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# ORAL EXFOLIATIVE CYTOLOGY – A REVIEW

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## ABSTRACT

As oral cancer is the 6th most common cancer worldwide. Only 50% survival rate is achieved so far. One of the main reasons for high mortality rate is due to late diagnosis and often patients come at the late stage of disease. In relation to the early diagnosis, oral exfoliative cytology technique which has higher benefits in diagnosis of various pre-cancerous and infectious diseases. There are various advancement have been explored in the field of oral cytology. As it is a simple and non-invasive technique, it can be done in mass screening programs and dental op when a suspicious lesion is noted. Understanding the importance and technique of exfoliative cytology can provide the best outcome in diagnosing. This review describes the overall importance of exfoliative cytology, techniques, applications and recent advances.

**KEYWORDS:** oral cancer, exfoliative cytology, pre-cancer, early diagnosis.

## 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. 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.[1]

**Cytology**

Cytology is the study of the cells. Cellular morphology reflects biological behaviour of the tissue & the host and Genetic & molecular biology of the cells.[1]

**Types of cytology**

Rubin in 1994, classified cytology into 3 types;

Exfoliative cytology, abrasive cytology and fine needle aspiration cytology.

**Rationale of exfoliative cytology**

**Types of exfoliation**

1. Natural Spontaneous Exfoliation: Natural process of continuous renewal of body tissues.
2. Artificial Surface Exfoliation: Occurs when surface of the mucosa or lesion is brushed, scraped or lavaged. Cells are better preserved & are younger than spontaneously exfoliated cells.[1,2]

Normal epithelium undergoes continuous exfoliation of its superficial cells as a part of physiologic turnover. The cells of the deeper layers are strongly adherence to each other under normal conditions. When the epithelium becomes malignant, the cells may lose their cohesiveness resulting in the exfoliation of the deeper cells along with the superficial cells.

**History of exfoliative cytology[1]**

| NAME | YEAR | DISCOVERY |
|---|---|---|
| **Walsh** | 1843 | Cancer cells in sputum sample. |
| **Lebert** | 1851 | Cancer cells - altered size of cells and nuclei |
| **Bealem** | 1860 | Attempted a cytological diagnosis of oropharyngeal cancer. |
| **Dudgeon** | 1927 | Devised a direct smear technique of surgical specimen for rapid diagnosis. |
| **Weinmann** | 1940 | Cytological examination of oral cellular keratinization. |
| **George N Papanicolaou** | 1941 | Started using "PAP test" as a routine procedure for early detection. |
| **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. |

**Indications**

1. A mucosal lesion that appears clinically innocuous & otherwise would not be biopsied.
2. Evaluation of an extensive mucosal lesion in which sufficient number of incisional biopsies not possible for adequate sampling

3. Follow-up for patients with a prior diagnosis of either a premalignant or malignant mucosal lesion
4. If the patient's medical status is too fragile for a surgical biopsy or if the patient refuses
5. Cytology should be considered only as an adjunct for any lesion involving oral mucosal surface if the diagnosis cannot be established by the history or clinical examination confirmed with histological evaluation of biopsied specimen.
6. Viral diseases like herpes simplex infection, herpes zoster infections.
7. Dermatological lesions like pemphigus vulgaris, benign familial pemphigus and keratosis follicularis. Pernicious and sickle cell anaemia

**Contraindications**

1. The Pap smear "works" only for surface lesions; while it “will not work" for submucosal masses.
2. Red & blistering lesions are candidates for Pap smears whereas white lesions are not included. If the lesion, however, is white, it is likely that the surface keratin will prevent obtaining deeper cells. Leukoplakia does not lend itself to cytological diagnosis because of scarcity of viable surface cells in the smear
3. Dry crusting lesions are not meant for oral cytology.
4. Evaluation of cytological smear is only an adjunct & cannot be considered as definitive diagnosis.[1][2]

**Advantages**

1. Painless and bloodless procedure
2. Non-invasive
3. Requires minimum armamentarium
4. Simple and quick chair side technique for dentists
5. It helps as a check against false negative biopsies.
6. It is especially helpful during post treatment follow-up and detection of recurrent carcinoma in previously treated cases.
7. It is valuable for screening lesions whose gross appearance does not warrant biopsy.
8. Post biopsy complications can be eliminated.
9. Useful for mass screening[3]

