Optical Coherence Tomography As An Alternative Method for Excisional Biopsy in Oral Pathology

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In vivo, non-invasive optical coherence tomography (OCT) allows high-resolution imaging of tissue surfaces and subsurface, with the potential capability for detection and mapping of epithelial pathologies. Our purpose was to evaluate the clinical capability of noninvasive in vitro OCT for fast diagnosis of oral malignancy. The OCT imaging analyzes were in complete accordance with the pathologist report. These data demonstrate the excellent capability of in vitro OCT for detecting and diagnosing oral malignancy in human subjects.

Key words: malignant tumors, non-invasive diagnosis, optical coherence tomography, real time imaging.

Oral cancer represents a global health problem with increasing incidence and mortality rates. There are a series of independent prognostic markers for this type of disease such as age, gender, immunological and nutrition status, size and location of the tumor, disease stage, nodal status, oncogene expression, proliferation markers and DNA content.

Oral cancer is one of the most common neoplasms, which accounts for 2-3% of all human malignancies [1]. Oral squamous cell carcinoma (OSCC) constitutes 90% of oral cancer [2].

One of the most important prognostic markers for oral squamous cell carcinoma is the tumor stage at diagnosis. Almost half of the oral cancers are diagnosed at advanced stages with 5 year survival rates ranging from 20% to 50%, depending on tumor sites [3]. Early detection is a cornerstone to improve survival and to reduce diagnostic delay. Diagnostic delay is most often categorized as:

1. Patient delay – the period between the patient first noticing a sign or symptom and their first consultation with a health care professional and

2. Provider/professional delay – the period from the patient's first consultation with a health care professional and the definite pathological diagnosis [4].

Recent evidence shows that early diagnosis can significantly decrease the morbidity associated with treatment and may improve overall long term survival [5]. Methodologically sound reports have been able to demonstrate that diagnostic delay was associated with an increased risk of recurrence and oral cancer mortality, even for tongue sites [6].

The efforts aimed at early diagnosis of oral cancer should be prioritized towards screening programs designed to detect the disease during its asymptomatic phases.

Current techniques require surgical biopsy of lesions. Benign lesions are often biopsied, reducing patient motivation to agree to further diagnostic biopsies in the future. Conversely, many lesions are only detected by biopsy at an advanced stage, when treatment options and outcome are far from optimal. A modality for the direct, non-invasive early detection, diagnosis, and monitoring of oral dysplasia and malignancy and for the screening of high-risk populations is urgently required to identify treatment needs at early, more treatable stages of pathological development. OCT is a new high-resolution optical technique that permits minimally invasive imaging of near surface abnormalities in complex tissues. It has been compared to ultrasound scanning conceptually [7]. Both ultrasound and OCT provide real time structural imaging, but unlike ultrasound, which utilizes sound waves, OCT is based on low coherence interferometry, using broadband light to provide cross-sectional high resolution sub-surface tissue images [8].

The main direction in optimizing these diagnostic techniques would be to reduce the second phase of the diagnostic delay by implementing the OCT investigation, to achieve a resolution comparable with histological sectioning.

Experimental part

The study was performed at the School of Dentistry, University of Medicine and Pharmacy "Victor Babes", Timisoara. The experimental design study was approved by the Local Ethics Committee and, after the presentation of the written protocol the patients signed their written participation agreement.

Two different biopsies of oral lesions were collected from patients hospitalized in the Oro-Maxilo-Facial Surgery Clinic. The excisional biopsy was achieved by the same

