Complementary Autofluorescence Imaging in the Diagnostic Evaluation of Oral Potentially Malignant Lesions: A Comparative Clinical Study

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Doina Chioran[1*], Octavia Balean[2*], Ramona Dumitrescu[2], Vlad Tiberiu Alexa[2], Adrian Moldoveanu[3,4], Antonis Perdiou[2,3], Tareq Hajaj[5,6]

[1]*Department of Anesthesiology and Oral Surgery, Research Center in Dental Medicine Using Conventional and Alternative Technologies, "Victor Babes" University of Medicine and Pharmacy, Eftimie Murgu Sq. No. 2, 300041 Timisoara, Romania*
[2]*Department of Preventive, Community Dentistry and Oral Health, Translational and Experimental Clinical Research Centre in Oral Health, University of Medicine and Pharmacy "Victor Babes", 300040 Timisoara, Romania*
[3]*Doctoral School, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania;*
[4]*Department of Surgery I, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania;*
[5]*Victor Babes University of Medicine and Pharmacy, Faculty of Dentistry, Department of Prosthesis and Dental Materials,2 Eftimie Murgu Sq, Timisoara 300041, Romania*
[6]*Research Center in Dental Medicine Using Conventional and Alternative Technologies, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy of Timisoara, 9 Revolutiei 1989 Ave, Timisoara 300070, Romania*
**These authors contributed equally to this work.*

Correspondence to:
Name: Ramona Dumitrescu
E-mail address: dumitrescu.ramona@umft.ro

Name: Vlad Tiberiu Alexa
E-mail address: vlad.alexa@umft.ro

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Abstract

Background/Objectives: Oral cancer remains a significant global health concern, with early detection being essential for improving patient outcomes. This study aimed to evaluate the clinical utility of autofluorescence imaging as a complementary tool to conventional examination for identifying potentially malignant oral lesions. Methods: Thirty patients with clinically suspicious oral mucosal lesions were randomly assigned to two groups: one examined under conventional white light (control group), and the other using both white light and autofluorescence (experimental group). All cases were subsequently biopsied, and histopathological analysis was used as the diagnostic reference standard. Results: The autofluorescence-assisted group demonstrated a higher proportion of histologically confirmed malignancies (93.3%) compared to the control group (75.0%), suggesting greater diagnostic alignment with biopsy outcomes. Autofluorescence facilitated enhanced visualization of lesion borders and subtle mucosal changes, supporting its role in improving clinical assessment. Conclusion: Autofluorescence imaging appears to be a useful adjunct in the evaluation of suspicious

oral lesions, offering better lesion detection compared to conventional examination alone. While not a substitute for biopsy, it may improve early identification and biopsy site selection. Further studies are needed to confirm these findings in larger populations.

Keywords: Oral cancer, autofluorescence imaging, adjunctive diagnosis, early detection, non-invasive diagnostic methods

INTRODUCTION

Oral cancer constitutes a significant global health burden, ranking among the most common cancers worldwide, particularly in regions with high tobacco and alcohol consumption. It encompasses a diverse group of malignant neoplasms that affect the lips, tongue, floor of the mouth, buccal mucosa, and oropharynx. The disease often presents asymptomatically in its early stages and may go unnoticed until it has progressed significantly, complicating treatment and diminishing survival rates. Risk factors include, but are not limited to, tobacco use, excessive alcohol intake, human papillomavirus (HPV) infection, poor oral hygiene, and chronic mucosal trauma. Early detection is critical, as it enables timely intervention, reduces morbidity, and improves overall prognosis. Therefore, increasing awareness and improving clinical screening strategies remain essential in combating the high mortality associated with oral malignancies [1,2].

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy affecting the oral cavity, representing approximately 90% of all cancers located in the head and neck region [3]. The prognosis of patients diagnosed with OSCC is largely dependent on the stage of the disease at the time of detection. When identified in its early stages, the therapeutic approach can be less invasive, and survival outcomes are significantly improved [4]. Despite advancements in diagnostic protocols and therapeutic modalities, the five-year survival rate for individuals affected by OSCC remains disappointingly low, with little improvement observed in recent decades, hovering around 50% [5].

Histopathological examination continues to be regarded as the gold standard in the diagnosis of oral cancer [6]. However, in clinical practice, performing a biopsy is not always straightforward. It may prove technically demanding, especially in cases with extensive or multifocal lesions where accurately selecting the most representative tissue sample is critical. Different areas within the same lesion can present distinct histopathological characteristics, potentially leading to diagnostic discrepancies or false negatives [5]. Moreover, beyond the technical challenges, biopsies often generate considerable psychological stress for patients. The fear of invasive procedures and possible unfavorable results may cause many individuals to delay or refuse this essential diagnostic step [7].

