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Margins of oral leukoplakia: autofluorescence and histopathology

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Abstract

Autofluorescence devices are widely used to examine oral lesions. The aim of this study was to see whether there were any signs of dysplasia, parakeratosis, or mucosal inflammation in the borders of homogeneous oral leukoplakia using autofluorescence, and we also compared clinically visible extensions with those detected by autofluorescence. Twenty patients with 26 homogeneous areas of oral leukoplakia were included in the study. After the clinically visible extensions of the lesion had been marked, we took a photograph through the autofluorescence device, which showed both borders in one picture. We then used photo-editing software to measure the size of the area of leukoplakia together with the area with loss of autofluorescence. We took 3 punch biopsy specimens: one from the leukoplakia, one 2.5 mm from its marked borders, and one from healthy mucosa. Seventy-eight biopsy specimens were examined by an experienced pathologist, and 95% CI calculated to assess the amount of parakeratosis. Spearman's rank correlation was used to assess the association with mucosal inflammation. Ten areas of leukoplakia were surrounded by normal green autofluorescence, and 16 were consistent with loss of autofluorescence with a mean size of 66%, which exceeded the clinically visible size of the area of leukoplakia. We calculated that there was a strong association between these entities and their surrounding areas, with loss of autofluorescence for parakeratosis. Some leukoplakias showed clinically invisible extensions during histopathological examination and autofluorescence. The technique described enables clinicians to measure the extent of these lesions beyond their visible margins. We found no dysplasia, which emphasises that autofluorescence detects non-dysplastic lesions caused by mucosal inflammation and parakeratosis.

Keywords: Oral leukoplakia; Autofluorescence; Photography; Punch biopsy; Velscope

Introduction

Oral leukoplakia was defined in 1978 by the WHO Collaborating Centre for Oral Precancerous Lesions as a white patch or plaque that cannot be characterised—clinically or histopathologically—as any other disease.[1] The 1994 update changed little: "Oral leukoplakia is a predominantly white lesion of oral mucosa that cannot be characterised as any other definable lesion. Some oral leukoplakia can transform into squamous cell carcinoma."[1,2] The annual transformation rate into squamous cell carcinoma (SCC) is assumed to be 1%–2.9%,[3,4] and it is strongly linked to the use of alcohol and tobacco, though it also appears without any of the known risk factors.[5,6]

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Many patients are not aware of oral leukoplakia, which commonly causes no symptoms, unlike other lesions such as oral lichen planus.[7,8] There is an argument in favour of the detection and histopathological identification of degrees of dysplasia in leukoplakia, in view of the potential for the progression of such lesions. "Premalignancy" is well established in uterine cervical disease, but neither the recording of oral leukoplakia in national databases nor a well-established treatment philosophy exists, although there is increasing evidence to suggest that leukoplakia with moderate to severe dysplasia should be treated rather than simply observed.

Oral leukoplakia may appear in the form of homogeneous leukoplakia with an apparent low malignant transformation rate, or as non-homogeneous leukoplakia, erythroleukoplakia, and verrucous leukoplakia with greater chances of malignant transformation.[9] The techniques that have been developed to diagnose mucosal lesions are scalpel incision biopsy, punch biopsy, and brush biopsy. Treatment ranges from observation to electrosurgery, photodynamic treatment, cryosurgery, laser treatment, and specific drugs.[10,11] Clinical appearance, anatomical site, and histopathological assessment are the usual methods of deciding on treatment.

Non-physiological alterations of the tissue can lead to loss of autofluorescence, which appears as a dark area during autofluorescence-assisted examination.[12–14] Koch et al. concluded that mucosal inflammation alone can lead to this happening.[15]

The aim of the present study was to investigate the borders of oral leukoplakia to look for signs of dysplasia, parakeratosis, and mucosal inflammation using an autofluorescence device, and compare clinically visible extensions with those detected by autofluorescence.

