Autofluorescence and Early Detection of Mucosal Lesions in Patients at Risk for Oral Cancer

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Abstract: Loss of autofluorescence as an early phenomenon associated with tissue degeneration seems to be promising for the diagnosis of oral cancer. The method seems to make visible early structural and biochemical alterations of the oral mucosa not always evident under direct inspection of the oral cavity.

For this reason, the margins of the mucosal lesions usually appear wider compared with direct visualization. Actual extension of the potentially malignant lesions must be precisely perceived to avoid any underestimation of the tumor. In this study, 32 patients at risk for oral cancer underwent autofluorescence test. Of these patients, 12 (group A) experienced potentially malignant diseases. The other 20 patients (group B) were previously operated on for oral cancer. In addition, 13 patients showed loss of autofluorescence (8 patients from group A and 5 patients from group B). Among these 13 patients, 12 were affected with lesions of relevance (in group A, 6 had squamocellular carcinoma and 2 had low-grade dysplasia; in group B, 2 patients had high-grade dysplasia, 2 had low-grade dysplasia, and 1 had an epithelial hypertrophy with inflammatory cells). Preliminary results seem to indicate that autofluorescence is a high-performing test for the individuation of oral cancer in populations at risk (sensibility up to 100% and specificity up to 93% in this study).

Key Words: Autofluorescence, oral cancer, dysplasia, flavin adenine dinucleotide, prevention

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Oral cancer has an incidence of 8.2 per 100,000 per year for males and 2.8 per 100,000 per year for females in the world. Some countries are more affected (oral cancer exceeds 30% of all cancers in the Indian subcontinent). More than 90% of these tumors are classified as "squamous carcinoma."[1] Age of onset is among those 50 and 70 years, although the last decades showed an increasing number of cases among the younger populations, mostly for tumors of the tongue.[1] Smokers have a 5-times higher risk of death from oral cancer compared with nonsmokers.[2,3] Excessive consumption of alcohol promotes oral cancer,[4,5] whereas association of tobacco smoke and alcohol has a synergic effect, being the risk of developing oral cancer 30 times higher.[6,7] Poor oral hygiene, improperly fitting dental prostheses, defective dental restorations, and misaligned or sharp teeth are considered able to promote oral cancer.[8] Viral DNA (human papillomavirus and hepatitis C virus) have been frequently demonstrated in oral carcinoma.[9,10]

According to literature data, premalignant lesions might turn into carcinoma in a percentage varying between 5% and 18% of cases, so that identification of leukoplakia, erythroplakia, lichen planus, and other potentially malignant disorders (PMDs), the main specific risk factors for oral cancer, is important to prevent the onset of tumors.[11,12]

At this time, no oral cancer screening tests on large populations is recommended; nevertheless, it may be useful to administer diagnostic tests at least on high-risk groups.[13] Currently, the recommended screening tests include a thorough history, physical examination, application of toluidine blue on suspected lesions, and definitive diagnosis by histologic examination.[14] Other tests available are chemiluminescent illumination and exfoliative cytology, but these tests are not supported by sufficient data to prove their usefulness.[15,16] Toluidine blue vital staining is instead recommended for its simplicity, low cost, noninvasiveness, and accuracy (sensitivity, 93.5%–97.8%; specificity, 73.3%–92.9%).[14,17–19]

Other promising techniques based on optical devices aiming at secondary prevention of the cancer of the oral cavity are now under development.[16,20] On 2006, a publication of the *Journal of Biomedical Optics* illustrated a device capable of showing up the reduction of oxidized flavin adenine dinucleotide (FAD) fluorescence from the tumor tissues (Fig. 1).[21] The device consists of a light source connected via optic fiber cable to the inspective device. Inside this portion, the light passes through an excitation filter, a convergent (collimator) lens, a dichroic mirror, and, finally, invests the oral mucosa. On its way back from the oral mucosa, the light passes through the dichroic mirror again, this time aiming at the eye of the examiner, than passes through an emission filter and a notch filter. In May 2008, the same *Journal* published a work on a new device based on the same principle: the improvements are given by easier transportability, binocular sight, and its combination with a video recording system.[22]

MATERIALS AND METHODS

We studied 32 patients for 12 months. Twelve of these patients were affected by PMDs or suspected cancerous lesions. The other 20 patients, who previously underwent surgical excision of cancer of the oral cavity, were observed during their follow-up.

