Biopsy and Histopathologic Diagnosis of Oral Premalignant and Malignant Lesions

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ABSTRACT

Accurate diagnosis of premalignant or malignant oral lesions depends on the quality of the biopsy, adequate clinical information and correct interpretation of the biopsy results. The purpose of this paper is to review the procedures for obtaining appropriate biopsy samples, and the criteria for diagnosing and grading dysplasias. The World Health Organization's description of the architectural and cytologic epithelial changes that characterize dysplasia is detailed, and guidelines for following up patients with premalignant and malignant lesions are provided. The benefits of using the centralized services and expertise of the British Columbia Oral Biopsy Service are also reviewed.

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Proper management of a patient with a premalignant or malignant oral lesion starts with an accurate diagnosis. The current gold standard for diagnosis is the histopathologic assessment of a tissue biopsy of the suspicious lesion. An accurate histopathologic diagnosis depends on the clinician doing an appropriate biopsy and providing adequate clinical information, and on the pathologist correctly interpreting the biopsy results. The purpose of this paper is to review the steps and procedures for obtaining appropriate biopsy samples, and the criteria for diagnosing and grading dysplasias.

How To Obtain an Appropriate Biopsy

An appropriate biopsy essentially contains tissue that is representative of the most severe or significant change in the lesion and is suitable for pathologic assessment. Achieving an appropriate biopsy involves 3 key factors: selection of the biopsy site, the procedures used and the proper submission of the biopsy sample.

Selection of the Biopsy Site

The biopsy site must be selected carefully to ensure that it yields accurate results. A suspicious oral lesion, particularly a large one, often varies in disease severity from one part of the lesion to another. For example, a lesion may have early invasive squamous cell carcinoma (SCC) in one part and mild dysplasia in another. An appropriate biopsy would include tissue from the worst part of the lesion (in this example, the early invasive SCC).

The worst part of the lesion may be determined from its clinical appearance, multiple biopsies and the use of adjunct visual tools. Choosing areas with nonhomogeneous leukoplakia or erythroplakia (e.g., a nodular, verrucous or indurated area; a reddish or ulcerated area) increases the likelihood that the biopsy will include the area with the most

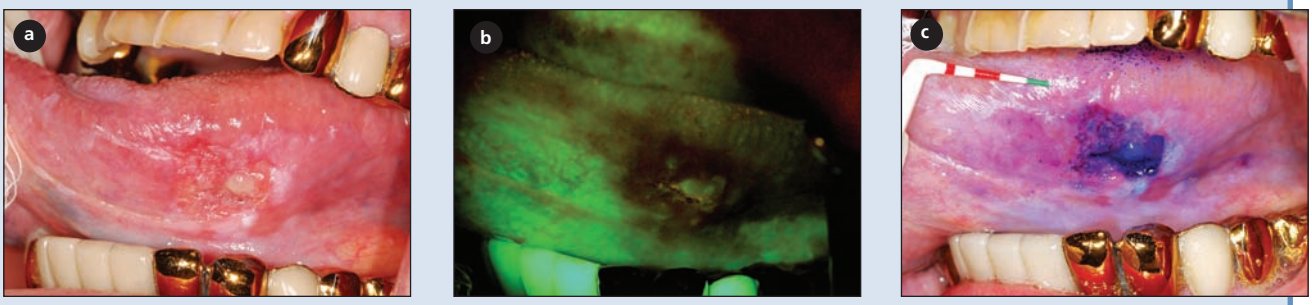

Figure 1: Use of fluorescence visualization and toluidine blue (TB) to select the biopsy site. **(a)** Clinical picture of a nonhomogeneous leukoplakia under white light. **(b)** The same lesion under direct fluorescent visualization. Note that the extent of the lesion, as seen by loss of green autofluorescence, is bigger than the clinical lesion seen by the unaided eye in Fig. 1a. **(c)** The same lesion stained with TB showing TB positivity with varying intensity of TB staining. In our experience, the area of the lesion with strong TB staining usually has worse histologic results than the less stained or negative area of the lesion.

severe disease (see Williams and others[1] in this issue for details). Taking biopsies from different parts of a lesion, particularly if the lesion is extensive or if it shows a variety of clinical presentations, can ensure reliable biopsy results. For example, for a 4-cm lesion, taking 2 biopsies from representative areas or those with different clinical appearances is justified. Using toluidine blue or direct fluorescence visualization (**Fig. 1**) can help a clinician highlight the most severe or significant change for biopsy.

