Oral Exfoliative Cytology:  
An Adjunct to Biopsy

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Oral exfoliative cytology is not a substitute for biopsy. Rather, it is a useful—although not essential—adjunct in the diagnosis of oral surface lesions due to cancer, viral disease, vesiculobullous dermatoses or fungal infection. Although this painless, atraumatic and simple technique for collecting a sample of superficial cells is used extensively in the diagnosis of less visible and accessible lesions, such as those in the uterine cervix or the lung, emphasis is here placed on its role in detecting and monitoring premalignant lesions and squamous cell carcinoma of the mouth. A recent review,¹ as well as papers advocating ² and questioning ³ the utility of oral cytology will lead to a more complete understanding of the field.

Indications for Oral Cytology

Exfoliative cytology not only aids in the differential diagnosis of an unidentified oral lesion or a probable benign lesion when the physician or dentist is reluctant to perform a biopsy, but also helps to detect carcinoma in situ and other premalignant lesions in suspicious red, velvety and granular-appearing areas. Less commonly, cytology may be employed to confirm a lesion strongly suggestive of cancer on a clinical basis when the patient refuses biopsy, when the referring physician or dentist wishes additional information or when the tentative cytologic findings may permit earlier hospitalization and treatment.

Thus, oral cytology is indicated in the diagnosis of:

An oral lesion which cannot be identified with clinical certainty. Cytologic examination may be required when the clinician is averse to biopsy for a seemingly innocuous lesion, such as a large oral ulcer or a small, mild erythematous area without induration, especially if the lesion is located in a region known to give rise to rapidly proliferating carcinomas with early metastases—the tongue, floor of the mouth and faucial regions. If, on the other hand, the physician feels biopsy is in order, cytology may provide useful additional information. Occasionally, carcinoma or carcinoma in situ will be detected in its early stages; most commonly, however, a suspicious lesion will prove to be inflammatory. If the abnormality persists in the face of negative cytology, biopsy is, of course, indicated.

An oral lesion thought to be benign. Cytology here aids in the differential diagnosis of viral lesions, dermatoses, such as pemphigus, specific infections, such as candidiasis, and, in the author's experience, in the detection of possible allergic reactions. Infrequently, cyto-

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I. Palatal ulcer in a 35-year-old white male. The ulcer is scraped with a tongue blade, the first step in making the cytologic smear. Although the lesion proved to be a single, major oral aphtha, it might have been cancer.

2b. The preparation is made by gently smearing the cells on the slide.

Ic. The preparation is fixed immediately, while still wet, by spraying with a fixative or placing it into a container filled with the fixative.

Id. Hansel-stained smear from this patient. * Note the many eosinophils, indicating the possibility of an allergic etiology. This patient was found to be allergic to several foods and drugs.

*Unless otherwise noted, all sections are stained with hematoxylin and eosin and all cytologic preparations are by the Papanicolaou method.

1e. If this lesion had been malignant, it might have shown cells similar to those seen in the above squamous cell carcinoma. Note the increased nuclear-cytoplasmic ratios, the irregular nuclear borders with periodic angularity and the hyperchromatic, large, irregularly-clumped, intranuclear chromatin—all indicative of cancer.

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logic examination reveals cancer despite strong clinical evidence to the contrary. For example, the same smear demonstrating the presence of candida may also show cells suspicious or positive for cancer; a subsequent biopsy and culture establishes the coexistence of candidiasis and squamous cell carcinoma.

A possible carcinoma in situ or less advanced premalignant lesion (dysplasia). A red, velvety, granular-appearing lesion or a white, keratotic (clinically leukoplakic) lesion (as well as any combination of these two types) may signal either carcinoma in situ or a less advanced premalignant lesion. Some investigators feel that oral cytology can detect suspicious or malignant cells regardless of the clinical appearance of the lesion. Others, the author included, believe that oral cytology most accurately detects cancer only in those red or ulcerated lesions where the epithelium is thinned or absent. When keratosis (clinical leukoplakia) is evident, dysplastic cells may be present only in the stratum germinativum, thus hampering evaluation by exfoliative cytology. Therefore, biopsy is indicated for keratotic (clinically leukoplakic) lesions of the dry vermilion border of the lip, as well as for nonidentified intraoral keratoses which do not resolve during a reasonable time after all irritants have been removed.

An oral lesion strongly suggestive of cancer on a clinical basis. In these situations, biopsy is, of course, mandatory before initiating definitive therapy. Nevertheless, oral cytology may be indicated when (a) a patient refuses biopsy but consents to cytology (a positive report may induce this patient to permit a biopsy); (b) a physician or dentist wishes additional proof of an obvious squamous cell carcinoma before referral, thus emphasizing the urgency of the situation to a recalcitrant patient; (c) a tentative cytologic confirmation of cancer may permit earlier hospitalization for a confirmatory biopsy just prior to surgery.