Each patient underwent traditional oral inspection followed by autofluorescence examination (AFE) of the entire oral cavity. The instrumentation consists of an LED lamp emitting at 450 nm and

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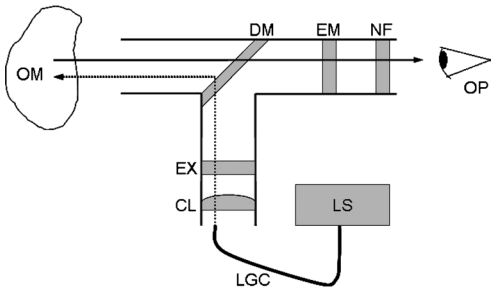

FIGURE 1. Original device for the detection of the oxidized FAD autofluorescence. Dotted arrow indicates light emitted from the light source (peak at 450 nm). Continuous arrow indicates light emitted from the oxidized FAD (peak at 515 nm). CL indicates collimator lens; DM, dichroic mirror; EM, emission filter; EX, excitation filter; LGC, liquid glass cable; LS, light source; NF, notch filter; OM, oral mucosa; OP, operator.

pass-band filters (peak at 515 nm; transmittance range, 470–610; compatible with the autofluorescence examination).

Patients positive for AFE underwent biopsy and histologic examination of the sampled lesion. Biopsies were performed, taking into consideration the margins of the lesion as indicated by the hypofluorescence halos rather than those evident at direct inspection.

Patients negative to the AFE were listed to be controlled every 3 months (group A) or continued normal postsurgery follow-up (group B). Pictures were acquired with Nikon D70 camera (Nikon Corp, Shinjuku, Japan) equipped with Nikkor AF Micro 60-mm lens and a pass-band filter.

RESULTS

Of the 12 patients belonging to group A, 8 were AFE-positive. Lesions of all 8 patients were considered histologically relevant and thus true positives. More precisely, 6 lesions were classified as squamous cell carcinoma of the oral cavity and 2 lesions were classified as low-grade dysplasia. Of the 20 patients belonging to group B, 5 patients were AFE positive. Lesions of 4 of these 5 patients were considered histologically relevant and true positives. In particular, 2 patients had low-grade dysplasia and 2 patients had high-grade dysplasia. The lesion of the patient considered false positive was affected by epithelial hyperplasia with inflammatory reaction (Table 1).

It was not possible to calculate sensitivity and specificity because of the impossibility to define the false negatives. However, we made the following observations:

- Among AFE-negative patients, no one developed new tumors during the study.
- Of the 12 true AFE-positive patients, only 9 were positive at the clinical inspection of the oral cavity.
- The clinical inspection of the oral cavity underestimated the actual margins of the lesion in 6 (66.6% of the cases) of 9 patients.

TABLE 2. Results (Group A + Group B)

	Pathology +	Pathology −	
AFE +	12 true positive	1 false positive	13
AFE −	0 false negative	19 true negative	19
	12	20	Total 32

Sensitivity, 100%; specificity, 95%; positive predictive value, 92%; negative predictive value, 100%.

- No AFE-negative patient showed any indication for biopsy.
- Seven patients were investigated with toluidine blue vital staining. All the toluidine blue–positive patients were AFE positive as well.
- Of 5 true-positive patients, only 3 were also positive to toluidine blue.[23]

If we were to consider true negatives, all patients who never had a clinically visible relapse and showed no signs of relapse/new lesions at the instrumental examinations during follow-up, we would obtain a sensitivity of 100%, a specificity of 93%, a predictive value of positive test of 92%, and a predictive value of negative test of 100% (Table 2).