If dentists are unsure about the most appropriate site to biopsy, they should refer the patient to a clinician specializing in the field because a biopsy from an inappropriately selected site could give both the patient and the dentist a false sense of security.

Biopsy Procedures

Clinicians use a number of biopsy techniques, including a scalpel, a punch biopsy, a laser or an electroknife.[2] For biopsy of mucosal lesions suspected of premalignancy or malignancy, particularly for excisional biopsies, the use of a laser or an electroknife should be avoided. These techniques may produce a coagulative artefact that hampers histologic interpretation of the samples, particularly the assessment of the margin. Punch biopsy has been shown to produce fewer artefacts than scalpel biopsy and is discussed here.[3]

The procedure for obtaining a punch biopsy involves the following steps (**Fig. 2**).

Selecting the Biopsy Site

Figure 2a details the selection of the biopsy site (see also the discussion above).

Administering Local Anesthesia

For a highly vascularized site (such as the tongue or lip) or lesion, anesthetics containing vasoconstrictors should be chosen to minimize bleeding (e.g., lidocaine containing epinephrine 1:50,000 or 1:100,000). The anesthetic should be administered to the area adjacent to the biopsy site (**Fig. 2b**) because direct injection of the anesthetic solution into the biopsy site can cause distortion artefacts in the specimen.

Determining the Size of the Biopsy

Biopsies of the mucosa should be at least 3 mm in diameter. Since biopsies shrink after formalin fixation, punch biopsies 4 or 5 mm in diameter are recommended to ensure an adequate sample size. The depth should be at least 2 mm. However, oral premalignant lesions and SCCs frequently need deeper biopsies because of the characteristic thickened epithelial lining and hyperkeratosis. For these lesions, the recommended depth is 4 or 5 mm. The bevel of the cutting edge, usually 1.5 mm (**Fig. 2c**), can be used as a depth guide.

Obtaining a Biopsy Sample with a Biopsy Punch

During a punch biopsy, the punch is gently inserted into the mucosa with a rotating motion to facilitate cutting the tissue to the appropriate depth (**Fig. 2d**). Tissue forceps and a scalpel are used to remove the biopsy sample (**Fig. 2e**). The biopsied tissue must then be placed on a piece of clean paper with the connective tissue surface (bottom layer) facing down for 1 minute (**Fig. 2f**) to ensure that the sample stays flat during fixation and to orient it properly for histologic examination (this is a critical step). The sample is then placed in 10% neutral buffered formalin fixative. The volume of fixative should be at least 20 times the volume of the sample to avoid improper fixation or autolysis. No other fixative should be substituted for the formalin fixative. Alcohol, surface disinfectant, local anesthesia solution or mouth rinse cannot fix the tissue properly for adequate histologic evaluation.

