

A Light Based Screening Method Based on Tissue Autofluorescence for Oral Precancerous Lesions: A Review

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ÖZET

Oral prekanseröz lezyonlarda doku otofloresansı esaslı ışıkla değerlendirme yöntemi: Derleme

Amaç: Oral mukozanın otofloresans özellikleri oral prekanseröz lezyonların erken tanısında artan bir ilgiyle karşılanmaktadır. Son dönemde ışık esaslı oral kanser görüntülemeye yardımcı cihazlar ağız muayenesi ve tanıyı desteklemek amacıyla geliştirilmektedir. Bu cihazlar özel olarak tasarlanmış ışık kaynağıyla ağız muayenesinde mukozayı belirginleştirmek için otofloresans özelliğini kullanmaktadırlar. Bu bildirinin amacı ışık esaslı bir otofloresan izleme yönteminin oral premalign ve malign lezyonlarda potansiyel fayda ve risklerini ortaya koymaktır.

Yöntem: Geniş literatür araştırması ve bu sistemlerden biriyle klinik tecrübe sistem değerlendirmesine olanak sağlamıştır.

Bulgular: Bu yazıda prekanseröz ve kanserli lezyonların belirlenmesinde yardımcı izleme yöntemlerinden bir tanesi değerlendirilmiştir. Sistemin özellikleri ve dezavantajları değerlendirilmiş, klinisyenin dikkatine sunulmuştur.

Sonuç: Günümüzdeki yenilikler malignite potansiyeli olan lezyonların erken tanısında yararlı olabilmektedir, ancak bunların tek başına diagnostik araçlar olmadığı, daha çok yardımcı araçlar oldukları düşünülerek dikkatle kullanılmaları gerekmektedir. Otofloresan kaybı mukozada displaziye belirlemekte rol oynamakla beraber bu lezyonlar klinik bilgi ve deneyimle değerlendirilmelidir.

Anahtar sözcükler: Otofloresans, velscope, prekanseröz lezyon

ABSTRACT

A light based screening method based on tissue autofluorescence for oral precancerous lesions: A review

Objective: Autofluorescence properties of oral mucosa has been gaining interest in the field of early diagnosis of oral precancerous lesions. To aid the oral cancer screening, light based systems have recently been developed in order to assist the oral examination and identification. These systems feature light sources with certain wavelength designed according to principles of tissue reflectance and autofluorescence to enhance the conventional oral examination. The purpose of this report is to address the potential benefits and potential risks of a light based autofluorescence screening method for potentially premalignant and malignant lesions.

Methods: Extensive literature review and personal clinical experience with one of these technologies enabled the evaluation of the system.

Results: This manuscript evaluated the use of one of the adjunctive screening methods to detect precancerous and cancerous lesions. The characteristics of the system were outlined, the limitations associated with autofluorescence screening were put forward for the clinician's consideration.

Conclusion: Recent technologies in the early detection of potentially malignant lesions may be beneficial, however they should be used with cautious since they are only adjunctive methods. Loss of autofluorescence seems to play a role for identifying mucosal dysplasia but lesions should be evaluated with clinical knowledge and experience.

Key words: Autofluorescence, velscope, precancerous lesion

INTRODUCTION

Oral Cancer

Oral cancer appears as the sixth most common malignancy worldwide, 90% of which consists of squamous

cell carcinoma. It has been reported that the incidence rate of oral and pharyngeal cancers is decreasing overall however, the incidence rate of cancers of the tongue, oropharynx and tonsil is increasing. Early diagnosis of oral cancer is therefore crucial to improve the patient's survival rate (1-3). However, because of the poor diagnosis of the

pre-malignant and malignant lesions, morbidity and mortality have not decreased over the past 50 years. In developed countries, the five-year survival was only 63% between 1999-2005. This poor prognosis is likely due to several factors. Oral cancer is frequently associated with the development of multiple primary tumors. The rate of second primary tumors in these patients, 3-7% per year, is higher than for any other malignancy (2-5).

