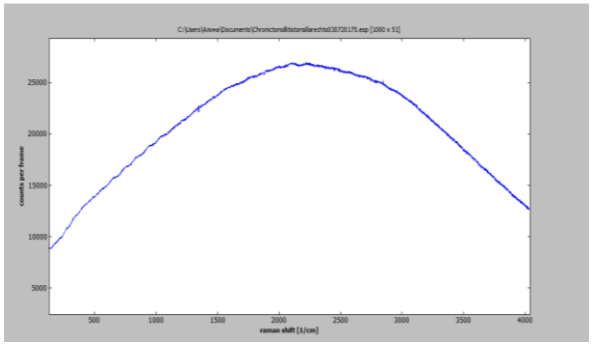


Pic. 1 Spectrum of fluorescence of bacteria.

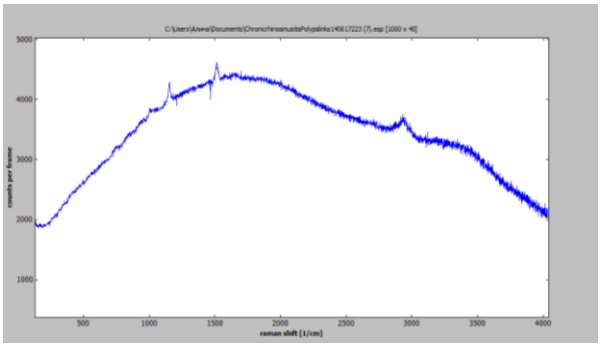
Ip - signal on wavelength of probing, If - signal on wavelength of fluorescence, S1 - intensity of fluorescence on wavelength 665 nm, S2 - intensity of fluorescence on wavelength 700 nm, A - maximal amplitude of fluorescence power, F - integral intensity (power) of fluorescence, measured in relative units,

S1/S2 - coefficient which is equal to ratio of fluorescence intensity at wavelength 665 nm to fluorescence intensity at wavelength 700 nm (aerobicity index); when additional peaks at different wavelengths are registered, S1/S2 is ratio of area under curve 1 to area under curve 2 on respective wavelengths what we call the normalized index of fluorescence; P - spectral half-width of fluorescence [1].

RESULTS OF OWN RESEARCH:

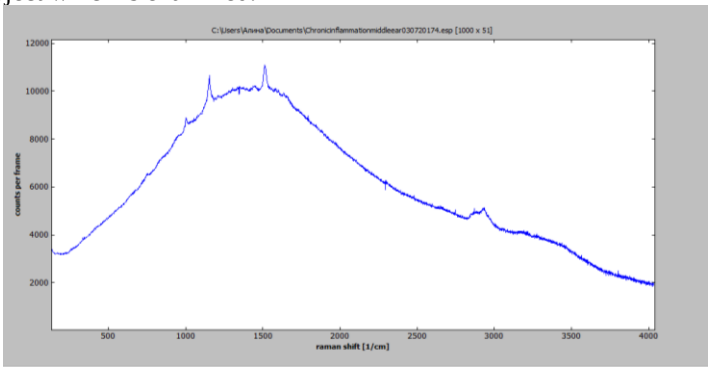


Pic. 2a (on the left) Spectrum of tissue of palatine tonsils of a patient with chronic tonsillitis in decompensated form. There are wavenumbers on the abscissa axis (1/cm) and intensity of fluorescence in absolute units on the ordinate axis.



Pic. 2b (on the right) Spectrum of pathologic substrate (polypous tissue) from maxillary sinus of a patient with polypous rhinosinusitis; it has been obtained after an endoscopic operation.

Form of the fluorescent curve, presence of additional peaks at certain wavelengths (wavenumbers), difference of values of aerobicity indexes, normalized indexes of fluorescence allow to prognosticate presence of an inflammatory process in the biologic object which is examined.

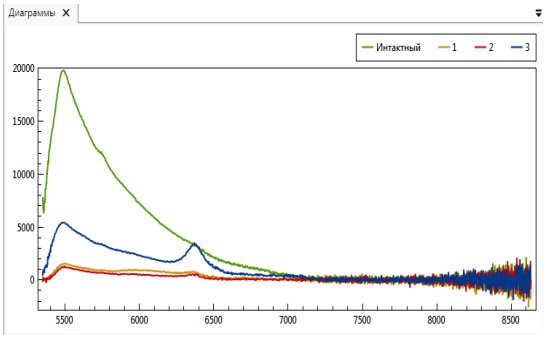


Pic. 3 Spectrum from surface of mucous tunic of middle ear in chronic purulent otitis media.