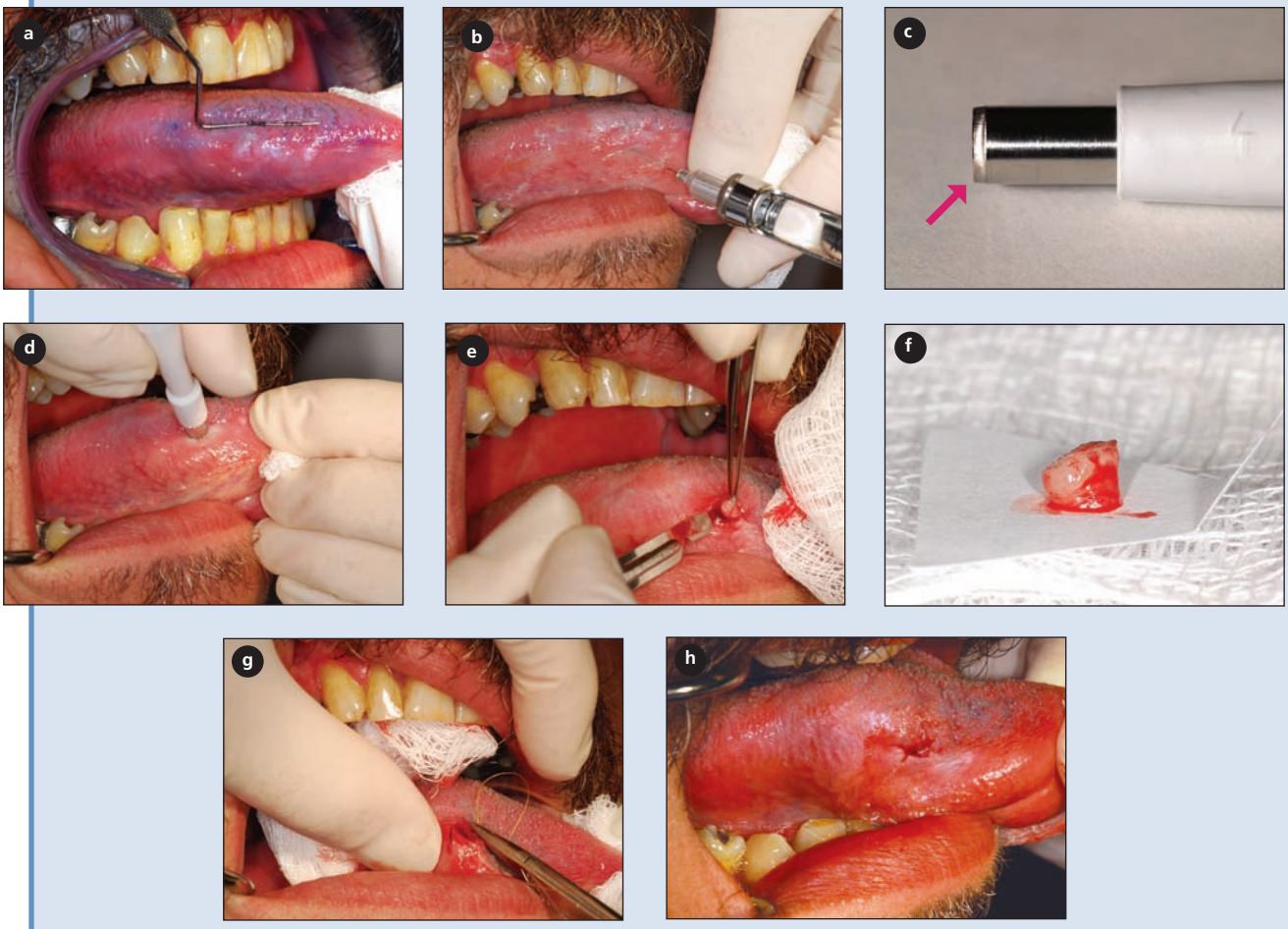

Figure 2: Steps in obtaining a punch biopsy. **(a)** Selection of the biopsy site with the use of toluidine blue staining as a visual aid. **(b)** Injection of local anesthetic. **(c)** Photo of a 5-mm punch illustrating the use of the 1.5-mm bevel (arrow) of the cutting edge to estimate depth. **(d)** Insertion of the punch into the tissue with a circular motion. **(e)** Use of a scalpel to obtain the biopsy sample. **(f)** Position of the tissue on a piece of paper with the connective tissue facing downward to prevent the sample from curling during fixation. **(g)** Suturing to close the biopsy wound. **(h)** Confirmation of hemostasis at the biopsy site.

Ensuring Hemostasis

If possible, the biopsy site should be sutured to close the wound and ensure proper hemostasis (**Figs. 2g** and **2h**).

Submission of the Biopsy

The biopsy sample should always be accompanied by pertinent clinical information, including past history of dysplasia or SCC, the patient's risk factors, the location of the lesion, and its appearance, size and duration. In addition, if multiple samples of the lesion are taken, each sample must be submitted in a separate, clearly labelled container. If possible, a colour photo of the lesion should be included to facilitate clinical and pathologic correlation. Finally, samples and accompanying documentation should be sent by courier to minimize delays in diagnosis and prevent freezing artefacts that can occur if the samples are placed in mailboxes or transported by carriers without temperature regulation in the winter.

The Meaning of "Dysplasia" When Used in a Pathology Report and Management

Pathologic evaluation of the presence and degree of epithelial dysplasia (mild, moderate, severe or carcinoma in situ [CIS]; **Fig. 3**) is used to assess the malignant risk of oral premalignant lesions. The risk of cancer is markedly different for low-grade (mild or moderate) than for high-grade dysplasia (severe dysplasia or CIS). Most low-grade dysplasias do not progress to cancer; high-grade dysplasia, however, often progresses if left untreated. Consequently, these dysplasias are often managed differently. In British Columbia, patients with high-grade dysplasias are referred for removal of the lesions, whereas

