REVIEW ARTICLE

ORAL EXFOLIATIVE CYTOLOGY: A REVIEW

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ABSTRACT: Oral mucosa undergoes continuous exfoliation of epithelial cells which can be evaluated for diagnosis of certain diseases. Exfoliative cytology is the microscopic examination of shed or desquamated cells from the epithelial surface usually the mucous membrane. The history of exfoliative cytology dates back to 19th century. Several staining procedures have been used for study of smears but PAP stain still remains as the stain of choice. Since exfoliative cytology is a painless, bloodless and minimally invasive procedure, it is well accepted by the patients and has diverse applications. Due to recent developments like cytomorphometry and molecular studies, the use of these techniques is re-emerging. This review highlights various aspects related to oral exfoliative cytology.

INTRODUCTION

Oral mucosa exhibits a rapid turnover of cells and these exfoliated cells have a valuable role in diagnosis of certain local and systemic diseases. Oral cavity reflects the various events occurring in the body and this is reflected by variations in the cytomorphology of the exfoliated cells.¹ Exfoliative cytology is the technique of microscopic examination of shed or desquamated cells from the epithelial surface usually the mucous membrane. It also includes the study of cells that have been collected by scraping the tissue surface or collected from body fluids such as sputum, saliva etc. Normally as a part of physiological turnover there is continuous shedding of the superficial cells. But in the cases of malignancy, the deeper cells which are strongly adhered in normal conditions also become loose and shed along with the superficial cells.²

HISTORICAL ASPECTS OF EXFOLIATIVE CYTOLOGY ²,³

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Description</th>
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<tbody>
<tr>
<td>Walsh</td>
<td>1843</td>
<td>First person to describe cancer cells in patient's sputum.</td>
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<td>Lebert</td>
<td>1851</td>
<td>Emphasized the altered size of cells and nuclei as a basis of diagnosing cancer.</td>
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<td>Dudgeon</td>
<td>1927</td>
<td>Devised a direct smear technique of surgical specimen for rapid diagnosis.</td>
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<tr>
<td>Weinmann</td>
<td>1940</td>
<td>Cytological examination of oral cellular keratinization.</td>
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<td>George N Papanicolaou</td>
<td>1941</td>
<td>Started using &quot;PAP test&quot; as a routine procedure for early detection.</td>
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Ziskin 1941 First person to report the use of exfoliative cytology in oral cavity.

| Morrison et al | 1949 | Cytological diagnosis of nasopharyngeal malignancies. |
| Montgomery and Von Hamm | 1951 | Used exfoliative cytology for the diagnosis of oral cancer. |

**RATIONALE OF EXFOLIATIVE CYTOMETRY**

The rationale of exfoliative cytology lies in the epithelial physiology. Due to physiological turnover, the normal epithelium undergoes exfoliation of its superficial cells. The cells of the deeper layer are adherent to each other normally. When there is any pathological condition, the cells may lose their cohesiveness and the cells in the deeper layer may also shed along with the superficial cells. These exfoliated cells as well as cells which are scrapped off by means of specific instruments, can be studied quantitatively or qualitatively.  

**TECHNIQUE**

The basic requirements for oral cytology are 1-2 glass slide, swab stick/icecream stick/metal spatula or cytobrush and Spray cyte or alcohol as a fixative. Before starting the procedure, explain the purpose of technique to the patient. The patient’s name, date and anatomic location of smear must be labelled on one side of glass slide with sticker or diamond marker. Any excess saliva in the area that will be smeared must be wiped off with a guaze piece. Vigorously scrape and rotate the cytobrush or swab stick. Spread it onto the glass slide-white film like layer on the glass slide should be seen. Spray the surface of the glass slide with spray cyte which acts as a fixative. Alcohol (95%) can also be used as a fixative. Fixation or preservation is one of the most important steps in the procedure. Drying of the cells prior to fixation will usually result in artifacts such as nuclear distortion and vacuolization. Send the fixed smear to the pathologist’s laboratory for interpretation.

**PAP STAIN- THE STAIN OF CHOICE**

The classic form of PAP stain involves five dyes in three solutions:-

- A nuclear stain, haematoxylin, is used to stain cell nuclei. The unmordanted haematein may be responsible for the yellow color imparted to glycogen.
- First OG-6 counterstain (-6 denotes the used concentration of phosphotungstic acid; other variants are OG-5 and OG-8). Orange G is used to stain keratin. Its original role was to stain the small cells of keratinizing squamous cell carcinoma present in sputum.
- Second EA (Eosin Azure) counterstain, comprising three dyes; the number denotes the proportion of the dyes, e.g. EA-36, EA-50, EA-65.
- Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia and red blood cells.
- Light Green SF yellowish stains the cytoplasm of other cells, including non-keratinized squamous cells. This dye is now quite expensive and difficult to obtain, therefore some manufacturers are switching to Fast Green FCF, and however it produces visually different results and is not considered satisfactory by some.
- Bismarck brown Y stains nothing and in contemporary formulations it is often omitted.

When performed properly, the stained specimen should display hues from the entire spectrum: red, orange, yellow, green, blue, and violet. The chromatin patterns are well visible, the cells from borderline lesions are easier to interpret and the photomicrographs are better. The staining results in very transparent cells, so even thicker specimens with overlapping cells can be interpreted. On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink at once.

The EA stain contains two mutually incompatible chemicals, Bismarck brown and phosphotungstic acid, which precipitate each other, impairing the useful life of the mixture and compromising the differential staining of eosin and light green.