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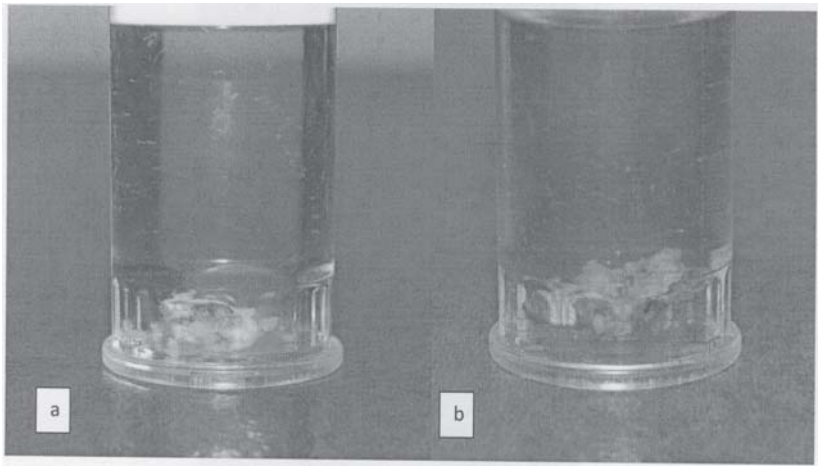

Fig. 1. Biopsy sample stored in 10% Formalin; a. biopsy from the suspicious lesion, b. healthy tissue

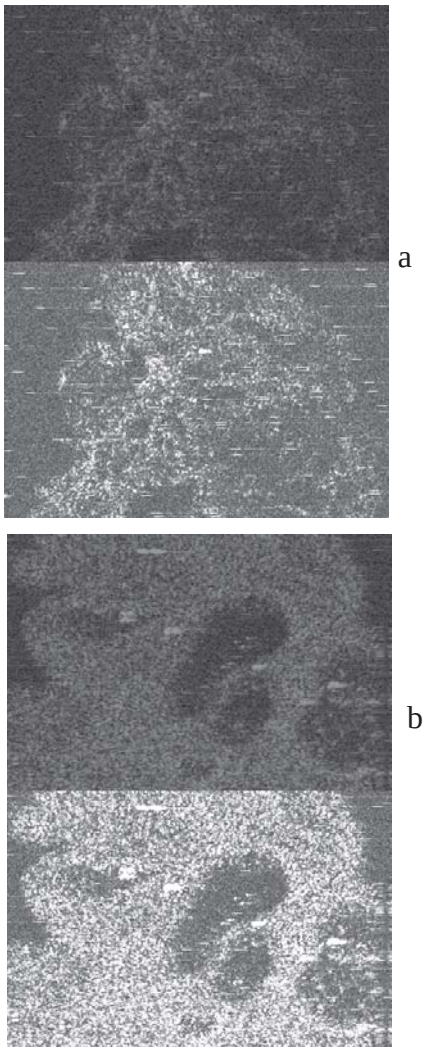

Fig. 2. OCT image of the buccal mucosa: a. healthy tissue, b. oral squamous cell carcinoma

surgeon, harvesting a sample of healthy tissue near the suspicious lesion and one from the lesion itself.

Both lesions were suspected of Oral Squamous Cell Carcinoma (OSCC). One of them was located on the buccal mucosa in the left superior part of the oral cavity. The other one was located in the right parotid.

The excisional biopsy was carried out according to the standard protocol and half of it was directed to the pathologist and the other half was assigned for OCT investigation. The samples were deposited in recipients containing 10% formalin (fig. 1). The OCT investigation was performed in the same day with the excisional biopsy for both samples and for both cases.

For the OCT imaging it was used a OCT *en-face* prototype (1300 nm). The calibration was performed as recommended by the manufacturer. The biopsy was positioned on the holder and the determination was performed by the same person for both samples, in both cases.

Results and discussions

Areas of OSCC of the buccal mucosa were identified in the OCT images by the absence or disruption of the basement membrane, an epithelial layer that was highly variable in thickness, with areas of erosion and extensive epithelial down-growth and invasion into the sub-epithelial layers (fig. 2). In the OCT image of the parotid tissue, the epithelium is highly variable in thickness, with areas of erosion; the basement membrane is not visible as a coherent landmark, presenting an irregular and unclear architecture of the lamina propria (fig. 3). These results were in accordance with the pathologist report.

