

<http://dx.doi.org/10.35630/2199-885X/2022/12/2.28>

IMPROVEMENT OF AUTOFLUORESCENCE IMAGING METHOD IN DETECTION OF CANCEROUS LESIONS OF THE ORAL MUCOSA

Received 04 February 2022;
Received in revised form 22 February 2022;
Accepted 24 February 2022

Ekaterina Gorbatova[✉] , **Marina Kozlova** ,
Larisa Dzikovitskaya , **Tamara Glybina** 

Central State Medical Academy, Department of President Affairs, Moscow, Russia

✉ Gorbatova_k@mail.ru

ABSTRACT — PURPOSE: to optimize the diagnostic method for detecting neoplasms of the oral mucosa.

MATERIALS AND METHODS: The study group included 32 people with lesions of the oral mucosa: lichen planus — 17; chronic mechanical injury (decubital ulcer) — 15. The authors have proposed a method for diagnosing erosive and ulcerative elements of the oral mucosa, which consists in staining pathological foci with a 1% solution of toluidine blue followed by autofluorescence imaging. The effectiveness of autofluorescence imaging by «AFS» stomatoscope, staining and a combination of two methods in visualizing the boundaries of precancerous lesions of the oral mucosa was determined.

RESULTS AND DISCUSSION: The technique of autofluorescence imaging by «AFS» stomatoscope, in 63.7% incorrectly diagnosed patients with cancer of the oral mucosa, in addition, 27.3% did not reveal malignant neoplasms. Marking the lesion elements with a 1% toluidine blue solution and their illumination with autofluorescence imaging, made it possible to establish a dark glow zone in those cases (27.3%) when imaging did not identify the pathological focus as an area without fluorescence. In all patients with malignant neoplasms of the oral mucosa diagnosed by autofluorescence imaging with staining, a truly positive result was confirmed by morphometric data.

CONCLUSION: Autofluorescence imaging with staining has a high sensitivity and specificity of 100%, and is 2 times more effective than autofluorescence imaging in the diagnosis of dysplasia of erosive and ulcerative elements of the oral mucosa.

KEYWORDS — autofluorescence imaging, optical diagnostic method, diagnosis for cancerous oral lesions.

INTRODUCTION

According to the literature, precancerous lesions of the oral mucosa range from 15.2 to 84.9% of all nosologies [1, 2]. This pathology of the mucous membranes of the oral cavity with the potential for the development of dysplasia, based on the classifica-

tion of A. L. Mashkillayson (1970) and other authors [3], includes: long-term non-healing ulcers, erosive-ulcerative forms of lichen planus, leukoplakia. Timely diagnosis of such conditions and neoplasms is the key to a favorable prognosis of the disease [4–6].

Detection of malignancy of precancerous conditions by standard methods is difficult due to the variability of the pathological process, expressed in a variety of morphological and morphometric disorders of the mucosa [7].

Studies conducted by A.E. Pursanova and co-authors (2015) showed that 42.8% of specialists differentiate early manifestations of cancer of the oral mucosa [8]. A.M. Avanesov and co-authors (2017) found that in 65% of cases, the dentist may suspect the presence of malignant neoplasms only on the third visit of the patient [9]. In the initial period, during the clinical examination of the oral mucosa, good visibility of the lesion elements does not give advantages therefore additional methods should be used [8–11].

To date, modern diagnostic tests that help primary care physicians to establish a disease of the oral mucosa and suspect the development of carcinogenesis include: autofluorescence imaging, luminescent imaging, staining with toluidine solution [3, 10–15].

The autofluorescence imaging technique is based on differences in the spectral composition and intensity of endogenous radiation of healthy tissues and lesions. As a result of disruption of metabolic processes, endogenous porphyrin (fluorophore) accumulates in tissues, due to this, when exposed to a light beam of the blue spectrum on the inflammatory zones, a red or maroon glow appears, the absence of a glow (dark spot) indicates malignization [10–15]. In dental practice today, this method is widely used to detect oncological diseases of the oral cavity that have manifestations in the form of erosive and ulcerative elements. According to a number of authors, the disadvantage of the method is low specificity according to the stated diagnostic criteria [14, 15].

In international practice, marking of lesion elements with a 1% toluidine blue solution is used to assess the size of a pathological focus. The principle of operation of the technique is based on the retention of

the dye in the intercellular space of damaged cells with impaired metabolic processes; binding to negatively charged mitochondrial membranes, affinity for DNA and sulfated mucopolysaccharides [3]. Sol Silverman et al., (2010) claimed a high (90%) accuracy of this diagnostic method.

Thus, the autofluorescence imaging methodology has disadvantages, and needs additional refinement. The test that involves staining with a 1% solution of toluidine elements of the lesion, despite its availability and high percentage of reliability, has not been widely used in practical dentistry.