In an effort to overcome these limitations, alternative diagnostic strategies have been proposed. Among them, exfoliative cytology and polymerase chain reaction (PCR) have attracted attention; however, their broader implementation is hindered by insufficient sensitivity in the case of cytology, and high operational costs in the case of PCR. Consequently, research has shifted toward the development of non-invasive, cost-effective, and clinically reliable methods for early detection of OSCC. One promising approach involves the identification of biochemical or optical changes in the oral mucosa induced by malignant transformation. Autofluorescence is an example of such a technique that exploits the intrinsic fluorescence properties of tissues, enabling differentiation between normal and dysplastic or malignant tissue without the need for external staining agents [8]. This distinction is based on variations in the emission of fluorescent signals at different wavelengths, which reflect structural and metabolic changes occurring in cancerous tissue [9]. Furthermore, diagnostic accuracy can be enhanced by incorporating fluorescent probes that specifically highlight either healthy or altered tissue when exposed to a particular light spectrum, improving the visualization of lesions and more accurately defining surgical margins [10].

From a broader perspective, oral cancer continues to pose a major public health concern globally. It is ranked among the top ten cancers in terms of incidence and remains a significant burden due to its aggressive nature and relatively poor survival outcomes. Dental practitioners play a central role in its early recognition and management, as they are often the

first healthcare professionals to observe suspicious lesions. Unfortunately, despite continued progress in clinical research and therapeutic innovations, survival rates for oral cancer have remained largely stagnant, reflecting the ongoing challenges that persist in its early diagnosis and control [11,12].

In recent years, a number of adjunctive diagnostic techniques have been explored with the aim of improving early detection. Among these, toluidine blue staining and autofluorescence imaging have shown promise. Toluidine blue, a cationic metachromatic dye, preferentially binds to areas with increased nucleic acid content, staining premalignant and malignant tissues a deep blue, which enhances the visual distinction from surrounding healthy mucosa. In parallel, chemiluminescence and autofluorescence technologies offer additional diagnostic support by detecting cellular changes typically associated with neoplastic transformation, such as nuclear enlargement and reduced collagen fluorescence within the connective tissue stroma [13–15].

Autofluorescence imaging, in particular, has emerged as a valuable tool for the early identification of OSCC and potentially malignant disorders of the oral mucosa. Its clinical application is increasingly being recommended to guide biopsy sampling and delineate resection margins during surgery for precancerous or early-stage cancerous lesions. Tissue regions undergoing malignant transformation often exhibit a noticeable loss or alteration of their autofluorescence profile, which can assist surgeons in identifying areas of occult tumor spread not easily visible under conventional light. When tissues are exposed to light within the 400–460 nm wavelength range, cancerous areas tend to appear darker compared to adjacent healthy mucosa. This contrast is primarily due to disruptions in normal metabolic activity and structural composition at the cellular level [15–17].

Despite the widespread use of conventional diagnostic methods, such as clinical inspection under white light, these approaches are inherently limited. They depend heavily on the clinician's experience and may be insufficient for detecting subtle or early-stage malignant changes. Moreover, distinguishing pathological from normal tissue based on visual cues alone can be particularly challenging in complex cases. In contrast, autofluorescence-based diagnostics provide an objective advantage by enhancing visualization of suspect areas through fluorescence loss, thus allowing for more accurate lesion detection and potentially facilitating faster and more precise assessment of tumor margins compared to traditional evaluation techniques [18].

Aim and objectives

The aim of this study was to evaluate the role of autofluorescence imaging as an adjunctive diagnostic tool in the clinical assessment of oral mucosal lesions suspected of malignancy. By comparing conventional visual examination with autofluorescence-assisted evaluation, the study sought to determine whether this non-invasive technique could enhance the clinician's ability to identify lesions with malignant potential more accurately.

MATERIAL AND METHODS

This observational, comparative study was conducted on a sample of 30 adult patients who presented with clinically visible lesions located in the oral and maxillofacial region, suggestive of malignant or potentially malignant transformation. The clinical evaluation and data collection were carried out in a single center by the same trained specialist, in order to ensure methodological consistency and eliminate variability related to inter-examiner interpretation.

This study was carried out in accordance with the ethical standards set forth in the Declaration of Helsinki. Approval for the research was granted by the Ethics Committee of

the University of Medicine and Pharmacy Timişoara (approval no. 59/25.11.2021). Prior to participation, all individuals received comprehensive information regarding the study's objectives, procedures, possible risks, and anticipated benefits, and provided written informed consent.