An invasive cancer may have different clinical signs; induration; persistent ulceration; tissue proliferation or destruction; red and white color; lack of mucosal mobility; progressive growth or enlargement of the affected site; pain or dysesthesia, paresthesia or loss of function; and cervical lymphadenopathy. Most oral squamous cell carcinomas exhibit one or more of these clinical signs, which often are identifiable on routine examination⁶.

The terms "pre-cancer", 'precursor lesions', 'pre-malignant', 'intra epithelial neoplasia' and 'potentially malignant' have been used in the international literature to broadly describe clinical presentations that may have a potential to become cancer. The World Health Organization (WHO) has periodically convened international workshops to redefine the term "precancer" and the various oral precancerous lesions. The most recent workshop, actually recommended the elimination of the term "precancer" and the use of the presumably more illuminating term "potentially malignant lesion" for oral lesions (7).

Oral Examination

Conventional oral examination (COE) for oral cancer involving visual inspection and palpation of oral lesions

under illumination has been deficient. While COE may help to detect the oral lesions, it may not discover all potentially premalignant lesions (4). Since malignant and benign lesions may not be clinically evident, the clinician cannot predict the biological character of the lesions evaluating their physical features alone (4,8,18).

In many cases, a biopsy is mandatory so that such lesions can be discarded. Histopathological examination and the classification of epithelial dysplasia still represent the gold standard in precancerous lesion risk evaluation.

Oral Cancer Screening

Lingen et al (4) defined the term as "Screening for disease has a precise definition and implies an ongoing, structured health care intervention designed to detect disease at an asymptomatic stage when its natural course can be readily interrupted if not cured. The important factor is that screening involves checking for the presence of disease in a person who is symptom-free".

There are some specific cancer screening programs which have been shown to reduce patient morbidity and mortality. Well-known Pap test for cervical cancer and mammography for breast cancer are used at any health care setting (4,8).

Early-stage lesions may be asymptomatic and resemble benign lesions whereas some alterations may not be readily evident in routine conventional white light oral examination.

In order to detect oral pre-malignancy and malignancy, new technologies with new diagnostic tools for localizing or emphasizing dysplasia have been proposed (4,9-18). Adjunctive screening devices are on the market in order to aid the clinicians with the detection of early cancerous changes or for the assessment of the biological characteristics of mucosal lesions (4,8,9,17-19).

Oral Autofluorescence

Autofluorescence technology has been already used in endoscopic instruments for bronchoscopy, esophageal examination, colonoscopy and skin evaluation¹⁸. In recent years, autofluorescence imaging has gained a growing interest in clinical practice for noninvasive imaging of the oral mucosa. With the help of different excitation wavelengths autofluorescence process occurs thanks to fluorochromes

Tissue Fluorescence and Dysplastic Progression

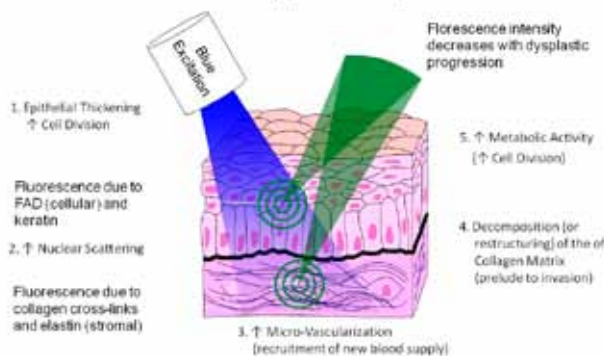


Figure 1: Tissue fluorescence and dysplastic progression.

located in the epithelial cell lining and submucosa (e.g. collagen, elastin, keratin, oxidized flavine adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide [NADH]). The normal intrinsic pattern of autofluorescence is modified by absorption and scattering (Figure 1). Light-based imaging of the tissues has been popular and has claimed to detect early dysplastic changes. The philosophy of this concept is the autofluorescence properties of oral mucosa. The discovery and harnessing of fluorescent proteins, together with the subsequent biological and medical research have been significant enough to have culminated in a Nobel Prize in Chemistry (18).