**Disadvantages**

1. Relatively less information than histological slides

2. Reliability of technique is limited, positive results are reliable but negative are not.
3. Only for epithelial cells, seldom used for evaluation of C.T lesion
4. It is only an adjunct and additional aid but not a substitute for biopsy.
5. Interpretation requires skilled and experienced cytopathologist.
6. Tumour grading cannot be assessed.
7. Cannot identify degree of differentiation of malignancy and extent of invasion

**Preparation of the lesion**

**Do's**

1. If covered with slough, clean the surface with gauze moistened with saline.
2. If lesion is tender, apply anaesthetic gel.
3. In keratotic lesions, fissured areas are best sites.
4. In thick keratotic lesions, remove the white area by scraping with curette till the pink tissue is seen.
5. Exudates must be treated like blood smears & spread on a glass slide with the edge of another glass slide.

**Don'ts**

1. Lesion should not be washed or dried
2. Lesion should not be wiped with cotton pellets
3. Swabs should not be used
4. Local anaesthesia may cause interpretive difficulties on cytology
5. Sharp instruments should not be used for scarping the lesion

**Factors influencing the appearance of exfoliated cells**

- Technique used in obtaining the cells
- Level of maturation at the time of exfoliation
- Nature of the parent tissue
- Medium used
- Interval between exfoliation & collection
- Type of fixative, processing & stain technique used

**Oral cytology methods**

1. Conventional Method
2. Liquid based Cytology.

3. Centrifugation Method

**1. Conventional Method**

Vigorously scrape with a wooden spatula or cytobrush over the entire lesion, spread the harvested cells onto the glass slide. Visible white, filmy debris seen on the glass slide, Spray the surface of the glass slide with the Spray-cyte or. Dip in 95% ethyl alcohol. Dry & Stain with Papanicolaou Staining (PAP)

**2. Liquid Based Cytology (Lbc)**

Use the cytological brush and scrape the lesional area, The brush with the scraped material was dipped and shaken in the suspending solution (20 ml of 95% ethanol, 6 ml of acetic acid, 74 ml of normal saline), Centrifuge the sample for 10 minutes at 2000 RPM. Obtained cell pellet is suspended in 95% alcohol. Suspension should be poured in the horizontal placed glass slide for 2 hrs. Allow the cells to Sediment in the slide. Dry & Stain with Papanicolaou Staining (PAP)

**3. Oral Washings/Oral Rinse Method**

Instruct the patient to firmly rub on the lesion using their tongue for 30 seconds, Instruct to swish with phosphate buffered saline (pH -7.2) and to expectorate into a sterile container. Label and centrifuge at 1,000 rpm for 5 minutes. Discard the supernatant fluid and prepare smears from the cell plug. Dry & Stain with Papanicolaou Stain [4]

**4. ORAL BRUSH BIOPSY**

Rinse the mouth thoroughly with mouth wash, use sterile gauge to clean the debris if present on the site of procedure. Deeply scrap the site of lesion (trans-epithelial) with sterile cytobrush, then smear is done with the cytology brush containing cells. Stain with PAP/sent to lab for computer analysis.

**STAINING**

**Requisite of ideal stain**

- Should allow evaluation of architectural patterns of the tissue fragments
- Permit proper evaluation of nuclear morphology.
- Help in the visibility of cytoplasmic characteristics.
- Visualization & identification of certain diagnostic features in the background Eg: Stroma & secretions.[5][6]

**PAPINOCOLAOU (PAP) STAIN**

Commonly employed method for staining cytological smear

| Stain | Cell/ cell organelle | Colour |
|---|---|---|
| Hematoxylin | Nuclei | Blue/Black |
| Light green SF | Cytoplasm | Blue/Green |
| OG 6 | Keratinizing cells | Pink/Orange |
| EosinY | Squamous cells, nucleoli, RBC's | Red/Pink |

Other stains includes - Hematoxylin and eosin, Romanowsky stain, May – Grunwald Giemsa, PAS, Zeihl-Neelson, Alcian blue, Mucicarmine, Feulgen reaction, Gram stain and Masson-fontana.