Oral cytology is also of value in monitoring patients following treatment for oral cancer. Periodic cytologic examinations of the treated and adjacent areas may detect suspicious or positive malignant cells before the reappearance of clinical signs. Since these patients may develop a new cancer at other sites more frequently than patients without a history of cancer, cytologic examinations should be done freely, even for an only slightly abnormal oral mucosa. Cytology may also be helpful in
Early squamous cell carcinoma in the floor of the mouth—which has been present for over five years—in a 60-year-old white female. A biopsy taken five years ago was interpreted elsewhere as benign "leukoplakia." However, review of the original sections demonstrated definite focal dysplasia. Because of the increasing size of the lesion and the patient's continued heavy use of alcohol and tobacco, cytologic smears and biopsies were performed.

monitoring patients following radiation therapy. An experienced oral or general pathologist or cytologist can usually differentiate between malignant cells and those showing radiation change. If cytology continues to be positive in areas of previous irradiation, biopsy may be indicated, despite the well-known risks involved.

In evaluating nonspecific inflammatory reactions or specific ones, such as candidiasis, which may occur in areas previously treated for cancer, cytology is also of value. If no suspicious malignant cells are encountered, the inflammation may be treated for a reasonable period of time. If, at the end of this time, the reaction is not resolving, biopsy may be indicated to rule out a recurrent or new cancer which can coexist with an inflammatory lesion. One may wish to wait somewhat longer before performing a biopsy on a previously irradiated area.

Of course, the detection of suspicious cells in treated areas following presumably curative therapy, alerts the clinician to suspect, and try to eliminate, such inimical habits as continued smoking and/or alcohol consumption. If cells positive for cancer continue to be found in the cytological preparations, biopsy and further therapy may be indicated.
Note the benign appearance of the surface cells from a patient with squamous cell carcinoma. This lesion presented clinically as an unbroken, small, keratotic plaque in the floor of the mouth.

Pitfalls of Oral Cytology

Although it is not known how frequently false negative findings occur, they are probably more common than false positives. Thus, a negative cytology does not preclude the presence of cancer and the clinician must be wary of a false sense of security which may militate against performing a biopsy.

Two of the more common causes of a false negative report include:

1. Cytology performed on keratotic (clinically leukoplakic) lesions with dysplasia in the deeper layers of the epithelium. Unless the keratosis (clinically leukoplakia) is associated with an ulcerated or erythematous area, cytology is not indicated; the cytology of surface cells of keratotic (clinically leukoplakic) lesions may not demonstrate dysplasia despite histologic demonstration of definite dysplasia or even invasive carcinoma.

5a. Another early squamous cell carcinoma of the floor of the mouth. The cells near the surface are more atypical than those in 3b and 4a.

4b. False negative smear taken prior to biopsy. Slight increases are seen in the chromaticity of some nuclei, but not enough to be designated as suspicious.

5b. Highly suspicious cytology from this same patient. The scraping was done much more vigorously than usual. Although nuclear detail is lacking, the extreme hypochromatism and the greatly increased nuclear-cytoplasmic ratios are suspicious.
6a. Most of the epithelial cells appear normal. However, just to the left and above center, note a clump of cells with three hyperchromatic nuclei. These findings could be due either to dysplastic changes or to inflammation, which is abundantly demonstrated.

6b. Although most of these cells appear normal, some demonstrate hyperchromatic nuclei with some chromatin clumping. These findings, as well as those in 6a, indicate the necessity for further cytologic studies or biopsy, depending on the clinical situation.

6c. A more suspicious slide showing hyperchromatic nuclei with chromatin clumping in keratinizing cytoplasm. The chromatin clumping in the nucleus of the cell to the right may be degenerative.

6d. Another highly suspicious smear. Note the chromatin clumping in enlarged nucleus with irregular, angulated borders.

6e. The nuclear changes in the small, keratinized cell in the center of the field are so marked that even in the face of the microorganisms and inflammatory cells, it is highly suspicious for cancer. Other epithelial cells also show nuclear changes, but none are so sharply defined as those in the keratinizing cell.

6f. Highly suspicious cells from squamous cell carcinoma of the tongue. The nuclear changes are compatible with carcinoma, but because of the degenerative cytoplasmic changes, it is difficult to positively identify these as malignant cells.
Although these cells occur in groups, sufficient detail is discernible to recognize the hallmarks of cancer—increased nuclear-cytoplasmic ratios, irregular and angular nuclear membranes with variation in staining of the membranes.

2. An improper technical procedure due to (a) a scanty smear—the physician or dentist either did not harvest enough cells from the lesion or did not transfer the cells to the glass slide; (b) a poor quality smear—perhaps the gross debris was not removed prior to obtaining the smear or immediate contact with the fixative may not have been made while the smear was still wet; (c) poor staining of the smear at the laboratory; or, (d) an improper interpretation.