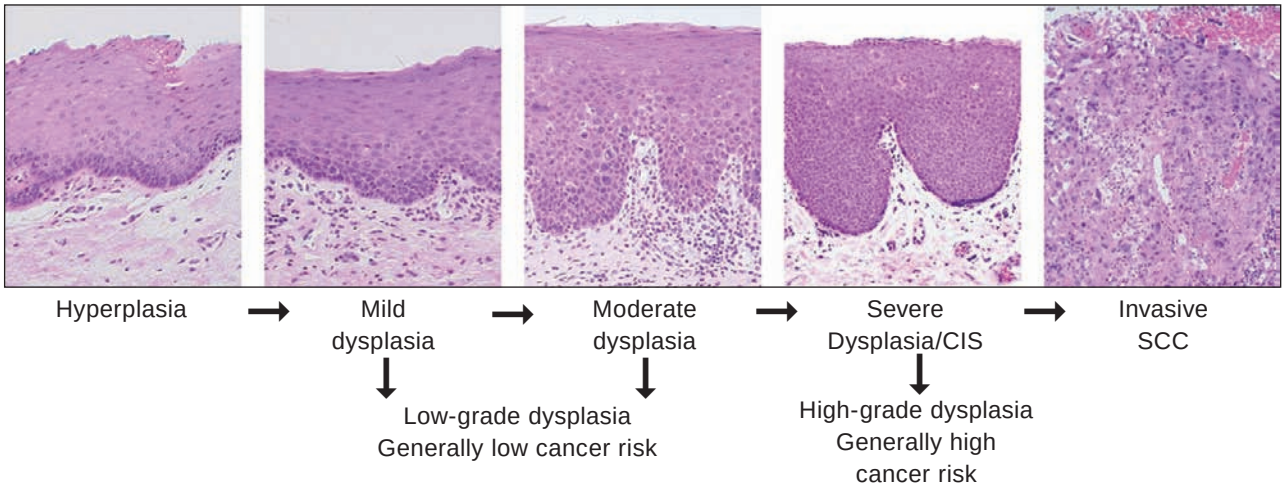

Figure 3: Histologic progression of oral cancer. Squamous cell carcinoma (SCC) is believed to progress from epithelial hyperplasia to an increasing degree of dysplasia to carcinoma in situ (CIS) and finally to SCC. Courtesy of BC OCPP (www.orcanet.ca).

those with low-grade dysplasias are assessed further with adjunct devices. In future, information accumulated from follow-up with technology will be used to differentiate low-grade dysplasias with a greater likelihood for cancer progression to guide early intervention and monitoring protocols for these lesions.[4]

Although diagnosis of invasive SCC is generally straightforward, pathologic diagnosis of oral premalignant lesions can be challenging. The goal of the following review of the diagnostic criteria for dysplasia is to remind dentists of the complexity of these criteria and grading dysplasia.

The World Health Organization (WHO)[5] has established criteria for dysplasia, including the architectural and cytologic changes in the epithelium.

The WHO's criteria for architectural changes in the epithelium:

- irregular epithelial stratification
- loss of polarity of basal cells
- drop-shaped rete ridges
- increased number of mitotic figures
- abnormal mitoses not limited to basal or parabasal layers
- premature keratinization in single cells (dyskeratosis)
- keratin pearls within rete ridges

The WHO's criteria for cytologic changes in the epithelium:

- abnormal variation in nuclear size (anisonucleosis)
- abnormal variation in nuclear shape (nuclear pleomorphism)
- abnormal variation in cell size (anisocytosis)
- abnormal variation in cell shape (cellular pleomorphism)
- increased nuclear–cytoplasmic ratio
- increased nuclear size
- atypical mitotic figures
- increased number and size of nucleoli
- hyperchromasia

Grading dysplasia depends on the extent of the involvement of the epithelial layers by the dysplastic changes.[6] In cases of mild dysplasia, cytologic and architectural changes are confined to the lower third of the thickness of the epithelium; in cases of moderate dysplasia, changes are seen in up to two-thirds of the thickness of the epithelium. In cases of severe dysplasia, the dysplastic changes fill more than two-thirds of the thickness, but less than the entire thickness of the epithelium. The dysplastic cells of CIS occupy the entire thickness of the epithelium (bottom to top changes), although the basement membrane is still intact. Invasive SCC involves dysplastic cells invading the underlying connective tissue stroma through the basement membrane.

