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Direct immunofluorescence testing results in cases of premalignant and malignant oral lesions

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Objectives. Oral premalignant and malignant lesions may mimic oral lichen planus (OLP) clinically and microscopically. OLP often shows basement membrane fibrinogen positivity on direct immunofluorescence testing (DIF). This study examined fibrinogen positivity in oral premalignant lesions and squamous cell carcinoma.

Study Design. The University of Florida Oral Pathology Biopsy Service records were searched for the years 2003 to 2013 for oral premalignant lesions and squamous cell carcinoma with concurrent DIF testing. Demographic, clinical, and DIF or histologic information was collected and analyzed.

Results. Sixty-eight fibrinogen positive lesions were identified within a total of 164 cases. Low-grade dysplasia and premalignant verrucous lesions made up the majority of the fibrinogen positive lesions (combined n = 43; 63.2%), and the most common locations in positive cases were the buccal mucosa, tongue, and gingiva. A lichenoid distribution of the inflammatory infiltrate significantly predicted fibrinogen positivity ($P < .0005$).

Conclusions. Fibrinogen positivity may be seen in premalignant and malignant oral lesions increasing the risk of misdiagnosis. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015;■:1-9)

Within the oral cavity, significant clinical and histologic overlap may occur among oral lichenoid lesions (OLL), oral lichenoid mucositis (OLM) and oral lichen planus (OLP), which will be referred collectively throughout this article as OLP for simplicity, and premalignant lesions or squamous cell carcinoma (SCCA). Both premalignant and malignant oral lesions and OLP may occur clinically as white, red and white, or ulcerated oral lesions, and the phenomenon of premalignant and malignant oral lesions exhibiting classically lichenoid features has been well established histologically.[1,2] This overlap may lead to a delay in the diagnosis and treatment of true dysplasia or SCCA of the oral cavity.

Direct immunofluorescence (DIF) testing has been established as an adjunctive diagnostic tool in differentiating OLP from similar appearing lesions.[3-6] Deposits of immunoreactants in OLP with "shaggy" basement membrane zone (BMZ) fibrinogen reactivity has been described as the best DIF indicator of the disease.[5] However, fibrinogen positivity at the BMZ is not specific to OLP and has been reported in other inflammatory disorders.[7]

Because of the potential for clinicopathologic overlap between OLP and oral dysplasia or SCCA, DIF is occasionally requested by the clinician for the purpose of differentiating OLP from other immune-mediated diseases, oral dysplasia, and SCCA, which may exhibit overlapping clinical and histologic features. Minimal information is found in the literature with regard to the DIF staining patterns of oral dysplasia or SCCA; thus, fibrinogen positivity on biopsy may contribute to diagnostic confusion in certain cases, especially those in which the level of premalignant features are mild or reactive.

The objectives of this study were to characterize the DIF results, in particular positive fibrinogen reactivity, in cases of oral premalignant lesions and SCCA in biopsy specimens, which were incidentally submitted for DIF testing because of clinical lichenoid appearance, and to characterize the clinical and histologic patterns. The aim of the study was to prove that fibrinogen positivity may be commonly found in premalignant and malignant oral lesions.

MATERIALS AND METHODS

Following institutional review board approval, the University of Florida Oral Pathology Biopsy service records were searched for the years 2003 to 2013 for

Statement of Clinical Relevance

Direct immunofluorescence findings may be positive in premalignant and malignant lesions, contributing to the confusion with oral lichen planus especially where overlapping clinical and histologic similarities are seen. Clinicopathologic correlation is essential in discriminating between these entities.