To avoid false-positive findings remember that atypical epithelial cells—similar in microscopic appearance to malignant or suspicious cells—may be seen in a variety of benign conditions such as herpetic infections and pemphigus vulgaris. Although unquestionably malignant cells can usually be differentiated from benign cells, "suspicious" cells found in malignant and premalignant states may be difficult to distinguish from epithelial cells which have become atypical due to inflammatory reactions. Sufficiently detailed clinical data will greatly aid the cytologist or pathologist in correctly evaluating the specimen.

**Procedures in Oral Cytology**

Oral cytology kits and history forms with detailed instructions are available.
# Utilizing the Oral Cytology Reports*

|                        | Negative for Cancer | Suspicious for Cancer | Positive for Cancer |
|------------------------|---------------------|-----------------------|---------------------|
| Clinically unidentified lesion | If lesion does not begin to regress within days to weeks after removal of irritants, biopsy. Observation time varies with appearance and location of lesion; e.g., lip lesions may be observed longer than tongue lesions. | If lesion does not begin to regress within days to weeks after removal of irritants, biopsy. Observation time would be shorter than for the same lesions with negative cytology. | Biopsy at once — by therapist, if possible. |
| Probable benign lesion (inflammation, trauma, erythema multiforme, etc.) | Continue to monitor lesion. If expected course is not followed, try to verify clinical diagnosis. Serial cytologic smears and/or biopsy may be indicated. | Most common cause of misleading “suspicious” evaluations. Rarely, however, cytology may indicate a squamous cell carcinoma coexisting with an inflammatory reaction; e.g., candidiasis. Treat “benign” condition. If “suspicious” cytology persists, biopsy. | Biopsy at once; or in the presence of a classical clinical picture (e.g., herpes simplex) only if positive cytology persists after clinical lesions have gone. |
| Possible precancerous lesions | If lesion does not begin to regress within days to two weeks after removal of irritants, biopsy. | One or more biopsies — at once, by therapist, if possible. | One or more biopsies — at once, by therapist, if possible. |
| Obvious clinical cancer (cytology unnecessary, but it may have been performed) | Biopsy at once — by therapist, if possible. | Refer to therapist. He might: (1) biopsy as outpatient; or (2) perform biopsy just prior to definitive procedure as frozen section. | Refer to therapist. He might: (1) biopsy as outpatient; or (2) perform biopsy just prior to definitive procedure as frozen section. |
| Follow-up after treatment of cancer | Continue monitoring patient unless suspicious clinical signs arise; in this event, biopsy. | Biopsy areas which are suspicious cytologically. | Biopsy positive areas prior to further therapy. |

*These suggestions are based on the author's personal experience and from the literature.
from most general and oral pathologists. Briefly, the main points to be observed are:
(a) Fill out the history form completely, adequately describing and identifying each lesion smeared.
(b) Have the complete armamentarium on hand—fixative, slides with frosted ends (two slides for each lesion), tongue blade or stainless steel spatula. The tongue blade may either be used as is or it may be broken into longitudinal halves so that there is a smooth and sharp edge for scraping the lesion. Be sure to soak the working ends of the tongue blade in tap water.
(c) Remove debris from the lesion and stroke the tongue blade firmly across the questionable area. Use a separate tongue blade soaked in its own container of water for each lesion smeared.
(d) Smear the collected cells evenly on a labelled glass slide.
(e) Immediately fix the slide by either: immersing it in 95 percent ethyl alcohol, or in equal quantities of 95 percent ethyl alcohol and ether; or by spraying it with commercial fixative or with a hair spray—Aqua-Net in its present formulation has been satisfactory in the author's experience.
(f) Allow the slides to air-dry and send them to an oral or general pathologist for processing and evaluation. The slides are usually stained by the Papanicolaou method after which each slide is carefully screened by a trained cytotechnologist, who marks those cells considered to be malignant or suspicious. The marked slides are then reviewed by the pathologist who makes the final evaluation. (In the author's opinion the single best oral cytology reference is the Atlas of Oral Cytology, by Medak, McGrew, Burkalow and Tiecke.)*

The final report should contain a general description of the types of cells present (epithelial, inflammatory, etc.) as well as an assessment of malignancy. The evaluation might be: (a) negative for cancer; (b) suspicious for cancer; or (c) positive for cancer. Many pathologists and cytologists now prefer this classification to the older I, II, III, IV, V classification.

References
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2. King, O. H., Jr.: Cytology—its value in the diagnosis of oral cancer. Dent. Clin. N. Amer. 15: 817-826, 1971.
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*May be obtained from U. S. Government Printing Office, Washington, D. C. 20402 as Public Health Service Publication No. 1949 (Price $4.75).