There are some light-based oral cancer screening aids which have been developed to identify the precancerous and cancerous lesions as early as possible. These light-based oral cancer screening aids use special light sources designed according to principles of tissue reflectance and tissue autofluorescence to enhance the oral examination process. Manufacturers of these devices claim that they may help the practitioner to visualize oral mucosal abnormalities that are not readily detectable with conventional operatory lighting, or that they can enhance the practitioner's ability to specifically identify potentially malignant lesions (6).

A direct visual screening device based on autofluorescence: Visually Enhanced Lesion Scope (VELScope[®]) "Narrow-emission tissue fluorescence"

VELscope (LED Dental, Burnaby, British Columbia, Canada) is an adjunctive device to aid the conventional oral examination in the identification of oral premalignancy which may not be apparent under conventional light (21). With the help of blue-light excitation (400–460 nm) provided by the unit, normal oral mucosa emits a pale green autofluorescence when viewed through the filter set incorporated within the hand-piece (Figure 2). Filtration is essential, as the intensity of the reflected blue-white light makes it otherwise impossible to visualize the narrow autofluorescent signal. The manufacturer of VELScope[®] indicates that abnormal mucosa exhibits decreased levels of autofluorescence appearing dark when compared with the surrounding tissue (6). On the other hand, loss of autofluorescence is not limited strictly to epithelial abnormalities and can be seen with prominent surface



Figure 2: VELscope device



Figure 3a: White light image of the angular mucosa of a 40-year-old heavy smoker patient with histopathologically confirmed dysplasia.

vascularity, including areas of inflammation, and melanin pigmentation (6,21). The device can be used during the surgery to identify the margins of the lesion for surgical excision (18,21).

Atypical or suspicious tissue exhibits decreased levels of normal autofluorescence and appears dark by comparison to the surrounding healthy tissue. An immature or dysplastic epithelial cell has much less NADH and FAD activity than a normal cell and so mucosal areas with such cells will not fluoresce, thereby appearing dark through the eyepiece (Figures 3a, 3b and 4a, 4b)(18,21,24).

Benign lymphoid tissues, such as tonsils are almost lack

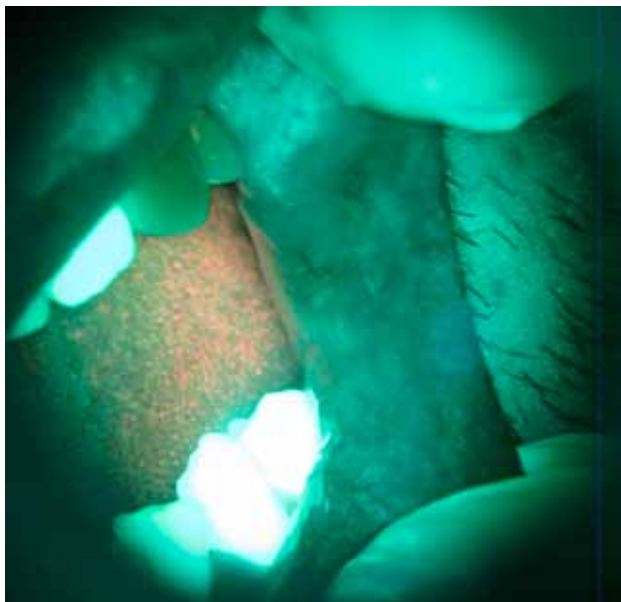


Figure 3b: Fluorescence image showing areas with slightly decreased autofluorescence.