Patients and methods

We obtained ethical approval (EK 192/11) and patients' written informed consent, and evaluated a consecutive series of 20 patients seen between 10/01/2011 to 09/30/2012 with suspected homogeneous oral leukoplakia and no history of oral cancer. None of the lesions had been biopsied previously. The inclusion criterion was persistence of the white lesion for 2 weeks after the removal of possible local irritants.

The same investigator examined all patients, and took detailed histories including details about smoking and drinking habits. The boundaries of the leukoplakic lesion were marked with a skin marker to distinguish between it and the surrounding area during the autofluorescence examination. A scale was placed near the lesion. A picture was obtained using an already-tested device that emits at 400–460 nm (VELscope®, LED Dental Inc., White Rock BC, Canada) connected to a camera (Finepix S3 Pro, Fuji Photo Film Co., Ltd, Tokyo, Japan; AF Micro Nikkor 105 mm, Nikon, Tokyo, Japan; Medical Nikkor 120 mm *f*/4.0 IF, Nikon, Tokyo, Japan). During the examination the camera was set according to the manufacturer's instructions, and the patient's eyes were protected with special eyewear.

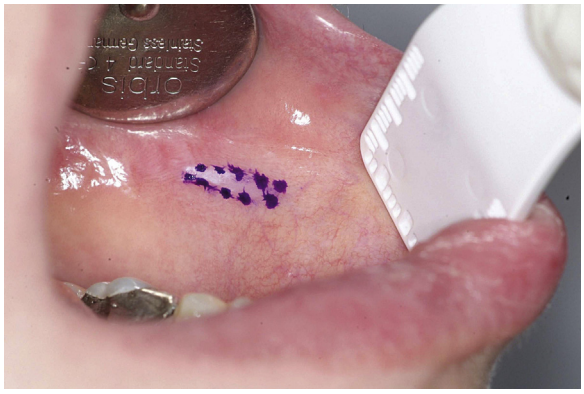

Fig. 1. Pronounced oral leukoplakia.

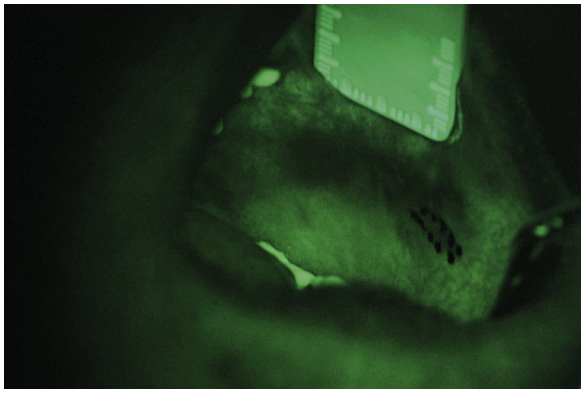

Fig. 2. Autofluorescence examination.

We used biopsy punches 3 mm in diameter and with a punch area of 7.07 mm^2 (Kai Europe GmbH, Solingen, Germany). Three punch biopsy specimens were taken under local anaesthesia (Ultracain® D-S 1:200.000, Aventis Pharma, Bad Soden, Germany). The first biopsy specimen was taken from the centre of the lesion, and the second one from outside the lesion 2.5 mm from its visible borders from the region around the leukoplakia. Finally, a third specimen was taken from an adjacent area that showed healthy mucosa on both clinical and autofluorescence examination, and was at least 10 mm from the border of the leukoplakia (Figs. 1–3). If required, a suture was placed to reduce the surface area of the wound. We then calculated the surface areas of the leukoplakia and the region around it with the help of photo-editing software (Adobe Photoshop CS4 Extended Version 11.0).

All specimens were fixed in 3.5% buffered formalin, embedded in paraffin, sectioned, stained with haematoxylin and eosin, and placed under a coverslip after dehydration (Fig. 4). The slides were examined for signs of dysplasia by an experienced pathologist. The mean value of parakeratosis was calculated after measurement of 5 representative areas of the slide. The mucosal inflammation was classified by the pathologist by the degree of density of inflammatory cells, starting with grade 0 (no inflammation) up to grade