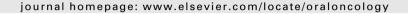Contents lists available at ScienceDirect

Oral Oncology

Novel quantitative analysis of autofluorescence images for oral cancer screening

Tze-Ta Huang [a,b], Jehn-Shyun Huang [a,b], Yen-Yun Wang [c,d], Ken-Chung Chen [a,b], Tung-Yiu Wong [a,b], Yi-Chun Chen [b], Che-Wei Wu [e,f], Leong-Perng Chan [e], Yi-Chu Lin [e,f], Yu-Hsun Kao [g], Shoko Nioka [i], Shyng-Shiou F. Yuan [c,d,h,j,*], Pau-Choo Chung [k,*]

[a] Division of Oral and Maxillofacial Surgery, Department of Stomatology, National Cheng-Kung University Medical College and Hospital, Tainan, Taiwan
[b] Institute of Oral Medicine, National Cheng-Kung University Medical College and Hospital, Tainan, Taiwan
[c] Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Translational Research Center, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Department of Otorhinolaryngology-Head and Neck Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[f] Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[g] Department of Oral and Maxillofacial Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[i] Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA
[j] Department of Obstetrics and Gynecology and Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[k] Department of Electrical Engineering, National Cheng Kung University, Tainan, Taiwan

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ABSTRACT

Objectives: VELscope® was developed to inspect oral mucosa autofluorescence. However, its accuracy is heavily dependent on the examining physician's experience. This study was aimed toward the development of a novel quantitative analysis of autofluorescence images for oral cancer screening.
Materials and methods: Patients with either oral cancer or precancerous lesions and a control group with normal oral mucosa were enrolled in this study. White light images and VELscope® autofluorescence images of the lesions were taken with a digital camera. The lesion in the image was chosen as the region of interest (ROI). The average intensity and heterogeneity of the ROI were calculated. A quadratic discriminant analysis (QDA) was utilized to compute boundaries based on sensitivity and specificity.
Results: 47 oral cancer lesions, 54 precancerous lesions, and 39 normal oral mucosae controls were analyzed. A boundary of specificity of 0.923 and a sensitivity of 0.979 between the oral cancer lesions and normal oral mucosae were validated. The oral cancer and precancerous lesions could also be differentiated from normal oral mucosae with a specificity of 0.923 and a sensitivity of 0.970.
Conclusion: The novel quantitative analysis of the intensity and heterogeneity of VELscope® autofluorescence images used in this study in combination with a QDA classifier can be used to differentiate oral cancer and precancerous lesions from normal oral mucosae.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant disease of oral mucosae and is closely associated with betel quid chewing, chronic smoking and alcohol consumption [1,2]. OSCC is also the sixth most common cancer worldwide, with an average five year survival rate of approximately 60% [3]. The incidence and severity of OSCC are increasing worldwide, especially in Taiwan [4]. From 1981 to 2010, the standardized mortality rates increased from 2.5 to 8.1 per 100,000 persons [5]. Currently, there are no reliable or easy approaches for early detection except conventional oral examination and biopsy by physicians. Although oral cancer develops in the superficial mucosa in the oral cavity and can be detected during a routine clinical examination, the screening rate for high-risk patients is only 25% in Taiwan [5]. Up to the present time, diagnosis of oral cancer has relied heavily on lesion biopsies, which are time-consuming and uncomfortable for patients [6]. Due to concerns about the disadvantages of biopsy,

* Corresponding authors at: Translational Research Center, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan (S.-S.F. Yuan), Department of Electrical Engineering, National Cheng Kung University, Tainan, Taiwan (P.-C. Chung).
E-mail addresses: yuanssf@ms33.hinet.net (S.-S.F. Yuan), pcchung@eembox.ee.ncku.edu.tw (P.-C. Chung).

non-invasive oral screening methods such as a brush biopsy, autofluorescence imaging, and toluidine blue staining have been applied [7–10]. Nevertheless, the physician's experience is critical when using current methods. The development of visual detection of chemoluminescence and tissue fluorescence for the diagnosis of oral cancer may improve detection and increase the capacity to identify potentially malignant lesions [11]. Tissue autofluorescence has been used in the screening and diagnosis of precancerous and early cancerous lesions of the oral cavity [12–16]. Changes in autofluorescence reflect complex alterations to fluorophores in tissue and structural changes in morphology. These autofluorescence changes are caused by alterations in structure (hyperkeratosis, hyperchromatin, and increased cellular and nuclear pleomorphism) and metabolism (concentration of flavin adenine dinucleotide-FAD and nicotinamide adeninedinucleotide-NADH of the epithelium and the composition of the collagen matrix and elastin of subepithelial stroma) [17]. During carcinogenesis, tissue structure and metabolism are changed. The Warburg effect shows that the aerobic glycolysis in cells will cause the generation of lactate [18], which boosts the production of NADH and raises the value of the NADH/FAD ratio [19,20]. Some studies have revealed that NADH intensity in cancerous and precancerous tissues is higher than it is in normal tissues [21]. The endogenous fluorophores that are most relevant for the diagnosis of precancerous and cancerous lesions are excited in the UV-A spectrum from 315–400 nm to emit visible violet-blue (400–450 nm). These endogenous fluorophores have properties that have been spectroscopically correlated with cancer [22]. The autoflorescence signal can be visualized directly by a human observer. Bright blue light (400–460 nm) excitation that yields emission of green autofluorescence imaging has been developed for inspecting oral mucosa and identifying lesions in the oral cavity using the VELscope® system [23]. The VELscope® system helps to detect lesions that would be otherwise unnoticed, but it crucially depends on a physician's degree of experience with direct visualization under autofluorescent light [24,25]. Because visualization solely depends on physician experience, there is a high possibility of human bias and error. Up to the present time, there has been no quantitative analysis of VELscope® images for oral cancer screening. In the current study, we introduced a quantitative analysis method for VELscope® autofluorescence images and applied quadratic discriminant analysis (QDA) to classify the experimental and control groups. This quantitative analysis method eliminates human bias and error while providing useful results for discrimination between oral lesions and normal mucosae.

