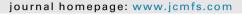
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Fluorescence based characterization of early oral squamous cell carcinoma using the Visually Enhanced Light Scope technique



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ABSTRACT

Objectives: Several diagnostic tools have been developed to assess benign and potentially malignant disorders of soft tissues. In this study, we aimed to assess the value of the VELscope[®] (Visually Enhanced Light Scope) imaging device as a technical tool to investigate malignant lesions of the oral cavity. *Material and methods:* In this retrospective study we analyzed the photographs of 90 patients who suffered from malignant oral soft tissue lesions or carcinoma in situ (CIS) from 2008 to 2014 in the Clinic of Oral and Maxillofacial Surgery of LMU in Munich.

Results: In 85.6% of the cases fluorescence quenching/loss could be detected. The average value for the colour red shows a significant difference in pathologic and physiologic tissues (p = 0.007) with a higher median for pathologic tissues. For the colours green and blue our measurements show significantly higher values in the healthy tissue (p < 0.001). The shade of red showed significantly higher values for pathologic tissues when compared to all three colours (p < 0.001). Furthermore, the shades of green and blue showed significantly lower values in the pathologic tissue (p < 0.001).

Conclusion: In the near future, VELscope[®] could help to a greater extent than visual observation alone in identifying the margins of tumor resections. VELscope[®] still lacks the ability to identify the overall risk level of oral lesions.

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1. Introduction

Oral cancer is the eleventh most common cancer worldwide according to the World Health Organization (Petersen et al., 2005). It accounts for nearly 3% of all cancer cases globally, with an incidence estimated at 274,000 new cases per year (IARC monographs on the evaluation of carcinogenic risks to humans 2012; Parkin, 2001). Oral squamous cell carcinoma (OSCC) accounts for 90% of these oral cancers (Silverman, 1998). OSCC may arise from oral potentially malignant disorders (OPMDs) such as leukoplakia, erythroplakia, or lichen planus (Warnakulasuriya et al., 2007). The most common

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treatment for OSCC is surgery (Forastiere et al., 2002). Early detection of premalignant lesions therefore offers a great potential benefit to patients. The differentiation of neoplastic tissue alterations from non-dysplastic epithelium represents a major concern of all surgical disciplines.

Recently, several diagnostic tools have been developed to assess benign and potentially malignant disorders. In particular, the use of autofluorescence imaging has gained interest in clinical practice for non-invasive and repeatable imaging of the oral mucosa. Autofluorescence is based on the excitation of different and specific endogenous fluorochromes in oral epithelium and submucosa (Awan et al., 2011). When viewed through a filter and irradiated, all fluorochromes emit light of the green spectral range with wavelengths between 375 and 440 nm (Betz et al., 2002). The VELscope[®] (Visually Enhanced Light Scope) device, available since June 2006, is a handheld system which uses blue light excitation between 400

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and 460 nm to cause tissue autofluorescence. The VELscope[®] is a certified medical device (No 41315446) approved by the American Dental Association for the detection of mucosal tissue abnormalities (CDT D0431). In the literature, there have been several studies displaying increased sensitivity and for malignant lesions by the combined use of autofluorescence and clinical examination of the oral cavity (Betz et al., 2002; Rana et al., 2012).

Under VELscope[®] exposure, normal tissue will fluoresce light green due to endogenous fluorochromes (Kulapaditharom and Boonkitticharoen, 2001). Dysplastic changes of epithelium cause loss of physiologic autofluorescence and the tissue appears dark (Betz et al., 2002; Lane et al., 2006). Loss of autofluorescence has been correlated with disease progression and has already been implemented in other tissue screening procedures such as cervical cancer (Richards-Kortum and Sevick-Muraca, 1996). In this study, we aimed to assess the value of VELscope[®] imaging as a technical device to determine the reliability of fluorescence loss in histologically proven OSCC.

2. Material and methods

2.1. Patients and data collection

In this retrospective study we analysed the photographs of 90 patients who suffered from malignant oral soft tissue lesions or carcinoma in situ (CIS) from 2008 to 2014 in the Clinic of Oral and Maxillofacial Surgery of LMU in Munich. The study was approved by the Ethical Committee of LMU Munich on the 7th of April 2014, and is listed under UE Nr. 042-14. Inclusion criteria were: age >18 and histologically verified CIS, squamous cell carcinoma, or adenocarcinoma.

2.2. Study variables

The parameters which were taken into account for the analysis were age, sex, cigarette or alcohol abuse, tumor location, and tumor classification according to the International Union Against Cancer (UICC) (Sobin et al., 2010), the stage and grade results acquired by PET-CT or CT according to German medical guidelines.

2.3. Analysis of the photographs

The fluorescence analysis was conducted on photographs taken routinely for treatment documentation. For this purpose the models EOS 7D Canon and D1X Nikon were used. In each photograph the oral epithelium lesion and surrounding tissue are depicted. We analyzed the differences of the fluorescence characteristics of different tissue types like masticatory mucosal tissue and epithelium. The camera properties were adjusted for optimizing green autofluorescence of each tissue type. The ISO Nr. was 4000 and shutter speed was 1/100 s, the 11 and 5.6 lens was used.

2.4. Measurements

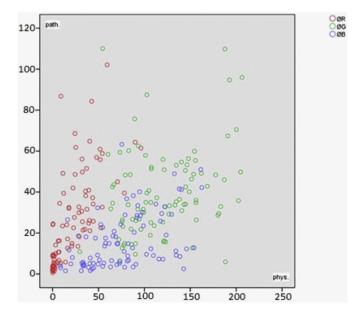
For analysis of our data the RGB System and Adobe Photoshop Elements 10 Editor were used. Photographs acquired during white light inspection showing epithelial lesions suspected for pathologic areas were chosen and compared to the photographs taken with the VELscope[®]. VELscope[®] images were obtained at an excitation wave length between 400 and 460 nm. On each photograph, 10 random measurement spots were chosen in the oral lesion and in the surrounding healthy tissue. This technique was described by several groups before (Roblyer et al., 2009; Schwarz et al., 2009; Lane et al., 2006). In Fig. 1 this concept is shown. For each measurement the same tissue type was selected. Then the average values for red, green, and blue were calculated. The result for each colour was added in a triplett. Thus we could calculate the share of each colour of the sum of all three. Furthermore, we calculated the share of each colour to the maximum intensity of 255 (see Table 1). Then the ratio for each colour was calculated in the pathologic and physiologic area.

2.5. Statistics

All statistical analyses were performed with IBM SPSS. The Ttest, Wilcoxon Signed Rank Test and Kolmogorov-Smirnov-Test were used. Significance was considered at p < 0.05. Additionally the directions of the different values for pathologic and physiologic

Table 1

Scatter plot displaying the distribution of the 3 colours in pathologic and healthy tissues. The colours blue and green are represented in physiological whereas red is displayed in pathological areas more frequently.



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