The aim of the study

is to optimize the method for diagnosis of oral cavity neoplasms

MATERIALS AND METHODS

In 2019–2021, 67 patients aged 35 to 87 years with diseases of the oral mucosa were examined at the Department of Dentistry, Central State Medical Academy (Moscow, Russia)

Inclusion criteria:

- erosive and ulcerative forms of lichen planus;
- individual elements of the lesion — erosion, ulcer, due to chronic mechanical trauma.

Exclusion criteria patients with diseases of the oral mucosa:

- infectious;
- allergic;
- benign tumors;
- keratoses.

Thus, the study group included 32 people (men — 13, women — 22) older than 45 years with the following lesions of the oral mucosa: lichen planus — 17; chronic mechanical injury (decubital ulcer) — 15.

The clinical examination consisted of standard methods: a survey, anamnesis collection, examination of the external status with mandatory palpation of the lymph nodes of the regional region. The examination of the mouth included a description of the condition of the mucous membrane of the lips, cheeks, hard and soft palate, gums, teeth, dentition, identification of factors of chronic mechanical trauma (dystopian teeth, sharp edges of teeth, fillings, orthopedic structures, the presence of dissimilar metals). The lesions of the oral mucosa were analyzed in detail according to the following scheme:

1. Appearance: type, localization; size; shape of edges; condition of surrounding tissues;
2. Palpation: painful/painless; consistency (soft-elastic/ tight-elastic), the presence of a seal;
3. Autofluorescence imaging is performed by a

fluorescent stomatoscope "AFS", the manufacturer "Polironik" (Russia) (Fig. 1 a, b).

With this device, it is possible to detect the localization of pathological processes due to the excitation of tissue fluorophores by light. In the light of a wave of a blue light beam, a healthy mucosa has a green or blue glow, foci of inflammation are red or maroon (due to endogenous porphyrins - waste products of pathogenic microflora), suspicious areas of the oral cavity will emit their own light with a longer wavelength, have a darker color and an uneven surface compared to other areas, which indicates the fact of accumulation of a large number of cells with enlarged nuclei in one place.

To visualize the size and boundaries of erosive and ulcerative elements of the lesion of the oral mucosa during biopsy, a method was patented (Gorbatova, E., Kozlova M., Ryabov, V. 2019 Visualization of oral lesions using autofluorescence imaging with staining in biopsy, 2722766. Rospatent, Russia):

1. The mouth is rinsed with water.
2. The staining of the lesion element of the oral mucosa (erosion or ulcers) is performed 1% toluidine blue solution with a sterile cotton swab, for 15 seconds.
3. The mouth is rinsed with water.
4. A 1% solution of acetic acid is applied to the lesion element of the oral mucosa with a sterile cotton swab, for 15 seconds.
5. The oral cavity is rinsed with water.
6. In natural light autofluorescence imaging Staining with subsequent autofluorescence imaging by "AFS" stomatoscope changes the intensity of fluorescence of pathological foci and gives a clear topography of the site of sampling of biological material, which ensures the reliability of histological examination.

For the final diagnosis, the lesions of the oral mucosa were taken for biopsy. Histological samples were examined in the Pathoanatomical Department of the Herzen Moscow State Medical Institute and Blokhin Russian Cancer Research Center (Moscow; Russia)

To evaluate the proposed method, a comparison of autofluorescence imaging using "AFS" stomatoscope, or autofluorescence imaging with staining with 1% toluidine blue solution and without staining was performed. The effectiveness was calculated using sensitivity and specificity.

Sensitivity (Sen.) was calculated by the formula:

$$\text{Sen.} = \frac{\text{truly positive}}{\text{true positive} + \text{false negative}} \times 100\%$$

Specificity (Sp.) was calculated:

$$Sp. = \frac{\text{truly negative}}{\text{false positive} + \text{truly negative}} \times 100\%$$

The values of the morphological conclusion were taken as an indisputable criterion for the diagnosis of cancer and the exclusion of false positive/negative results.

The obtained results were processed by the method of descriptive statistics, the method of variance analysis (Student's *t*-test). The differences were considered significant in cases when the probability of belonging to one of the communities did not exceed $p = 0.023$.

RESULTS AND DISCUSSION

Analysis of the dental status of all patients showed that in 95% of all cases, the local cause of the occurrence or exacerbation of the existing disease of the oral mucosa was a permanent injury of various origins, mainly sharp edges of destroyed and dystopian teeth, orthopedic structures.

During autofluorescence imaging by "AFS" stomatoscope, in 32 patients with erosive and ulcerative changes of the oral mucosa in 36.4% of cases, a "false positive" identification of the development of carcinogenesis occurred, a positive result was confirmed in 27.3%, a false negative response — 27.3%, a true negative — 9.0%.