**Methodical modification[7]**

| Name | Year | Work |
|---|---|---|
| Gladstone | 1951 | Use of sponge biopsy |
| Schneider & Cowson | 1952 & 1960 | Variants of staining techniques |
| King | 1963 | Use of frosted glass slides |
| Staats and Goldsby | 1963 | Use of metal spatula |
| Sandler | 1964 | Removal of keratotic layer with a curette |
| Dumbach | 1980 | Smear curettage |
| Mehrotra | 2008 | Tooth brush to harvest cells in the resource challenged settings. |

**Grading of the smear[8,9]**

| Grade | Description | Inference |
|---|---|---|
| Class 1 | Normal | Only normal cells are observed. |
| Class 2 | Atypical | Presence of minor atypia due to inflammation. No signs of malignancy. |
| Class 3 | Intermediate | Wider atypia suggestive of severe dysplasia, carcinoma-in-situ or cancer. |
| Class 4 | Suggestive of cancer | Shows few epithelial cells with malignant changes. Biopsy is mandatory. |
| Class 5 | Positive for cancer | Cells show characteristics malignant changes. Biopsy is mandatory. |

**Class 1 smear**

- Normal nuclear/cytoplasmic ratios.
- The blue/green staining indicates that those cells were acquired from deeper layers (diagnostic).
- The red/orange cells were acquired from superficial layers (not diagnostic).

Inference - "Normal, no atypical cells seen".

**Class 2 smear**

– There may be slight atypia that is assumed to be the result of inflammation.
– The nuclei are of normal size and shape. There are, however, scattered inflammatory cells and some subtle atypical changes in the upper left cell.
Inference - "A few atypical cells; probably inflammatory."

**Class 3 smear**

– The nuclei of these cells are abnormally large (altered nuclear/cytoplasmic ratio) indicating that they may be malignant.
– Changes occur in superficial cells (red/orange) is worrisome.

Inference - "Atypical cells seen; may be malignant."

**Class 4 smear**

– Many nuclei almost fill the cells (altered N/C ratio).
– Cells are of different sizes and shapes (pleomorphism).
– Biopsy is the required next step.
Inference - "Many atypical cells; probably malignant."

**Class 5 smear**

– Features of anaplasia are prominent. There is altered N/C ratio, pleomorphism, and enlarged/multiple nuclei.
– The lesion is malignant.
Inference - "Most cells are atypical; definitely malignant."

**Cytopathology of oral carcinoma (CLASS III, CLASS IV)**

**A. Nuclear abnormalities**

**a) Size of the nucleus**

Disproportion enlargement of the nucleus.

Variation of size of one nucleus to another (Anisonucleosis)

**b) Irregular shapes:** Outline is crenated with sharply indented edges. Extreme elongation, sharp contour, angular shape, budding and spheroidal projections from the nuclei.

**c) Multinucleation:** Nuclei are hyperchromatic, closer together and difficult to distinguish from one another.

In benign condition: multinucleation shows uniform distribution of chromatin and uniformity in the size and shape of the nuclei.

- Abnormal mitosis.
- Nuclear hyperchromatism: Excessive synthesis of DNA in nuclei of cancer cells imparts basophilia to the nuclei.
- Aberrant chromatin pattern.
- Prominent and multiple nucleoli.
- Nuclear predominance.
- Altered nuclear cytoplasmic ratio.
- Degenerative changes of the nuclei.

**B. Cytoplasmic abnormality**

a) Scanty cytoplasm ‘

b) Vacuolization and inclusions

c) Altered stained characteristics

**C. Cell as whole**

a) Enlargement : Anisocytosis , Anisonucleosis

b) Bizarre shapes: Tadpole like cells, cannibalism, and multinucleation.

**Study of smear**

Reporting the slide

Method of analysis of the slide

- Qualitative Method
- Quantitative Method

**Reporting the slide**

- Quality of fixation and staining
- Cellular details
- Concentration of cellular material
- Architectural patterns of tissue fragments
- Stromal characteristics
- Background – bloody, inflammatory, necrotic, excess stroma, colloid, mucin[9]

**Method of analysis of the slide**

Qualitative method of analysis of cytological smear - Assessment of cellular and nuclear atypia, Staining patterns, nuclear aggregation, Chromatin clumping and Application of immunochemical methods.

Quantitative method of analysis of cytological smear - Analysis of cellular and nuclear morphology, Cellular & nuclear diameter & area and nuclear cytoplasmic ratio.

Cellular Morphology - Cell Size, cell Shape and cell type.

Nuclear characteristics - Nuclear size, nuclear shape, Chromatin pattern, nuclear membrane and nucleoli.

Cytoplasmic characteristics - Origin of cell/ cell type, Texture – vacuolization and inclusions and functional state.