Clinical examination and biopsy are still two standard methods to determine the nature of suspicions oral mucosa lesion. The biopsy remains the gold standard to determine the nature of an oral lesion. This can be uncomfortable, time consuming, and expensive. In addition, many patients presenting with oral leukoplakia show larger or multicentric lesions due to field cancerization, which further complicates a curative treatment [9]. However, the patient may refrain from biopsy due to the discomfort associated with this invasive procedure. Moreover, the non-uniform appearance of premalignant and malignant lesions may complicate the localization of the biopsy site, which is crucial in the histopathological verification of oral cancer. Other available clinical techniques, such as vital tissue staining with toluidine blue, cytological observation of collected exfoliated cells or molecular analysis, have been developed as additional tools for the early recognition of malignant lesions. These techniques require more advanced technical training and skills to prevent false-positive and false-negative results [2]. Therefore, development of suitable biomarkers needs to be improved to identify early malignant oral lesions, especially in at-risk populations. Plasma biomarkers are useful for diagnostic, prognostic and therapeutic determination of various cancers. Detection of tumors at early stages would improve the survival rates of OSCC patients [10]. However, the complexity of tumor progression and the variability of plasma protein in different patients make the identification of such markers more difficult. Currently, using traditional methods for analysis and treatment, patients are often diagnosed at later stages and the survival rates are

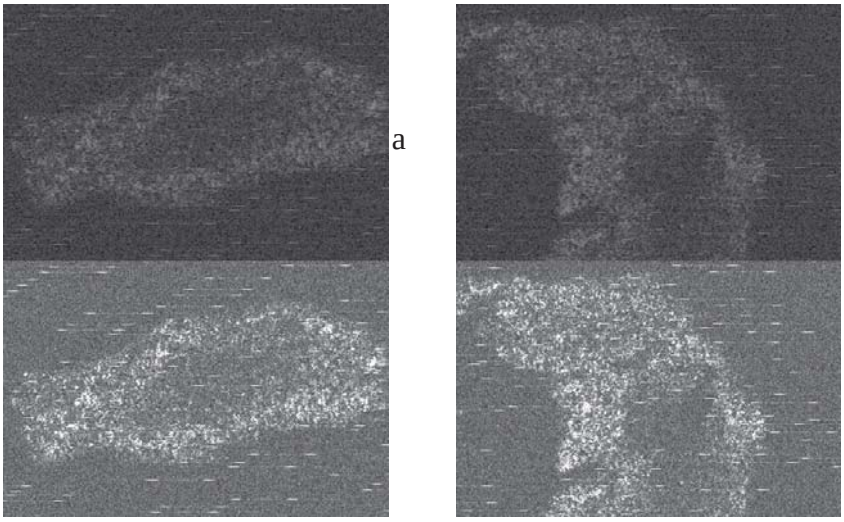

Fig. 3. OCT image of the parotid: a. healthy tissue, b. oral squamous cell carcinoma

substantially lower than for those diagnosed at an early stage.

The non-invasive nature of this imaging modality coupled with a penetration depth of 2–3 mm, high resolution (5–15lm), real-time image viewing, and capability for cross-sectional as well as 3D tomographic images, provide excellent prerequisites for *in vivo* oral screening and diagnosis. Several OCT systems have received FDA approval for clinical use, and OCT is deemed by many as an essential imaging modality in ophthalmology. *In vivo* image acquisition is facilitated through the use of a flexible fiber optic OCT probe. The probe is simply placed on the surface of the tissue to generate real-time, immediate surface and sub-surface images of tissue microanatomy and cellular structure, while avoiding the discomfort, delay and expense of biopsies.

Conclusions

Apart from excisional biopsy, there are no validated methods for detecting malignant transformation *in vivo*. In this respect, optical coherence tomography (OCT) seems to be a highly promising imaging modality. This non-invasive technique could provide images of tissue in situ and real time, without the need for surgical biopsy and multiple specimens processing.

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