Thus, when conducting the autofluorescence imaging technique in 63.7%, the test did not have reliable information regarding the diagnosis of patients with oral mucosal cancer, the same percentage of detection (27.3%) and non-detection (27.3%) of this pathology was also established. Otherwise, half of the patients with suspected possible malignancy of the lesion elements were not detected.

Marking the lesion elements with a 1% toluidine blue solution and their illumination with an «AFS» stomatoscope revealed a zone of dark glow in those cases (27.3%) whereas only autofluorescence imaging failed to identify the pathological focus as an area without fluorescence.

All erosive and ulcerative lesions that intensively absorbed the dye and had a black halo of glow were subsequently confirmed as malignant neoplasms according to morphometric studies. (Fig. 2 (a, b, c, d)).

All patients who were diagnosed with the condition of the oral mucosa by the autofluorescence imaging method with staining had truly positive results of the presence of malignant neoplasms, confirmed by histological results. In addition, the identification of the sampling site for the biopsy took place, which was an important advantage of this method.

Analyzing the results obtained, it should be noted that autofluorescence imaging method with staining

has a high sensitivity of 100%, 2 times more effective than autofluorescence imaging in detecting dysplasia of erosive and ulcerative elements of the oral mucosa. The specificity of the diagnostic autofluorescence test was 25%, with staining — 100% ($p=0.023$).

The difficulty of interpreting the results of the diagnosis of autofluorescence imaging is due to the fact that the presence of porphyrins is also characteristic of inflammatory processes. A number of experimental and clinical studies have confirmed the fact that fluorophores were present in areas of ischemia and hypoxia of tissues, while morphologically altered cells characteristic of malignant or benign neoplasms were not detected [16]. This method can determine the prevalence of a pathological focus and it is impossible to analyze the depth of the lesion.

Autofluorescence imaging can be recommended as a diagnostic test to identify the presence of pathology of the mucous membranes of the oral cavity.

Autofluorescence imaging by "AFS" stomatoscope with marking of elements with 1% toluidine blue solution more effectively visualizes the zone of altered fluorescence and determines the site for biopsy. The described methods should be used by general dentists in case of suspected malignancy of the oral mucosa as a first opinion. At the second level of examination, the results of autofluorescence must be confirmed by morphometric examination of the pathological focus.

CONCLUSIONS

1. The proposed method of topography of erosive and ulcerative lesions of the oral mucosa with subsequent autofluorescence imaging by "AFS" stomatoscope allows us to clearly visualize the zones of altered fluorescence, the boundaries of the pathological focus and intact tissue for biopsy, which ensures a reliable result of histological examination.

2. The diagnostic technique of autofluorescence imaging with staining is 2 times more effective than autofluorescence imaging in determining neoplastic changes in the tissues of the oral mucosa.

REFERENCES

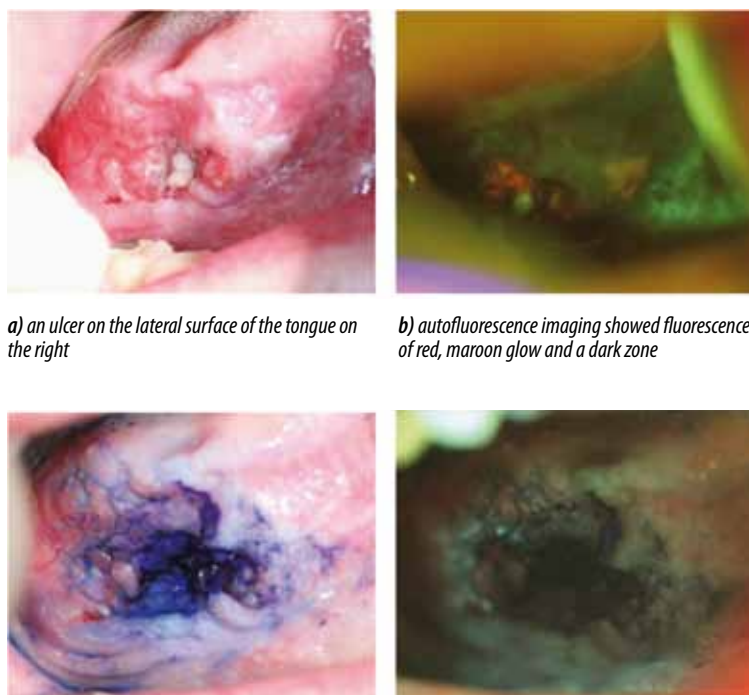
1. GILEVA O. S. ET AL. Precancerous diseases in the structure of pathology of the oral mucosa // Problems of dentistry. – 2013. – No. 2. – p. 1–9.
2. ISKAKOVA M. K., ZARKUMOVA A. E., NURMUKHAMBETOVA G. K. The proportion of diseases of the oral mucosa among common dental diseases // Bulletin of the Kazakh National Medical University. - 2017. – No. 3. – p. 163–167.
3. SILVERMAN S., EVERSOLE L.R., TRUELOVE E.L. Essentials of Oral Medicine, 2010, 467 p.
4. ZAZULEVSKAYA L. YA., RUSANOV V. P., VALOV