Characteristic peaks at 1100/cm, 1500/cm and 3000/cm are detected which are typical for an inflammation; peaks are additionally visualized at 750/cm and 1000/cm too. One can see on Pictures 2a, 2b and 3 that spectra in chronic tonsillitis, chronic rhinosinusitis (including polypous process in sinuses) and in chronic purulent otitis media differ. In addition, after analysis of spectra, which had been obtained from surface of biologic tissues in inflammatory diseases of ENT organs, and after treatment of results by means of

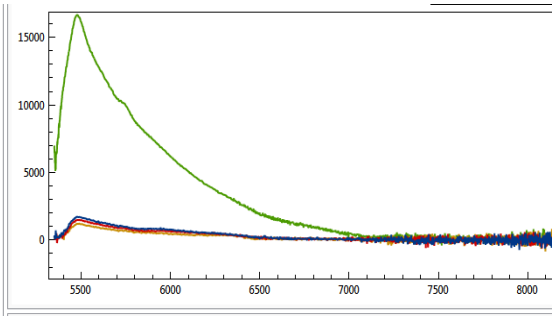
statistical methods a registration of peaks at wavenumbers 1100/cm, 1500/cm and 3000/cm was detected; the above mentioned peaks were not registered in health (when there was no disease, i.e. in healthy volunteers).

Therefore we can conclude that an inflammatory process is characterized by similar spectral data but they have their distinctive features inside the examined groups; they should be analyzed by use of principal components method, method of projection to latent structures, linear discriminant analysis and nonparametric methods of statistics. Pictures and tables below present graphic and digital data of spectral characteristics of tissue of palatine tonsils and microbial cultures from surface of a pathologic focus of a patient with chronic tonsillitis in decompensated form.



Pic. 4a (on the left) Spectral characteristics from surface of palatine tonsils of patient N with chronic tonsillitis in decompensated form (CT DF).

There are wavenumbers on the abscissa axis (1/cm) and intensity of fluorescence in absolute units on the ordinate axis. Green color marks the spectrum from surface of an intact point (skin of inner surface of patient's forearm); red color marks the point 1 (upper pole of palatine tonsil); yellow color marks the point 2 (lower pole of palatine tonsil); blue color marks the point 3 (lacuna of palatine tonsil).



Pic. 4b (on the right) Spectra of tissue of healthy volunteers' palatine tonsils.

Designation of index	AI 3	NIF 3	AI 2	NIF 2	AI 1	NIF 1	AI intact	IF* intact
Value of index (tissue of palatine tonsils in chronic tonsillitis)	1.25	0.335	1.63	0.0601	1.95	0.0614	1.77	1.03E+07

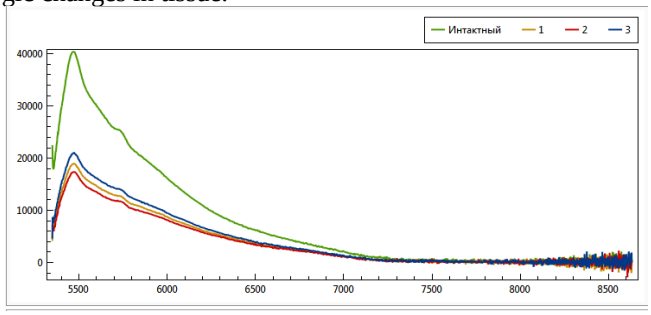
* IF - intensity of fluorescence

Table 1a Values of aerobicity indexes (AI) and normalized index of fluorescence (NIF) in examined points in CT DF.

One can see on the Picture 4a that an additional peak is registered at the value 6350/cm; this peak is typical for hypoxia in tissues and this fact had been revealed in previous studies [1]. The above mentioned peak indicates content of protoporphyrin IX in tissues, this protoporphyrin accumulates in tissues in hypoxia, in particular, in a long inflammatory process; no such phenomenon is detected in health (Pic. 4b).

We proved by use of statistical methods that this peak (665-670 nm in terms of a wavelength unit) is registered in all patients with chronic inflammatory diseases of ENT organs. However, to conduct differential diagnostics a more detailed and deep analysis of processes is needed, which take place on microlevel in tissues, this analysis must be

accompanied by evaluation not only of morphometric indexes but also of the metabolic ones and microbial factor as well as of physiologic changes in tissue.