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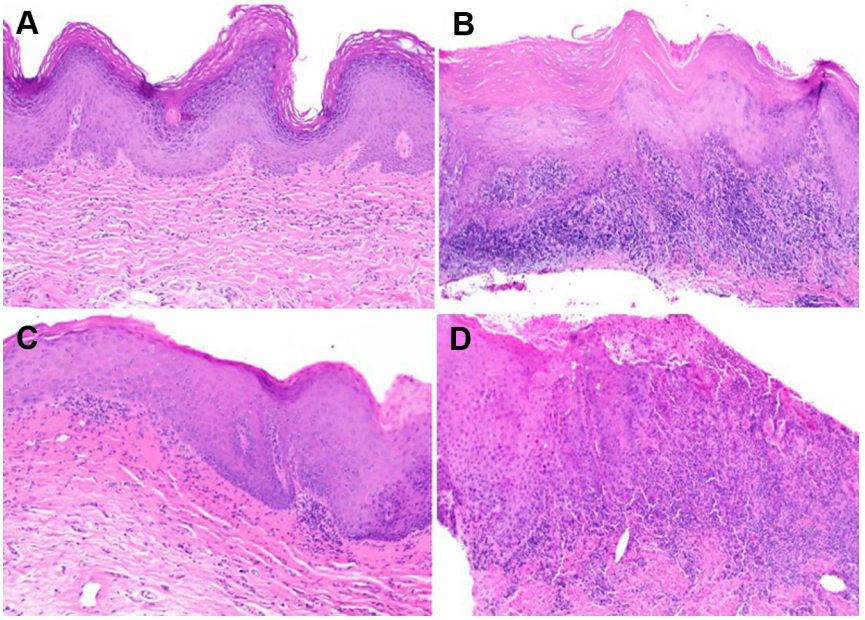

Fig. 1. Inflammatory pattern. **A**, minimal inflammation, grade 0 (hematoxylin&eosin, original magnification ×10); **B**, diffuse lichenoid, grade 1 (hematoxylin&eosin, original magnification ×10); **C**, focal lichenoid, grade 2 (hematoxylin&eosin, original magnification ×10); **D**, nonlichenoid, grade 3 (hematoxylin&eosin, original magnification ×10).

cases with specific diagnostic codes that also had DIF studies performed. These diagnostic codes included verrucopapillary hyperkeratosis (VPHK), dysplasia, atypical squamous epithelial proliferation (AEP), SCCA, and verrucous carcinoma (VC). Pathology reports were reviewed for all cases containing these codes, as well as age, gender, diagnosis, DIF findings, and biopsy location, were recorded. Archival hematoxylin and eosin (H&E) prepared slides were re-examined by two study participants (LM and SF) to confirm the sign-out diagnosis. All cases were originally examined and signed out by three of the study participants (IB, DC, and MI). Four of the study participants (IB, DC, MI, and SF) were board-certified oral and maxillofacial pathologists at the time of the study and the remaining study participant (LM) was at the time a senior oral and maxillofacial pathology resident. Cases in which the searched diagnosis and DIF were from different anatomic locations were excluded from the study.

The cases were divided into six diagnostic categories: (1) VPHK (the term utilized at our institution for lesions compatible with the hyperkeratosis to early verrucous hyperplasia stages of proliferative verrucous leukoplakia, showing verrucoid thickening of the epithelium and orthokeratin or parakeratin layers),[8] (2) low-grade dysplasia (LGD; atypia or dysplasia to mild dysplasia), (3) high-grade dysplasia (HGD; moderate to severe dysplasia), (4) AEP (defined as lesions exhibiting architectural features of malignancy but not meeting criteria for frank carcinoma), (5) SCCA, and (6) VC. Cases which were diagnosed as dysplasia in addition to VPHK were categorized on the basis of their degree of dysplasia, rather than being included in the VPHK category. During the slide review by two study participants (SF and LM), the case was reassigned to the most appropriate category if the original diagnosis rendered was borderline between two of the designated diagnostic categories of the study. If no general agreement were to be found between the original sign-out diagnoses and those of the diagnostic reviewers, the case was to be dropped, although no cases fell into this category within this study.

Multiple cases from the same patient were only included if the biopsies were from different sites within the oral cavity. If more than one biopsy was found for the same patient at the same site, the initial biopsy was included in the study, and subsequent biopsies were excluded in order to avoid counting the same lesion multiple times. Since the original slides of frozen DIF sections were not available for review (except for five cases), the DIF findings were recorded on the basis of the pathology report sign-out.

The original DIF was performed at the in-house laboratory of the University of Florida College of Dentistry oral pathology biopsy service, utilizing frozen tissues cut into 4-micron sections and air dried, acetone fixed, washed with buffer, treated with protein block (Dako North America, Inc., Carpinteria, CA), incubated with antisera conjugated immunoglobulin G (IgG), IgM, IgA, C3, and fibrinogen (Thermo Scientific, Lab Vision Corporation, Fremont, CA), and then rinsed with distilled water and coverslipping.