Int. J. Oral Maxillofac. Surg. 2011; 40: 155–161
doi:10.1016/j.ijom.2010.10.022, available online at http://www.sciencedirect.com

International Journal of
Oral & Maxillofacial Surgery

Clinical Paper
Head and Neck Oncology

The utility of toluidine blue staining and brush cytology as adjuncts in clinical examination of suspicious oral mucosal lesions

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P. Güneri, J. B. Epstein, A. Kaya, A. Veral, A. Kazandı, H. Boyacioglu: The utility of toluidine blue staining and brush cytology as adjuncts in clinical examination of suspicious oral mucosal lesions. Int. J. Oral Maxillofac. Surg. 2011; 40: 155–161.
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Abstract. The objective of this study was to investigate the utility of toluidine blue and brush cytology in patients with clinically detected oral mucosal lesions. Clinical examination of 35 patients was completed before toluidine blue application, oral brush cytology and scalpel biopsy. Lesions were photographed before and after stain application; followed by brush cytology. All findings were compared with histopathologic results. Severe dysplasia and carcinoma-in-situ were determined as 'positive'; no dysplasia and mild to moderate dysplasia were defined as 'negative'. The sensitivity, specificity, positive and negative predictive values of clinical examination and toluidine blue were the same: 0.923, 0.433, 0.414, and 0.929, respectively. Those of brush cytology were 0.923, 0.517, 0.462, and 0.938. The concordance of all methods was 30% for benign and 61% for malignant lesions. Adjuncts identified 92% of carcinoma-in-situ and squamous cell carcinoma as confirmed by histopathology, in contrast to clinical findings alone in which 62% of these lesions were identified ($p = 0.046$). In conclusion, adjunct diagnostic methods decreased the level of uncertainty for the diagnosis of oral malignancies and lichenoid dysplasias when applied as adjuncts to clinical examination.

Keywords: oral cancer; squamous cell carcinoma; toluidine blue; brush biopsy.

Accepted for publication 26 October 2010
Available online 26 November 2010

It is anticipated that diagnosis of high-risk oral premalignant lesions (OPLs) and early stage cancer decreases the morbidity of treatment and mortality due to oral squamous cell carcinoma (OSCC)[14,29]. OPLs and oral premalignant disorders may present clinically as leukoplakia, erythroplakia[8,32], ulceration[16], oral submucous fibrosis[23], and oral lichen planus[1,6].

The malignant risk cannot be determined using standard clinical examination. Adjunct methods and devices have been introduced to improve detection, thereby promoting diagnosis[8,9,16,19,28,29,36]. The most evaluated adjunct for lesion detection is toluidine blue (Tblue). Tblue is a metachromatic dye that binds to deoxyribonucleic acid and retention has been associated

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with loss of tumor suppressor gene (TSG) loci on specific chromosomes. TSG loss has been shown to predict progression of OPLs to cancer. The utility of Tblue in the identification of OPLs and early diagnosis of OSCC[13,15,21,22,28,46], to assess margins of OPLs and SCC of the lesions before biopsy, to assist in biopsy site selection[4], and to accelerate the decision to biopsy has been examined. Retention of Tblue is also seen in ulcerated and potentially inflammatory lesions although the pattern of staining may be different, thus, retention may result in a false-positive outcome. In order to assist clinicians, a 2 week review of lesions not felt to be at high risk of cancer at first evaluation is suggested[22].

Oral exfoliative cytology has been evaluated since the 1950s as a method to collect epithelial cells in order to examine cell morphology under a light microscope. Exfoliative cell collection using a bristle-brush (brush cytology) has been reported to obtain a full thickness collection of epithelial cells including basal epithelial cells[18]. It is promoted as a fast, inexpensive and well-tolerated method that may reduce or increase the need for biopsies in clinically benign lesions[18,23,35,36]. While a number of reports have shown the efficiency of brush cytology for early detection of OSCCs[18,19,36], others reported large numbers of false-positive and potentially false-negative results[8,15,26,31] ranging between 30–84%[33] and 63% for dysplastic lesions[27].

The goal of the present study was to examine the utility of Tblue and brush cytology in patients with clinically detected oral mucosal lesions by comparing the results of Tblue application and the characteristics of brush cytology with the findings of scalpel biopsy.