Materials and methods

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital (IRB No: B-ER-103-347) and Kaohsiung Medical University Chung-Ho Memorial Hospital (IRB No: KMUH-IRB-20140285), Taiwan, and was conducted in the Division of Oral and Maxillofacial Surgery and Department of Otorhinolaryngology-Head and Neck Surgery with the written informed consent of all the enrolled participants. In the study, we aimed to differentiate oral cancer and precancerous lesions from normal oral mucosae using VELscope® autofluorescence images. Clinical data was collected from each patient, and the pathological reports were also recorded. The normal oral mucosa group was recruited from patients who had received a routine oral examination and did not have habits including smoking, alcohol consumption, or betel quid chewing.

The enrolled patients were instructed to clean the oral cavity using a mouth rinse with normal saline 3 times for 2 min each time. A digital camera (Nikon, Coolpix P7700) was used to take the white light image of the lesion first. The patient was then asked to wear eye goggles while using the VELscope® device to take an autofluorescence image of the lesion. VELscope® was developed by LED Medical Diagnostics (White Rock, BC, Canada) and has a range of visible light in the 400–460 nm wavelength range that causes fluorescent excitation of mostly flavoprotein compounds including FAD in oral mucosae. Emission light from normal oral mucosae is in a range of green light.

The collected image data were stored in folders and included the patients' chart numbers and enrollment dates. The data were subsequently saved in a server.

The lesion in the image was chosen as the region of interest (ROI) to avoid any noise produced by teeth, prosthesis, or filling materials. The ROI was selected by the principal investigator, who was also the patients' attending physician. After selecting the ROI, we calculated the average intensity of RGB and gray channels using the following equation, where I(x, y) represents the intensity of R, G, B or gray channels, and n is the number of pixels in the ROI. The average intensity of the image is defined as

$$\mu = \frac{\sum I(x,y)}{n}. \tag{1}$$

The average intensity of the ROI in autofluorescence images from the digital camera could be measured using the above equation. However, the standard deviation of ROI was also used to represent the image heterogeneity. The image heterogeneity was defined as where μ is the mean value calculated by [1]; I(x, y) is the intensity of the levels of R, G, B or gray, and n is the number of pixels in the ROI.

$$\sigma = \sqrt{\frac{\sum (I(x,y) - \mu)}{n}} \tag{2}$$

Linear discriminant analysis (LDA) is a method frequently used in pattern recognition for medical imaging, especially in pathological examinations, to find a linear combination of features that separates several classes of objects [26]. Quadratic discriminant analysis (QDA) is an extension of LDA. It is used to separate classes of objects according to quadric surface, which makes it more flexible than an LDA [27]. The QDA classifier was chosen as a classification algorithm because the features we used are simple and independent.

The QDA assumes that each class of data is distributed with multivariate Gaussian distributions, where the probability density function (PDF) of Gaussian distribution for class k is:

$$f_k(x) = \frac{1}{\sqrt{(2\pi)^{p/2}|\varepsilon_k|^{1/2}}} e^{-\frac{1}{2}(x-\mu_k)^T \varepsilon_k^{-1}(x-\mu_k)}, \tag{3}$$

where p is a dimension; μ_k is the mean of class k, and ε_k is the covariance of class k.

In the QDA, the decision boundary of class k and class m has the same PDF value, which means $f_k(x) = f_m(x)$. The formula of decision boundary is defined as δ_k:

$$\delta_k(x) = -\frac{1}{2}\log \varepsilon_k - \frac{1}{2}(x - \mu_k)^T \varepsilon_k^{-1}(x - \mu_k) + \log \pi_k, \tag{4}$$

Where π_k is $\dfrac{number\ of\ samples\ in\ class\ k}{total\ number\ of\ samples}$. \hfill (5)

We used the above equations to get a boundary of the input classes and then calculated the sensitivity and specificity.

Results

The participants were enrolled from December 2014 to February 2016 after providing written informed consent. All the partici-