Patients were randomly assigned into two equal groups of 15 individuals each. Randomization was performed using a computer-generated randomization sequence to ensure unbiased allocation. Stratification was applied based on lesion size and smoking status, two factors known to influence lesion behavior and diagnostic complexity. Allocation concealment was ensured through sealed, opaque envelopes, which were opened only after obtaining informed written consent from each participant.

The control group underwent standard clinical examination using conventional white light illumination. The protocol included visual inspection, palpation of the lesion, and documentation of clinical characteristics such as site, size, surface appearance, consistency, and associated symptoms (e.g., local pain, dysphagia, or spontaneous bleeding).

In the experimental group, patients received the same standard clinical evaluation, with the addition of adjunctive autofluorescence imaging performed at the same appointment. A handheld autofluorescence device was used, designed to detect subtle biochemical and structural changes in the oral mucosa by analyzing tissue autofluorescence patterns under specific wavelengths of light. This method served to enhance the visualization of lesion margins and tissue abnormality, providing supplementary guidance to the clinician during the diagnostic decision-making process.

Eligibility criteria for study inclusion required that patients presented with primary tumors in the oral or maxillofacial region, without prior treatment history for malignancies of the head and neck region. Patients undergoing or having previously received chemotherapy, radiotherapy, or surgical excision for such conditions were excluded. Additional exclusion criteria included the presence of current oncological treatments for other conditions, a history of malignancies in any location, or refusal to provide informed consent.

All patients were evaluated using a standardized clinical form that included sociodemographic variables (age, sex, place of residence), lesion-specific information (topography, duration, morphological aspect), and known risk factors such as tobacco use, alcohol intake, and the presence of systemic comorbidities (e.g., type II diabetes, arterial hypertension).

Following the clinical evaluation, data from both study groups were compiled and organized in tabular format. Descriptive statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Frequency distributions were calculated for categorical variables (e.g., gender, smoking, alcohol use), and means and ranges were computed for continuous variables such as age. Comparative analysis between the control and experimental groups was based on these descriptive summaries, allowing the identification of patterns and group characteristics relevant for interpretation.

RESULTS

The gender distribution within the two study groups was relatively balanced, with a slight male predominance observed in both. In the control group, 60.0% of participants were male (n = 9) and 40.0% were female (n = 6). Similarly, in the experimental group, 66.7% were male (n = 10) and 33.3% were female (n = 5). The mean age of participants was 55.6 years (range: 42–69) in the control group and 56.4 years (range: 41–71) in the experimental group, with no relevant differences in age distribution between groups.

In terms of residential background, the majority of patients in both groups came from urban areas (66.7% in the control group and 73.3% in the experimental group), while the

remaining participants were from rural communities. This urban predominance may reflect patterns of healthcare access and referral pathways for suspicious oral lesions.

Regarding lesion characteristics, the most frequent anatomical sites included the lateral border of the tongue (33.3%), the buccal mucosa (26.7%), and the floor of the mouth (20.0%). Most lesions presented as ulcerative or exophytic formations. Over 70% of patients in both groups reported lesion persistence longer than four weeks prior to evaluation, often associated with symptoms such as localized pain, difficulty in chewing or swallowing, and occasional bleeding.

Exposure to recognized risk factors was also documented. Smoking was more prevalent in the experimental group (73.3%) compared to the control group (53.3%), while alcohol consumption was reported by 40.0% of experimental and 33.3% of control participants.

Table 1. Distribution of comorbidities among participants in the control and experimental groups

Comorbidities	Control Group		Experimental Group	
	Yes	No	Yes	No
Smoking	53.3% (n = 8)	46.7% (n = 7)	73.3% (n = 11)	26.7% (n = 4)
Alcohol intake	33.3% (n = 5)	66.7% (n = 10)	40.0% (n = 6)	60.0% (n = 9)
Type II diabetes	20.0% (n = 3)	80.0% (n = 12)	26.7% (n = 4)	73.3%(n = 11)
Arterial hypertension	46.7% (n = 7)	53.3% (n = 8	60.0% (n = 9)	40.0% (n = 6)

In order to gain a more comprehensive understanding of the baseline health characteristics of the study population, the presence of several frequent comorbidities known to influence oral health and cancer risk was systematically documented in both the control and experimental groups. Among these, tobacco use emerged as the most prevalent risk factor. A higher proportion of individuals in the experimental group reported active smoking (73.3%) compared to those in the control group (53.3%). This difference may reflect varying degrees of cumulative exposure to carcinogenic factors within the study sample and could potentially correlate with more advanced or aggressive lesion behavior.