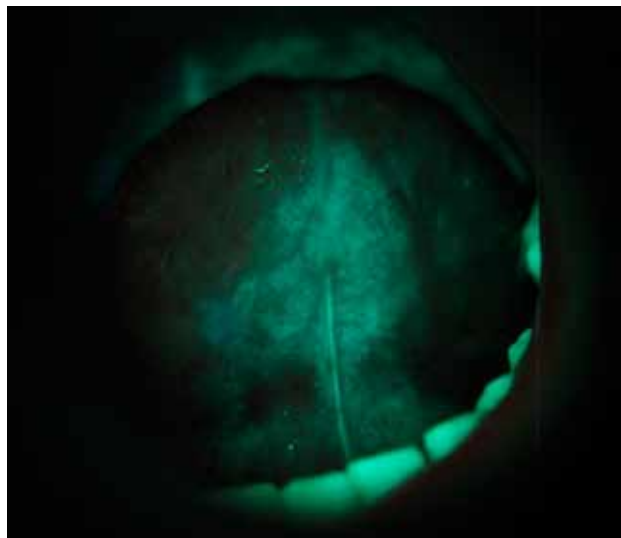


Figure 4b: Fluorescence image showing minimum loss of fluorescence.



Figure 4a: White light image of the leukoplakia on the ventrum of the tongue.

of collagen and leukocytes may lack the autofluorescence molecules influenced by the wavelengths of the oral devices. They appear dark when viewed with the VELScope[®] (18). Bacteria using different fluorescent cytosol molecules give off a red, pink or orange fluorescence (Figures 5a and 5b). Fungal microorganisms, such as candida, may fluoresce yellow or yellow/orange.

Many studies have assessed the sensitivity and specificity

of autofluorescence screening for the detection of dysplastic and malignant lesions from normal oral mucosa (22, 23, 25-35). Using histology as the gold standard, the device demonstrated varying sensitivity and specificity percentages.

Lane et al. (25) showed a high sensitivity (98%) of VELscope[®]. Awan et al. (33) determined 84,1% sensitivity and 15,3% specificity in distinguishing dysplastic lesions. Rana et al. (34) evaluated the sensitivity and specificity as 100% and 74% respectively. Contrary to those findings, Farah et al. (29) indicated the sensitivity as 30% and specificity as 63%. Scheer et al. (30) showed that the sensitivity was 100% and the specificity was 80,8% whereas positive predictive value was 54,5% and negative predictive value was 100%. The system needs to be evaluated in more number of studies with larger groups.

Despite its applicability, the system is expensive ranging from \$4,000 to \$7,000, and color interpretation is difficult, which could lead to an erroneous diagnosis. False positive screening results may happen due to lack of experience and lack of knowledge about oral mucosal diseases(36).

Other Light-based oral precancer screening devices

Vizilite Plus with TBlue system (Zila Pharmaceuticals, Phoenix, Arizona,U.S.), Identafi (Trimira-Remicalm, Houston,TX, USA), Microlux/DL (AdDent Inc, Danbury, Connecticut) and Orascoptic DK (Orascoptic, Middleton, WI) are other light-based systems on the market.



Figure 5a: Invasive squamous cell carcinoma on palatal mucosa

ViziLite imaging device has been on the market more than twelve years and it has been approved by FDA to use in the oral cavity and works with the emission of light from a chemical reaction between hydrogen peroxide and acetylsalicylic acid inside a capsule light stick. Thus, rinsing the mouth with 1% acetic acid solution for one minute is recommended when ViziLite / ViziLite Plus (ViziLite system combined with Toluidin Blue) are used. Its specificity is low as 14 % (18,19,31).

Identafi, MicroLux DL and Orascopic are other devices on the market claiming to have a capacity to enhance the identification of abnormal oral mucosa. The studies reflecting their clinical use, sensitivity and specificity are needed to assess their role in the detection of mucosal abnormalities (4,18,31).

A most preferred method for aiding the early detection of cancer is to have both low false negative rate and low false positive rate, however this has not seemed to be possible with the mentioned systems (4,31,35).