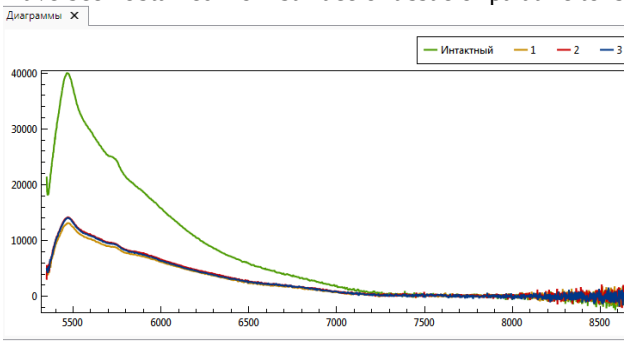


Pic. 5a Spectral characteristics of microbial colonies (Streptococcus parasanguis 10*6 CFU/ml) of a patient with CT DF (biologic material was taken from surface of palatine tonsils and incubated on a special nutrient medium with use of blood agar).

The table below summarizes indexes of spectral characteristics of microbial colonies of culture Streptococcus parasanguis 10*6 CFU/ml on blood agar (biologic material from surface of palatine tonsils of patient N with chronic tonsillitis in decompensated form); Э - designation of indexes of pure culture, it is calculated as difference between indexes of nutrient medium + colonies and nutrient medium in pure form.

Streptococcus parasanguis 10*6 CFU/ml	AI 3	NIF 3	AI 2	NIF 2	AI 1	NIF 1	AI intact	IF intact
Culture on blood agar	1.52	0.567	1.51	0.476	1.52	0.51	1.51	2.33E+07
Blood agar	1.48	0.73	1.47	0.702	1.47	0.573	1.52	2.27E+07
Э	0.04	0.16	0.04	0.23	0.05	0.06	0.01	0.06E+07

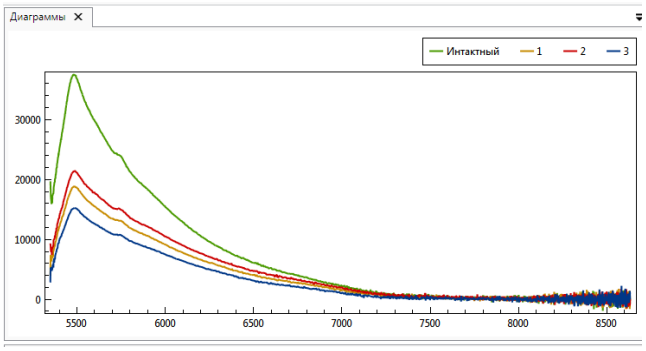
Table 1b Aerobicity indexes and normalized indexes of fluorescence - digital equivalent of spectral characteristics of colonies Streptococcus parasanguis 10*6 CFU/ml in pure form. Obtained data are considerably lower than equivalent spectral characteristics which have been obtained from surface of tissue of palatine tonsils (Pic. 5a).



Pic. 5b Spectral characteristics of microbial colonies (Staphylococcus aureus 10*6 CFU/ml) of a patient with CT DF (biologic material has been taken from surface of palatine tonsils and incubated on a special nutrient medium with use of chocolate agar).

Staphylococcus aureus 10*6 CFU/ml	AI 3	NIF 3	AI 2	NIF 2	AI 1	NIF 1	AI intact	IF intact
Culture on chocolate agar	1.59	0.394	1.53	0.411	1.54	0.382	1.54	2.2E+07
Chocolate agar	0.947	0.0205	2.05	0.0188	1.25	0.0184	1.54	2.31E+07
Э	0.64	0.37	0.52	0.39	0.29	0.36	0	0.11E+07

Table 1c Aerobicity indexes and normalized indexes of fluorescence - digital equivalent of spectral characteristics of colonies Staphylococcus aureus 10*6 CFU/ml in pure form.



Pic. 5c Spectral characteristics of colonies of microbes Staphylococcus aureus 10*6 CFU/ml + Staphylococcus aureus 10*6 CFU/ml from surface of palatine tonsils of patient N with CT DF.

Staphylococcus aureus 10*6 CFU/ml + Staphylococcus aureus 10*6 CFU/ml	AI 3	NIF 3	AI 2	NIF 2	AI 1	NIF 1	AI intact	IF intact
Culture on chocolate agar	1.49	0.454	1.4	0.662	1.41	0.57	1.47	2.23E+07
Chocolate agar	0.947	0.0205	2.05	0.0188	1.25	0.0184	1.54	2.31E+07
ℨ	0.54	0.43	0.65	0.64	0.16	0.55	0.07	0.08E+07

Table 1d Aerobicity indexes and normalized indexes of fluorescence - digital equivalent of spectral characteristics of colonies Staphylococcus aureus 10*6 CFU/ml + Staphylococcus aureus 10*6 CFU/ml. Obtained data in all 3 cases (Tables 1b, 1c and 1d) considerably differ from spectral data which have been obtained from surface of palatine tonsils (Pic. 5a); this fact proves that an important part is played not only by microbial factor but also by physiologic processes, which take place in tissue, as well as by its morphometric and metabolic characteristics.