Alcohol consumption was also assessed and found to be relatively comparable between groups, being reported by 40.0% of participants in the experimental arm and by 33.3% in the control group. While alcohol intake was not substantially different between cohorts, its presence in conjunction with tobacco use remains clinically relevant, given the synergistic effect of these risk factors in the development of oral malignancies.

Regarding systemic comorbidities, type II diabetes mellitus was present in 26.7% of participants in the experimental group and in 20.0% of those in the control group. Arterial hypertension was slightly more frequent in the experimental group (60.0%) than in the control group (46.7%). Although these conditions were not the primary focus of the study, their relatively balanced distribution reinforces the internal comparability of the two cohorts. Such alignment strengthens the validity of the study design by reducing the likelihood that observed diagnostic differences could be attributed to variations in general health status rather than the diagnostic approach employed (Table 1).

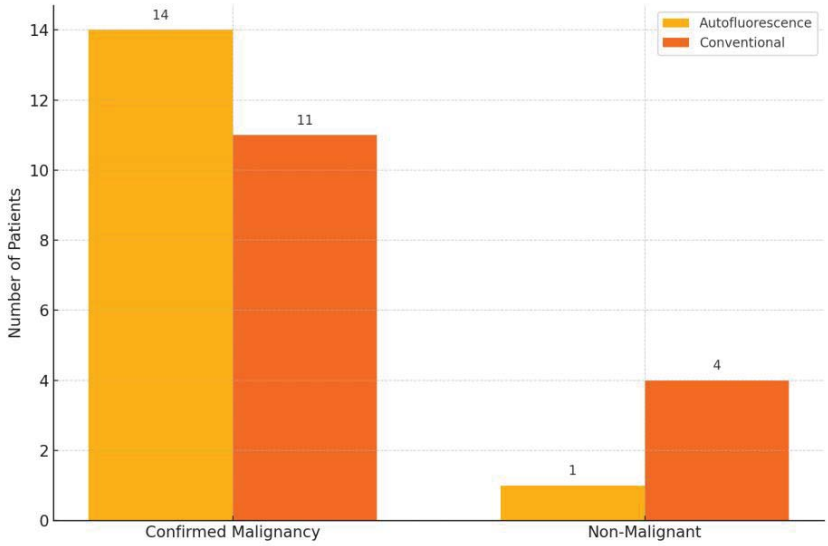

Figure 1. Comparison of histopathological outcomes between autofluorescence-assisted and conventional clinical assessment

A comparative analysis of the two diagnostic strategies revealed a marked difference in the proportion of lesions confirmed as malignant following histopathological evaluation. In the experimental group, where autofluorescence was used in conjunction with conventional white-light examination, 14 out of 15 lesions (93.3%) were diagnosed as malignant, while only one lesion (6.7%) was identified as non-malignant. This high level of agreement between clinical assessment and biopsy results suggests that autofluorescence may enhance diagnostic precision by improving the visualization of early or subtle mucosal changes that could otherwise be overlooked. Conversely, in the control group assessed solely by traditional clinical inspection under white light, 11 lesions (75%) were confirmed as malignant and 4 (25%) were benign, indicating a lower correlation between clinical suspicion and histological confirmation.

Figure 1 illustrates the distribution of confirmed malignancies versus benign outcomes across both groups, emphasizing the improved diagnostic performance observed when autofluorescence is employed as an adjunct. The contrast between the two approaches highlights the clinical relevance of integrating such non-invasive optical tools into standard diagnostic workflows, with the aim of refining lesion characterization, improving biopsy targeting, and ultimately facilitating earlier and more accurate identification of malignant oral pathology.

DISCUSSIONS

The present study investigated the utility of autofluorescence imaging as a non-invasive, adjunctive method for the clinical assessment of oral mucosal lesions suspected of malignancy. The results suggest that this technique may enhance the diagnostic accuracy of conventional visual examination by improving the early detection of lesions with malignant potential. In the experimental group, where autofluorescence was employed alongside white-light inspection, a higher proportion of histologically confirmed malignancies was observed compared to the control group, where clinical evaluation relied solely on standard inspection. This difference supports the hypothesis that autofluorescence facilitates the identification of subclinical or poorly demarcated changes in the oral epithelium—changes that might otherwise escape detection. While histopathological examination remains the gold standard,

the incorporation of autofluorescence as an adjunctive screening tool may assist clinicians in selecting optimal biopsy sites and prioritizing cases for further intervention, especially in settings where diagnostic delays or resource limitations are common.