New Technologies

In order to detect the oral cancer earlier, new technologies seem to be promising. Some of them are; the

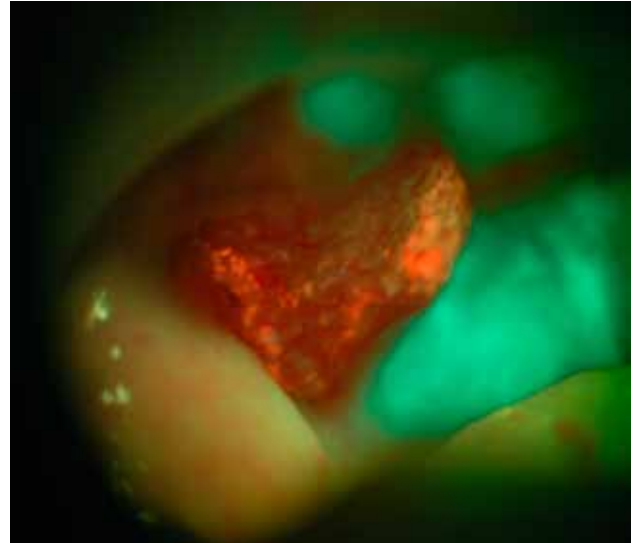


Figure 5b: With secondary infection and red glow from bacteria using different fluorescent cytosol molecules under fluorescent light

use of saliva in oral cancer screening; the use of DNA aneuploidy analysis; loss-of-heterozygosity analysis; identification of bacterial markers in biofilms; identification of individual or multiple protein biomarkers revealed via tissue biopsies; the use of in vivo molecular probes and paints; and the use of other imaging modalities (6).

Chromosomal aberrations are key events in the initiation and progression of cancer. DNA-aneuploidy is considered to be a marker for the neoplastic transformation of cells. DNA-Image-Cytometry is used to detect the cytometric equivalent of chromosomal aneuploidy, which is called DNA-aneuploidy. This method gives information about the occurrence and number of abnormal stemlines, polyploidization of euploid or aneuploid stemlines, cell cycle fractions and occurrence of rare aneuploid cells with an abnormally high DNA content (11-15). Detection of loss of heterozygosity is another method in the detection of oral cancer. When the presence of heterozygosity at a genetic locus in an organism's germline DNA, and the absence of heterozygosity at that locus in the cancer cells are detected, the loss of heterozygosity is determined. Recent studies have shown this method as promising (6,8).

Saliva testing for specific mRNAs has been increasingly searched for the early diagnosis of oral cancer. Cell-free nucleic acids & proteins in saliva are used for the analysis. Molecular markers are mainly the changes in the cellular DNA, altered mRNA transcripts and altered protein markers.

All the biomarkers were significantly altered in oral cancer and found to be useful as a supportive tool for diagnosis, prognosis and post-operative monitoring. These are; CycD1, Ki67, MMP-9, OGG1, Maspin, Proangiogenic/proinflammatory cytokines; IL-1, IL-6, IL-8, increased telomerase activity, presence of p53 autoantibodies, altered levels of reactive nitrogen species and antioxidants, HPV for HPV related cancer, DNA hypermethylation, salivary mRNA, transferrin and salivary biomarkers (Actin, myosin) (37-39).

Biofilms are bacterial communities that colonize the mouth in the form of dental plaque and recently their role in oral cancer can have been searched using with some techniques including DNA-DNA hybridization, 16S rRNA gene sequencing, denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, denaturing high-performance liquid chromatography and pyrosequencing. New oral cancer screening tests based on salivary counts of different species of bacteria may help with screening compliance (37,38). The mentioned modalities will play important roles in future clinical practice.

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CONCLUSION

Although oral mucosa screening using autofluorescence characteristics has been gaining interest over the few years, there is still a debate on its utility. Oral cancer screening is a part of a clinical oral examination that includes obtaining a patient history and an oral cancer risk assessment. Whenever an oral mucosal lesion is detected, re-evaluation in up to 14 days to see either persistence or healing of the lesion is essential. Only a definitive test examining cells or tissue can determine the biologic behavior of a lesion. When a definitive diagnosis is needed, a clinician should perform a surgical biopsy with subsequent specimen processing and histopathological examination.

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