The healthy mucosa emits a weak green fluorescence (Fig. 2). In Figure 3, we compare various lesions as they seem to direct visualization and autofluorescence examination.

During our experience, interesting facts emerged. Autofluorescence results may be adversely affected by the consumption of licorice or coffee. A simple oral rinse with water and/or mouthwash can avoid false-positive detections (Fig. 4).

Hyperkeratosis of oral lesions shows an increase in autofluorescence (Fig. 5).[24] In such cases, it is possible to see hypofluorescence halos around the main lesion. To demonstrate the superiority of the autofluorescence examination is beyond the scope of this study. If we compare the AFE performance with the results obtained from standard inspection of the oral cavity, we observe that there is no significant difference in the number of lesions diagnosed by the 2 methods (2-tailed Fisher exact test, $P = 0.2174$). This is probably because our sample population was too restricted. Further studies on larger samples of population may find statistically significant differences. However, if we take as reference the ability to discriminate real margins of the lesion, the mere inspection is less effective than AFE (2-tailed Fisher exact test, $P = 0.0090$; Table 3).

DISCUSSION

Identification of the high-risk population and early treatment are perhaps 2 effective ways to control oral cancer.

Knowledge of risk factors allows us to identify the population who should undergo a screening test. Currently, the most common and recommended method for screening is physical examination, vital staining with toluidine blue, and, if positive, incisional

TABLE 1. Results: 8 of 12 Patients From Group A and 5 of 20 Patients From Group B Were AFE Positive

Group A: Patients With PMD		Group B: Patients in Postsurgical Follow-Up	
8 true positive	6 oral squamous cell carcinoma	4 true positive	2 high-grade dysplasia
	2 low-grade dysplasia		2 low-grade dysplasia
0 false-positive	No lesions observed at the histologic examination	1 false positive	Epithelial hyperplasia and inflammatory reaction

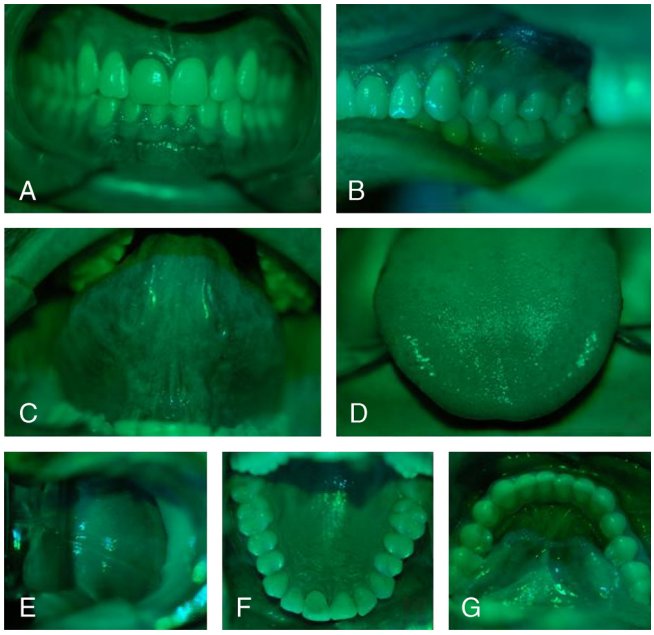

FIGURE 2. Healthy oral mucosa as seen during autofluorescence examination: gingiva (A, B); tongue (C, D); buccal mucosa (E); hard palate (F); and floor of the mouth (G).

or excisional biopsy for definitive diagnosis in patients experiencing PMDs of the oral cavity. Any oral lesion that does not spontaneously regress or does not respond to the usual therapeutic measures after a period of 2 weeks should be considered potentially malignant; histologic examination is always recommended in these cases.