CONCLUSIONS:

The data, which have been obtained during the study, allow to conclude that fluorescent methods of diagnostics are promising ones in respect of rapid diagnostics of inflammatory diseases in vivo, they also are informative for evaluation of character of an causative agent and its concentration per gram tissue in an inflammatory process during examinations in vitro [8, 9].

It should also be noted that the technique of fluorescent spectrometry, which is performed by means of hardware-software complexes EnSpectr R532 and EnSpectr M405, is highly sensitive and highly specific. Use of the presented technology allows to register spectral characteristics of a causative agent or associates of causative agents in a certain inflammatory process in vitro and that allows to considerably reduce time of diagnostics and to prescribe a rational treatment in time. However, during use of these methods the main problem is the fact that when spectral data are registered, we receive a generalized signal which characterizes not only the microbial component but also characterizes together anatomic, histologic and physiologic indexes of tissue itself.

Thus, development of algorithms of rapid diagnostics of inflammatory diseases in otorhinolaryngology is absolutely necessary not only for timely and quick diagnostics but also for conducting of an effective therapy as well as for monitoring of the disease on its different stages. In this connection, fluorescent methods are interesting and they must be developed and studied for introduction and use in clinical practice.

REFERENCES:

1. Aleksandrov M. T. Laser clinical biophotometry: Theory, experiment, practice: Technosphere; 584 p., 2008 (in Russian).
2. Borisova O. I., Aleshkin V. A., Pimenova A. S., Kriukov A. I., Kunelskaia N. L., Gurov A. V., Shadrin G. B., Tovmasian A. S. Efimov B. A., Kafarskaia L. I. Microbial composition of microflora of fauces in patients with tonsillar pathology // Infection and immunity, 2015, Vol. 5, No. 3, p. 225–232 (in Russian).
3. Kulichenko T. V., Patrusheva I. S., Redina A. A., Lazareva M. A. Modern diagnostics and treatment of acute streptococcic pharyngitis and tonsillitis in children and adults // PF. 2013. No. 3 (in Russian).

4. Sineva E. L., Pankova V. B., Sarancha E. O. Prevalence and structure of diseases of ENT organs in children of industrial regions // Bulletin of Otorhinolaryngology, 2, 2015 (in Russian).
5. Anders Jensen, Helena Fagö-Olsen, Christian Hjort Sørensen, and Mogens Kilian. Molecular Mapping to Species Level of the Tonsillar Crypt Microbiota Associated with Health and Recurrent Tonsillitis. PLoS One. 2013; 8(2): e56418., doi: 10.1371/journal.pone.0056418
6. Joseph Smolsky, Sukhwinder Kaur, Chihiro Hayashi, Surinder K. Batra, and Alexey V. Krasnoslobodtsev. Surface-Enhanced Raman Scattering-Based Immunoassay Technologies for Detection of Disease Biomarkers, Biosensors (Basel). 2017; 7(1). pii: E7. doi: 10.3390/bios7010007.
7. McQueenie, Ross Stevenson, Ross Benson, Robert MacRitchie, Neil McInnes, Iain Maffia, Pasquale Faulds, Karen Graham, Duncan Brewer, James Garside, Paul. Detection of inflammation in vivo by surface-enhanced Raman scattering provides higher sensitivity than conventional fluorescence imaging (2012) . Analytical Chemistry, 84 (14). pp. 5968-5975. ISSN 0003-2700
8. SimonatoL.E., TomoS, MiyaharaG.I, NavarroR. S, VillaverdeA.J. Fluorescence visualization efficacy for detecting oral lesions more prone to be dysplastic and potentially malignant disorders – a pilot study. Photodiagnosis Photodyn Ther. 2017 Mar;17:1-4. doi: 10.1016/j.pdpdt.2016.10.010. Epub 2016 Oct 29.
9. Supriya Srinivasan, Vinay Bhardwaj, Abhignyan Nagasetti, Alicia Fernandez-Fernandez and Anthony J. McGoron; Multifunctional Surface-Enhanced Raman Spectroscopy-Detectable Silver Nanoparticles for Combined Photodynamic Therapy and pH-Triggered Chemotherapy Journal of Biomedical Nanotechnology Vol. 12, 2202–2219, 2016.