The outcomes of our study are consistent with the findings of Antonis et al., who emphasized the diagnostic potential of autofluorescence in distinguishing malignant and premalignant oral lesions from clinically normal mucosa. Their study explored the mechanism of tissue fluorescence loss in neoplastic regions and highlighted its relevance in guiding early diagnosis. While their focus was primarily on controlled, in vitro device testing, our research applied the technique directly in clinical practice, demonstrating its ease of integration into routine consultation workflows[12]. This real-world validation provides an added dimension to their theoretical and laboratory-based insights, reinforcing the notion that autofluorescence can be a practical, accessible enhancement to standard oral cancer screening protocols.

The findings also align closely with those reported by Tamošiūnas et al., who evaluated the effectiveness of autofluorescence and chemiluminescence as complementary diagnostic methods in detecting oral potentially malignant disorders (OPMDs). Their results showed improved visualization of early-stage lesions when these optical adjuncts were employed, a benefit that we similarly observed in our patient population[8]. Importantly, while their study highlighted the usefulness of such tools in broader screening initiatives, our work extends the implications to targeted diagnostic encounters, demonstrating that autofluorescence can refine lesion assessment in more focused clinical contexts. Together, both studies underscore the value of such technologies in enhancing the precision of initial evaluations and informing timely decisions regarding biopsy and referral.

Further support for our conclusions is provided by the systematic review and meta-analysis conducted by Santos et al., which assessed both autofluorescence and fluorescent probes in the early detection of OPMDs. Although their findings indicated only moderate sensitivity and specificity for autofluorescence devices such as VELscope®, they also pointed out the variability introduced by examiner interpretation and the lack of standardized clinical protocols[19]. Our study partially addresses these concerns by employing a single, experienced operator and a consistent diagnostic approach, thereby reducing subjectivity and increasing the reproducibility of observations. Moreover, while their meta-analysis suggested that autofluorescence should be regarded as an auxiliary technique rather than a replacement for histopathology, our clinical experience reinforces this perspective by illustrating how autofluorescence can add meaningful value without supplanting traditional diagnostic procedures.

A more technologically advanced approach was presented by Huang et al., who developed a dual-channel autofluorescence imaging system capable of quantifying metabolic activity through measurements of NADH and FAD fluorescence. Their research focused on evaluating the redox status of oral tissues, which allowed for a more objective and quantitative differentiation between normal, premalignant, and malignant regions [20]. While our study relied on subjective visual assessment of fluorescence loss, it demonstrated that even basic, real-time visual tools can provide significant diagnostic insight when applied systematically. The two studies represent complementary perspectives on the future of oral cancer diagnostics: ours affirms the current value of accessible tools for clinical practice, while Huang et al. point toward future integration of advanced imaging and metabolic profiling for even greater precision.

Despite the encouraging outcomes, a number of limitations must be acknowledged. The relatively small sample size (n = 30) restricts the statistical power and external validity of the study. Furthermore, clinical examinations were performed by a single investigator, which, while ensuring consistency, does not account for inter-observer variability that could arise in

larger, multi-practitioner settings. Another limitation is that the autofluorescence evaluation was based solely on subjective visual interpretation, without the support of software-assisted quantification or photographic documentation, which could have provided additional validation. Additionally, the absence of long-term follow-up data precludes assessment of lesion progression or recurrence, factors that would be essential in evaluating the prognostic value of autofluorescence findings.

Nevertheless, these limitations do not diminish the practical contributions of the study. The integration of autofluorescence into real-world consultations, the correlation with histopathological outcomes, and the consistency of results across different anatomical locations support its utility as a reliable adjunctive tool. The simplicity of the technique, combined with its non-invasive nature, make it particularly attractive for use in general dental practices, community screening programs, and in settings with limited access to specialized diagnostic services. Future studies with larger cohorts, objective fluorescence quantification, and long-term monitoring are warranted to further validate these findings and potentially establish standardized protocols for broader clinical adoption.

CONCLUSIONS

This study demonstrates that the integration of autofluorescence imaging into routine clinical assessment of oral mucosal lesions can enhance the early detection of potentially malignant conditions. By facilitating better visualization of subclinical or poorly demarcated abnormalities, autofluorescence serves as a valuable adjunct to conventional white-light examination. The higher proportion of histologically confirmed malignancies in the autofluorescence-assisted group supports its diagnostic utility and reinforces its potential as a non-invasive tool to guide clinical decision-making, especially in primary care or resource-limited settings.

Although histopathology remains the gold standard for diagnosis, the use of autofluorescence may streamline patient triage, improve biopsy site selection, and reduce diagnostic delays. Importantly, this technique offers practical benefits without increasing procedural complexity, making it an accessible innovation for general dental and medical practitioners involved in oral cancer screening.

Conflicts of Interest

The authors declare no conflict of interest.

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