**Applications of oral exfoliative cytology**[10-12]

**Acute and chronic inflammation**

Potentially malignant disorders – leukoplakia and erythroplakia

Physical and chemical injury

Gingival and periodontal diseases

Skin lesions associated with oral mucosa – lichen planus, erythema multiformae

Viral lesions – viral inclusion bodies in herpes simplex and zoster

Fungal lesions – candidiasis

Bacterial lesions – TB granuloma, Actinomycosis and syphilitic ulcer

Diabetes mellitus type 2

Radiation and chemotherapy

**Most Common Non-invasive Methods for the Diagnosis of Oral Squamous Cell Carcinoma (OSCC).**

| S.no | Principle | Test |
|---|---|---|
| 1. | Vital stain | 1.Toluidine blue test |
| 2. | Oral brush biopsy | 1.Conventional oral biopsy<br>2.Oral brush biopsy with computer assisted analysis |
| 3. | Saliva based oral cancer diagnosis | 1.Genomic substances<br>2.Transcriptomic substances<br>3.Proteomic substances |
| 4. | Light based detection system | 1.Chemilumniscent (Microlux/dl, Vizilite plus)<br>2.Tissue fluorescence imaging (Velscope) |
| 5. | Optical biopsy | 1.Raman Spectroscopy<br>2.Tissue Fluorescence Spectroscopy<br>3.Elastic Scattering Spectroscopy |

| | | |
|---|---|---|
| | | 4.Differential Path-length Spectroscopy<br>5.Nuclear Magnetic Resonance Spectroscopy<br>6.Confocal Reluctant Microscopy(crm)<br>7.Optical Coherent Tomography and<br>Angle-Resolved Low Coherent Interferometry (A/LCI) |
| 6. | Biomarkers | 1.DNA – analysis |
| 7. | Laser capture micro dissection | |

**Methods in development- newer in exfoliative cytology[13]**

Laser capture micro dissection, Lab-on-a-chip sensor technique, DNA image cytometry, saliva based oral cancer diagnosis, molecular analysis, microscopy and spectroscopy

## CONCLUSION

Oral cytology can be used for various purposes in pathological conditions and other potentially malignant disorder and there are several researches are done to make this technique in an advanced level. Cytomorphologic studies, quantification of nuclear DNA content, identification of various tumor markers and molecular analysis of exfoliated cells in titanium implants combined with IHC are the current evolving trends in the field of exfoliative cytology.

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**Conflicts of interest**

There are no conflicts of interest.

## REFERENCES

1. Aneja T, Guleria M, Mahajan A, Agarwal L. Oral exfoliative cytology : a review, 2017; 2(1): 1–6.
2. Shafer WG, Hine NK, Levy BM. A text book of Oral Pathology. 6th ed. New Delhi: Elsevier, 2009.
3. Sivapathasundharam B, Kalasagar M. Yet another article on exfoliative cytology. Journal of Oral & Maxillofacial Pathology., 2004; 8(2): 54-57.
4. Nanci A. Cytoskeleton, junctions and fibroblasts. In: Ten Cate AR, Dolan J, Arnold R, eds. Ten Cate's Oral Histology. Development, Structure and Function. 5th ed. St. Louis, Mo, USA: Mosby Elsevier, 1985; 54–78.

5. Kuyama K, Sun Y, Taguchi C, Endo H, Wakami M, Fukumoto M, et al. A clinico-pathological and cytological study of oral candidiasis. Open Journal of Stomatology, 2011: 212–7.
6. Mulki S, Shetty P, Pai P. Oral rinse-based cytology and conventional exfoliative cytology: A comparative study. J Cancer Res Ther [Internet], 2015; 11(1): 129.
7. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. 2006; 9: 1–9.
8. Ganjare A, Bagul N, Kathariya R, Oberoi J. 'Cell junctions of oral mucosa'-in a nutshell. QScience Connect, Jul 31, 2015: 7.
9. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. Journal of the Royal Society of Medicine, Dec., 1985; 78(12): 995-1004.
10. Rivera C, Núñez-de-Mendoza C. Exfoliative cytology of oral epithelial cells from patients with type 2 diabetes: cytomorphometric analysis. International journal of clinical and experimental medicine, 2013; 6(8): 667.
11. Patton LL, Rcsed FDS, Epstein JB, Frcd C, Rcsed FDS, Kerr AR.. J Am Dent Assoc [Internet], 2008; 139(7): 896–905.
12. Fedele S. Diagnostic aids in the screening of oral cancer. Head & neck oncology, Dec, 2009; 1(1): 5.
13. Chai RC, Lambie D, Verma M, Punyadeera C. Current trends in the etiology and diagnosis of HPV-related head and neck cancers. Cancer medicine, Apr, 2015; 4(4): 596-607.