Materials and methods

Thirty-five patients with oral mucosal lesions identified by the Orofacial Lesions Council of Ege University, İzmir, Turkey, were seen for further evaluation. Informed consent was obtained from the patients and thorough clinical head, neck and intraoral examinations were completed before Tblue application, oral brush cytology and scalpel biopsy. The clinical appearance, location and size of each lesion were recorded on a standard form; thus, all evaluations were performed on the same area of each lesion. All clinically identified lesions underwent biopsies irrespective of the findings with Tblue staining and the results of brush cytology. Scalpel biopsies were performed under local anesthesia following Tblue staining and brush cytology, without any significant delay (not more than 2 weeks) between the 3 methods of investigation. For 12 lesions, repeat evaluation was conducted after a 2 week period, while the remaining patients were evaluated in one visit. All clinical examinations were performed by the same examiner (P.G.) who is experienced in evaluating oral mucosal lesions and therefore the clinically suspicious nature of the lesions was affirmed in advance. Surgical biopsies were performed by an experienced oral and maxillofacial surgeon.

Clinical examination

A photograph of each lesion was obtained before and after the procedure using a digital camera (Olympus Camedia C-2500 L, Melville, NY, USA). An example of the lesions that were evaluated in this study is presented in Fig. 1. Lesions selected for further examination with Tblue staining and brush cytology were homogenous and nonhomogenous leukoplakia[2,26], reticular[1,2,38] or erosive/ulcerated lichenoid lesions[37], and superficial ulcerations suspicious of malignancy.

Toluidine blue staining

To decrease false-positive rates, a waiting period of 10–14 days after the initial clinical examination was conducted for lesions not highly suspicious of cancer. Potential causative agents (factors related to traumatic or inflammatory changes, including ill fitting dentures, non-hygienic/defective restorations, orthodontic brackets, cheek biting) were treated to prevent false-positive results with staining at follow-up. At recall, examination and tissue testing were conducted, including Tblue staining and brush cytology and tissue biopsy. Tblue was prepared as an oral rinse[22], since there is no pharmaceutical grade Tblue available in Turkey. Toluidine blue rinse (1%) was compounded at Faculty of Pharmacy, Ege University, as follows[21,26,31]: 1 g tolonium chloride powder (Merck KGaA, Darmstadt, Germany); 10 ml acetic acid (Merck KGaA, Darmstadt, Germany); 4.19 ml absolute alcohol (Merck KGaA, Darmstadt, Germany); and 86 ml of distilled water[31] without flavoring. The pH value of the solution was 4.5. One hundred milliliters of 1% acetic acid rinse was prepared by adding 1 ml glacial acetic acid to 99 ml distilled water[31]. The oral rinsing protocol was: 20 s pre-rinse with 30 ml of 1% acetic acid; 20 s water rinse; 20 s rinse/gargle with 10 ml of the 1% tolonium chloride solution; 20 s post-rinse with 30 ml of 1% acetic acid (twice); a final water rinse.

Each lesion was photographed before and after application of the Tblue and findings were recorded on the standard form. The pattern of dye retention and the intensity of stain retention were recorded (2, dark blue staining; 1, minimal blue staining; 0, no blue staining). Occasionally, normal mucosa also appeared light blue, but this staining was not interpreted as positive.

Brush cytology

Brush cytology was performed using a Cytobrush Plus GT (Medscand Medical AB, Malmo, Sweden) which was rotated on the lesion site with pressure, until pinpoint bleeding was observed. The harvested cells were transferred to a slide (SuperFrost Plus; Menzel, Braunschweig, Germany) by a 360° turning and rolling motion with the brush and the slides were

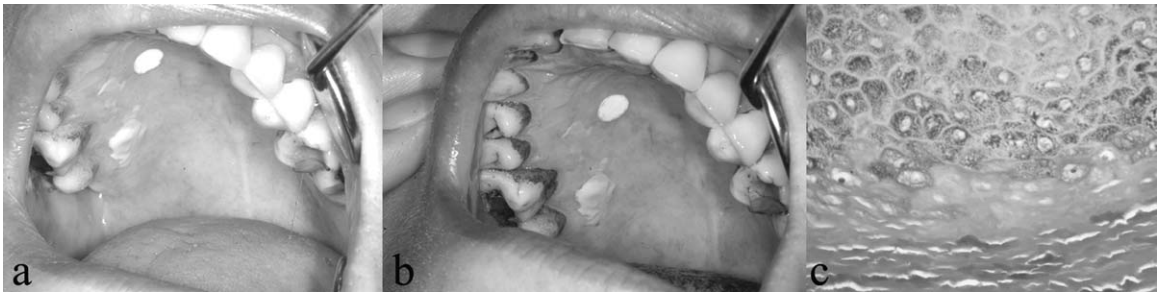

Fig. 1. A white hyperkeratotic lesion that was evaluated using clinical examination, Tblue staining, brush cytology and scalpel biopsy.

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