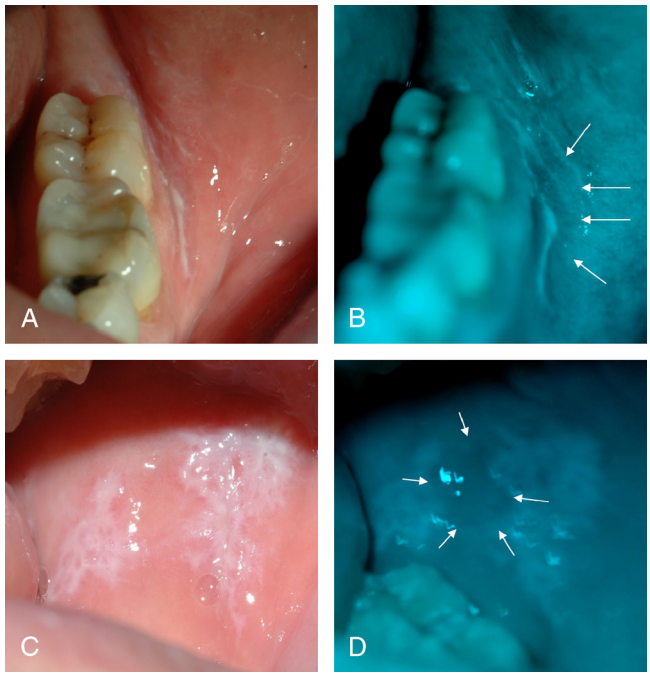

FIGURE 3. Hypofluorescence halos in patients affected with low-grade dysplasia. A, Patient affected with oral lichen planus. B, Same patient showing hypofluorescence of the buccal mucosa (arrows). C, Patient who previously underwent biopsy for leukoplakia: mucosa of the left cheek. D, Intralesional hypofluorescence halo on apparently healthy mucosa (arrows).

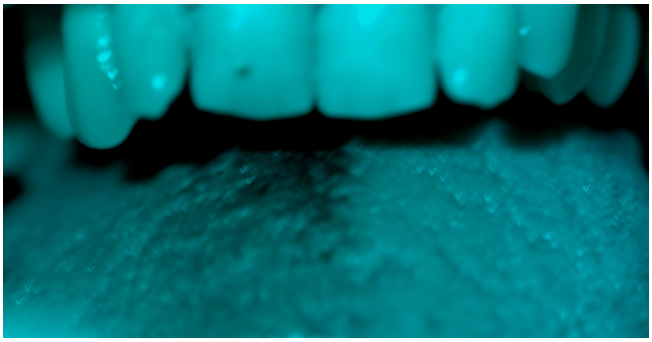

FIGURE 4. False-positive autofluorescence examination in a patient who consumed licorice 60 minutes before undergoing the test.

Because the early detection depends very much on the awareness and experience of the examiner, it may be a useful objective method, with low costs, high sensitivity, which reduces the gap of uncertainty related to the operator and allows, where possible, to highlight early degenerating epithelium with high accuracy.

Although direct visualization of the oral cavity allows recognizing only a small fraction of the spectral characteristics that differentiate healthy and cancerous lesions, optical methods based on tissue autofluorescence have improved our ability to detect early cancerous lesions in tissues such as lung, uterine cervix, and oral cavity.[25–30]

The fluorophore of our interest is FAD, which, in its oxidized form, emits a light of 515 nm wavelength (green) if enlightened with a light of 450 nm wavelength (blue violet).[31]