The complexity of these diagnostic criteria arises from a number of factors. The assessment of dysplasia is subjective and open to interpretation. For example, there is no consensus about the amount of alteration in the intensity of chromatin staining required for the classification of hyperchromatism. Cellular changes in response to trauma or inflammation can resemble dysplastic changes and confound diagnosis. Studies about the rela-

tive weight of each histologic criterion or combination of criteria for predicting the risk of cancer progression in oral premalignant lesions are scarce. This scarcity is largely due to a shortage of longitudinally derived specimens or those with a known outcome that could be used to train pathologists or oral pathologists. This lack is also due to the difficulty of objectively quantifying change in individual cells.

As a consequence, accurate pathologic grading of dysplasia requires ample experience. Furthermore, experiences grading dysplasia in one organ are not necessarily applicable to grading dysplasia in another organ. For example, a pathologist with extensive experience grading uterine cervical dysplasia may feel uncomfortable grading oral lesions. A pathologist who has limited experience with oral dysplasia may well underestimate the degree of change in an oral dysplasia. If possible, samples from a suspicious oral premalignant lesion should be sent to or reviewed by an oral pathologist or a pathologist who specializes in conditions of the head and neck.

The clinician must discuss the case with the pathologist if the diagnosis is inconsistent with clinical findings. If the sample was not diagnosed by an oral or head-and-neck pathologist, the clinician could request a second opinion from such a specialist.

Necessary Follow-up for Dysplasia

All oral dysplasias must be followed up at least annually, even if the lesion has been completely excised (i.e., no clinically visible lesion remains), and regardless of whether the patient has stopped using tobacco products. Increasing evidence shows that even when excision is confirmed both clinically and histologically, molecular clones of altered cells may remain and later give rise to further dysplasia or SCC. It is critical that the site of the previous dysplasia be followed regularly, even when it appears clinically normal. Semi-annual follow-up is preferable. The lesion should be biopsied again if clinical changes become evident.

Because exposure to carcinogens such as tobacco smoking has a field effect (the whole oral cavity is exposed), the appearance of dysplasia at one oral site frequently indicates a markedly increased chance of dysplasia or SCC at other oral sites. These lesions may already exist, but may not be clinically apparent at the time of the examination. For this reason, the follow-up examination should include not only the site of the previous dysplasia, but the entire oral cavity as well.

Examinations should be meticulous to check for early subtle clinical changes. In our dysplasia clinics, we regularly use adjunct visualization tools (toluidine blue and direct fluorescence visualization) in the follow-up of patients with dysplasia to facilitate the identification of subtle clinical changes in patients with oral premalignant lesions or cancers.

British Columbia Oral Biopsy Service

Centralized oral biopsy services in Canada and the United States can play a critical role in the diagnosis and management of oral premalignant lesions. In British Columbia, the provincial Oral Biopsy Service (OBS), which serves dentists, and ear, nose and throat surgeons throughout the province, deals with more than 4,000 cases per year. The OBS also functions provincially as a consulting service for all pathologists examining oral lesions. The OBS provides expertise, centralization of samples, a central referral centre and better communication.

Expertise

OBS's pathologists are oral or head-and-neck pathologists who are experienced in the diagnosis of oral premalignant lesions. Because of the OBS's link to an ongoing longitudinal study in the British Columbia Oral Cancer Prevention Program (BC OCPP), the pathologists have experience reading the multiple biopsies of more than 600 patients with oral premalignant lesions who are being followed in the BC OCPP oral dysplasia clinics. This unique experience allows the pathologists to compare progressing and nonprogressing dysplasia over time.

Centralization of Samples

The OBS's centralized service ensures that all of a patient's biopsy samples are located in the same pathology laboratory. Centralizing samples allows the pathologist to compare the biopsy samples taken over time to determine whether a lesion is progressing or regressing.

Central Referral Centre

In British Columbia, the provincial OBS plays a critical role in referring patients and guiding treatment for oral premalignant lesions and oral cancer. Dentists, through the BC OBS, have the option of referring patients with a diagnosis of low-grade dysplasia (mild or moderate dysplasia) and those with a diagnosis of high-grade lesions (severe dysplasia or CIS) to the appropriate low-risk or high-risk oral dysplasia clinic.[7]

Better Communication

The close rapport between the oral and head-and-neck pathologists and clinicians facilitates communication, streamlining the diagnosis and management of oral lesions.

Conclusion

Diagnosis and risk assessment of oral premalignant lesions require a team effort from both clinicians and pathologists. Centralized services such as a centralized biopsy service or dysplasia clinic play crucial roles in facilitating the diagnostic process.

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