- K. M. Oncological alertness of dentists-the key to preventing cancer of the oral mucosa //Bulletin of the Kazakh National Medical University. – 2012. – No. 1. – p. 201–204.
5. ZYKOVA E. A. Oncological alertness in diseases of the oral mucosa //Healthcare of Ugra: experience and innovations. – 2016. – No. 3. – p. 49–55.
 6. AMKHADOVA M. A., SOYHER M. I., CHUYANOVA E. YU. Onconstentiveness in the practice of a dentist // Medical alphabet. – 2016. – Vol. 2. – No. 9. – p. 6–9.
 7. TKACHENKO T. B., GAIKOVA O. N. Features of the oral mucosa in various age periods of human life (morphological study) //Institute of Dentistry. – 2008. – Vol. 3. – No. 40. – p. 70–73.
 8. PURSANOVA A. E. ET AL. Evaluation of the effectiveness of therapy of precancerous diseases of the oral mucosa using autofluorescence diagnostics //Modern problems of science and education. – 2018. – No. 4. – p. 151–151.
 9. AVANESOV A.M., GVOZDIKOVA E. N. Determination of dental risk groups for radiation mucositis in patients with squamous cell carcinoma of the oropharyngeal region on the background of radiation therapy // Radiation diagnostics and therapy. – 2017. – No. 2. – p. 66–66.
 10. GAZHVA S. I. ET AL. Direct visualization of autofluorescence of tissues as a method of early diagnosis of pathological conditions of the oral mucosa //Modern problems of science and education. – 2014. – No. 6. – p. 1237–1237.
 11. SHKAREDNAYA O. V. ET AL. Optimization of early diagnosis of pathological conditions of the oral mucosa //Modern technologies in medicine. – 2017. – Vol. 9. – No. 3. – P. 119–125.
 12. BULGAKOVA N. N., VOLKOV E. A., POZDNYAKOVA T. I. Autofluorescence stomatoscopy as a method of oncoscreening of diseases of the oral mucosa //Russian Dental Journal. – 2015. – Vol. 19. – No. 1. – p. 27–30.
 13. KRIKHELI N. I. ET AL. Results of autofluorescence stomatoscopy of lichen planus as a screening method for detecting precancerous and cancerous changes in the oral mucosa //Russian dentistry. – 2016. – Vol. 9. – No. 4. – p. 13–17.
 14. NAGI R., REDDY-KANTHARAJ Y., RAKESH N., JANARDHAN-REDDY S., SAHU S. Efficacy of light based detection systems for early detection of oral cancer and oral potentially malignant disorders: Systematic review //Med Oral Patol Oral Cir Bucal. – 2016. – V. 21. – No 4. P. 447-455. 10.4317/medoral.21104
 15. NALLAN C. S. ET AL. A Meta-analysis on efficacy of auto fluorescence in detecting the early dysplastic changes of oral cavity. Chaitanya K., Sunanda Chavva, Elizabeth Surekha, Vedula Priyanka, Mule Akhila, Hari Kiran Ponnuru, and Charan Kumar Reddy // South Asian J Cancer. – 2019. – V. 8. – No 4. P. 233–236. doi: 10.4103/sajc.sajc_336_18
 16. LITVINOVA K. S., ROGATKIN D. A. Catalogue of spectral characteristics of the main fluorophores of human and animal tissues //Collection of materi-



a) visualization pathological foci of oral mucosa b) Stomatoscope "AFS"

Fig.1. Visualization of pathological foci of oral mucosa by stomatoscope "AFS", "Polironik" (Russia)



a) an ulcer on the lateral surface of the tongue on the right

b) autofluorescence imaging showed fluorescence of red, maroon glow and a dark zone

c) visualization of the ulcer boundaries after staining with 1% toluidine blue solution

d) there is no fluorescence in the pathological focus after staining and autofluorescence imaging. In a morphometric study, neoplastic changes of cells were established, on the basis of the histological examination (No. 1532/2019), the final diagnosis was made – "squamous cell carcinoma"

Fig. 2. Clinical picture, patient B., 81. Autofluorescence imaging (a, b), autofluorescence imaging with staining (c, d)

als of the III Eurasian Congress on Medical Physics and Engineering "Medical Physics-2010". Moscow: Publishing House of Moscow State University. – 2010. – pp. 141–143.