The advantages of PAP staining as follows:
- The dehydration and clearing solutions help in causing cellular transparency. This detects the overlapped cells and their individual morphology better, which otherwise would be confused for a giant cell, or a bi or multinucleated cell
- It leads to a differential staining for different degrees of differentiation, green-blue cytoplasm for basal cells and yellow-orange for a spinous or granular cell.
- Then like some other techniques of good standing it do have stability over long periods, stability of color and of course the better reproducibility of results.

**INTERPRETATION OF SMEARS**

The cells of different layers of epithelium can be identified based on their staining characteristics.
CLASSIFICATION OF CYTOLOGIC SMEARS

- Class I (Normal)- Indicates that only normal cells were observed.
- Class II (Atypical)- Indicates the presence of minor atypia, but no evidence of malignant changes.
- Class III (Indeterminate)- This is an in-between cytology that separates cancer from non-cancer diagnosis. The cells display wider atypia that may be suggestive of cancer, but they are not clear-cut cancer and may represent precancerous lesions or carcinoma in situ. Biopsy is recommended.
- Class IV (Suggestive of cancer)- A few cells with malignant characteristics or many cells with borderline characteristics. Biopsy is mandatory.
- Class V- (Positive for cancer) Cells those are obviously malignant. Biopsy is mandatory.

INDICATIONS

- Mucosal lesions that appear clinically innocuous and otherwise would not be biopsied.
- Microbial diseases like herpes simplex infection, herpes zoster infection.
- Dermatological lesions like pemphigus vulgaris, benign familial pemphigus, keratosis follicularis.
- Pernicious and sickle cell anemia.
- Evaluation of extensive mucosal lesion when it is not possible to do enough incisional biopsies for adequate sampling.
- Follow up for patients with prior diagnosis of either a malignant or premalignant mucosal lesion.
- If the patient’s medical status is too fragile for a biopsy or if the patients refuses.

CONTRAINDICATIONS

- Majority of benign lesions do not let themselves to cytologic smears.
- Lesions having an intact surface like fibroma must never be smeared.
- Leukoplakia does not lend itself to cytologic diagnosis because of scarcity of viable surface cells in the smear.

ADVANTAGES

- Painless and bloodless procedure.
- Noninvasive.
- Requires minimum armamentarium.
- Simple and quick chairside technique for dentists.
- It helps as a check against false negative biopsies.
- It is especially helpful in follow-up detection of recurrent carcinoma in previously treated cases.
- It is valuable for screening lesions whose gross appearance is such that biopsy is not warranted.
- Post biopsy complications can be eliminated.
- Useful for mass screening.
Has potential for early detection of malignant lesions.

DISADVANTAGES 4,5

- Relatively less information than histological slides.
- Reliability of technique is a limitation, positive results are reliable but negative are not.
- Suitable only for epithelial cells, seldom used for evaluation of C.T lesion.
- It is only an adjunct and additional aid but not a substitute for biopsy.
- Interpretation requires skilled and experienced cytopathologist.
- Tumor grading cannot be assessed.
- Cannot identify degree of differentiation of malignancy and extent of invasion.

RECENT ADVANCES

Molecular Analysis of Exfoliated cells-changes occur at the molecular level before they are seen under the microscope and before clinical changes occur. LOH and other molecular changes, including changes at p16, p53 and cyclin D, can be assessed in exfoliated cells.12,13

Exfoliative cytology was also studied in patients with titanium implants. Metal-like particles were observed inside and outside epithelial cells and macrophages in cytological smears of peri-implant mucosa of both patients with and without periimplantitis. The concentration of titanium was higher in the peri-implantitis group as compared to the group without periimplantitis.14

Ogden et al. suggested that quantitative techniques, based on the evaluation of parameters such as nuclear area (NA), cytoplasmic area (CA), and nucleus-to-cytoplasm area ratio (NA/CA), may increase the sensitivity of exfoliative cytology for early diagnosis of oral cancers, since these techniques are precise, objective and reproducible.15

Cowpe et al. demonstrated that exfoliative cytology is capable of detecting malignant changes, through estimation of NA/CA using the planimeter method in Papanicolaou-stained smears.16

Archival cytology slides can also be used for HPV DNA detection with ISH. The diagnosis of metastatic lesions usually is determined by fine-needle aspiration. Human papillomavirus (HPV) is now being considered as a causative agent in a subset of HNSCC. Ki 67 has been studied in oral cytological smears using Immunocytochemistry to evaluate the nature of lesion and response to treatment. Sharma et al evaluated Ki-67 expression in cytologic scrapes from oral squamous cell carcinoma before and after 24 Gray radiotherapy in 43 patients. Ki-67 expression was seen in an extremely small number of cells. Only 10 tumours showed positive cells, and the labeling index in them varied from 0.1 % to 0.01 %. After 24 Gray irradiation, no case showed Ki-67 positive cells.17 The validity of
oral cytology for analyzing the number of keratinised cells and the nucleolar activity (AgNORs) in smoking patients has recently been demonstrated.\(^8\)

**CONCLUSION**

Oral exfoliative cytology is a very simple chairside test requiring minimum of armamentarium. Although it is not a substitute to biopsy yet it can be used as an excellent adjunct. With the advent of recent advances like cytomorphometry, its value as a diagnostic and predictive aid has certainly increased. Awareness must be spread regarding its importance so that it can be routinely used by dentists.

**REFERENCES**


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