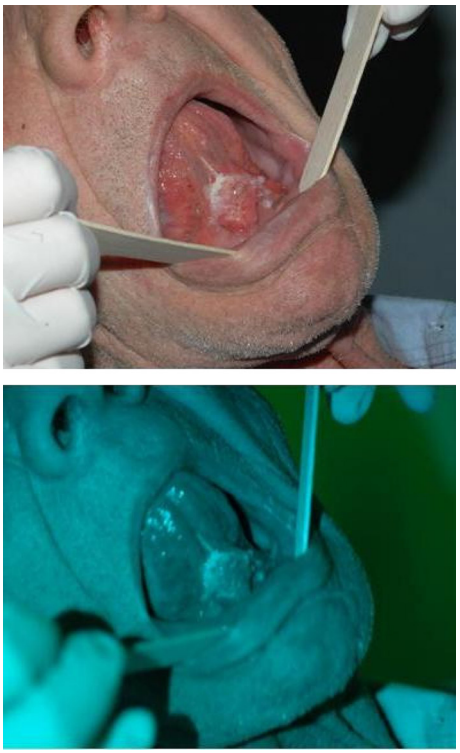

FIGURE 5. Hypofluorescence in a patient with hyperkeratotic lesion of the tongue and the floor of the mouth.

TABLE 3. 2 × 2 Contingency Table

Diagnosis	Test +	Test −	Margins	Free	Infiltrated
AFE	12	0	AFE	9	0
Inspection	9	3	Inspection	3	6

Left column compares the ability to detect a lesion between AFE and standard inspection of the oral cavity. Two-tailed Fisher exact test, $P = 0.2174$ (difference not statistically significant).

Right column compares the ability to identify the actual margins of the lesion of the 2 methods. Two-tailed Fisher exact test, $P = 0.0090$ (statistically significant).

In the tumor tissue, there is loss of fluorescence of the oxidized FAD irradiated with blue violet light. The cause of this phenomenon is not known with certainty, but this probably lies in a combination of several phenomena. The tumors are generally associated with angiogenesis, which could lead to an increase in the absorption of exciting light (hemoglobin strongly absorbs light at 420 nm). Also, disarrangement of the extracellular matrix and thickening of the epithelium after tumor growth seem to reduce the signal.[32]

In this study, we analyzed autofluorescence of the oral cavity on 32 patients who were selected because of their high risk of developing oral cancer. All of the 12 patients in group A (affected with at least 1 PMD) also had 1 or more of the following risk factors for oral cancer:

1. History of tobacco smoking
2. History of alcohol drinking
3. Chronic traumatism of the oral cavity mucosa

The 20 patients of the follow-up group (group B) were considered at risk because of their previous oral cancer, which is a renowned risk factor for oral cancer itself.[33,34]

The results of this study seem not realistic. In fact, sensitivity and specificity of autofluorescence examination were found to be both very high. However, it should be considered that the study was conducted on a selected population of patients at risk, with precancerous conditions, cancerous lesions, or in postsurgery follow-up for cancer of the oral cavity. The reason for such favorable results in the sensibility and sensitivity of this method should be kept in mind. Moreover, multiple focal areas of dysplasia in the oral cavity are not uncommon, especially in those patients with bad habits.[34]

Patients with low-grade dysplasia were considered true positives in this study because of the impossibility of determining whether a lesion considered low-grade dysplasia at the pathologic investigation has an evolutionary nature or not. It is known that some of the cells observed in tissues with dysplasia have such genomic aberrations that are inevitably destined to an increasing chromosomal instability.[35]

It is of great importance to identify lesions in the preclinical or early stage. Indeed, prognosis is heavily influenced by stage at diagnosis. However, 68% of cases are diagnosed in stage III–IV, when invasive treatments are needed, and prognosis is particularly poor.[33]

CONCLUSIONS

Our study aims to demonstrate the validity of the autofluorescence examination as a method for easy and low-cost screening for oral cancer in high-risk patients. Autofluorescence not only proved itself capable of identifying lesions that were impossible to see during clinical evaluation but also allowed us to identify early stage cancer. Moreover, the limits of the lesion examined by means of autofluorescence were often wider than those showed at clinical examination.

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