

## Oral Exfoliative Cytology

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### ABSTRACT

Exfoliative cytology is a simple, non-invasive diagnostic technique which could provide as an adjunct in early diagnosis of oral premalignant and malignant lesions. Cytology is cheaper and easy procedure that can be carried out at outdoor patient department to diagnose malignancy at early stage. It's a microscopic examination of shed or desquamated cells from the epithelial surface usually the mucous membrane. It also includes the study of those cells that have been collected by scraping the tissue surface or collected from body fluids such as sputum, saliva etc. Aging, smoking, alcohol consumption, systemic diseases such as anaemia, diabetes mellitus, radiotherapy, chemotherapy, pregnancy, menstruation, medications are the other possible factors that could contribute to the morphometric changes in the cells. Oral exfoliative cytology is the simple, sensitive and valuable adjuvant for gold standard Biopsy.

**Key words:** oral exfoliative cytology, premalignant, malignant, cytobrush.

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## **Introduction:**

Cancer is latinized from the Greek word 'Karkinos', meaning crab, denoting how carcinoma extends its claws like a crab into adjacent tissues.<sup>1</sup> Cancer being a genetic disorder involves multiple alterations of the genome progressively accumulated during a protracted period, the overall effect of which surpasses the inherent reparative ability of the cell. In the course of its progression, visible physical changes are taking place at the cellular level (atypia) and at the resultant tissue level (dysplasia). These alterations include genetic changes, epigenetic changes, surface alterations, and alterations in intercellular interactions. The sum total of these physical and morphological alterations are of diagnostic and prognostic relevance and is designated as 'precancerous' changes. These changes are ultimately involved in driving cells further along the path to neoplastic transformation.<sup>2</sup>

Oral cancer is currently the sixth most common malignancy in the world. In India it is the most common malignancy among men and one of the five most common malignancies among women.<sup>3</sup> The diagnosis of precancer is primarily based on morphology and its grading on histology (dysplasia). Despite the fact that this estimation is subjective and therefore

carries a low prognostic value of an impending malignancy, is still widely practiced to assess the risk of malignant potential of such lesions. Because of this inherent discrepancy, such lesions may well be designated as potentially malignant.<sup>2</sup>

Oral exfoliative cytology is a simple and non-invasive diagnostic technique that could be used for early detection of oral premalignant and malignant lesion.<sup>4</sup> Quantitative parameters like morphometry are objective and reproducible and may be important in cytological evaluations in these lesions. Cytobrush sampling is more frequently used nowadays for exfoliative cytology, since it maximizes the number of cells obtained, and facilitates their uniform distribution onto the microscope slide, thus probably improving sensitivity.<sup>5</sup>

## **Classification of Papanicolaou:**<sup>6</sup>

CLASS I: (Normal) indicate that only normal cells were observed.

CLASS II: (Atypical) indicate minor atypia but no evidence of malignant changes.

CLASS III: (Intermediate) the cells display wider atypia that may be suggestive of cancer, but they are not clear cut.

CLASS IV: (Suggestive of cancer) few epithelial cell with malignant character or many cells with border line characteristic.

CLASS V: (Positive cancer) cells those are obviously malignant.

### **Advantages of Pap Smear:**<sup>7</sup>

1. It is painless and simple
2. Does not cause bleeding
3. Does not need anaesthesia
4. Can detect cancer and precancer
5. Can identify non-specific and specific inflammations
6. Can be carried out as an outpatient procedure

### **Causes of Unsatisfactory Smears**<sup>7</sup>

Unsatisfactory smears can be due to non-representative / inadequate samples or due to poor quality of preparation (thick smears, extreme admixture with blood, delayed fixation, over staining etc). Attention to matters of technique regarding the procedure and preparation of smears will considerably reduce the number of unsatisfactory smears received in a cytology lab.

### **Artifacts Due To Faulty Techniques**

1. Delay in processing may lead to degenerating smear picture with loss of cell morphology and plenty of bacteria in the smear background.
2. Delay in fixation may lead to Air-drying artifacts - pale stained nuclei, lack of differential cytoplasmic staining, cytoplasmic and nuclear eosinophilia.
3. Contamination from other smears and cell from effusion smears to

other slides should be avoided. All the alcohol and xylene solutions should be filtered every day using Whatman No.1 filter paper. The fixative should be filtered after each use.

### **Properties of Cytologic Fixatives**<sup>7</sup>

1. Do not excessively shrink or swell cells.
2. Do not distort or dissolve cellular components.
3. Inactivate enzymes and preserve nuclear details.
4. Kill microbes.
5. Improve optical differentiation and enhance staining properties of the tissues and cell components.

### **Staining Methods In Cytology**<sup>7</sup>

#### **Papanicolaou Staining Method**

Papanicolaou staining method is the routine staining procedure used in cytopathology laboratory. This technique is named after Dr. George N. Papanicolaou, the father of exfoliative cytology and is devised for the optimal visualization of cells exfoliated from epithelial surfaces of the body. It is a polychrome staining reaction designed to display the many variations of cellular morphology showing degree of cellular maturity and metabolic activity. The use of the Papanicolaou stain results in well stained nuclear chromatin, differential cytoplasmic counterstaining and cytoplasmic transparency.

### Steps of staining procedure

- a. *Fixation:* The cytology smears are fixed in 95% ethyl alcohol or in other substitutes for a minimum of 15 minutes.
- b. *Nuclear staining:* It is done by using haematoxylin stain. Harris haematoxylin or its modified form is used in Papanicolaou staining in regressive method, in which we deliberately over stain with haematoxylin and remove the excess stain by using a differentiating solution such as acid alcohol (0.05% HCl in 70% ethyl alcohol) or 0.05% aqueous solution of HCl alone. As haematoxylin is used in an acid pH, a pink colour will form and it is not stable. In order to make it stable, the compound is brought to alkaline pH (bluing) by treating with a weak alkaline solution. Running tap water which is slightly alkaline (pH 8) is used as bluing solution in small laboratories. Ammonium hydroxide solution (15 ml of ammonium hydroxide 28-30% weight/volume to 985 ml of 70% ethanol) can also be used.
- c. *Cytoplasmic staining:* Cytoplasmic stains are OG-6 and EA-36. Both are synthetic stains and OG-6 is a monochrome stain while EA-36 is a polychrome stain.
- d. *Dehydration:* Rinse the smears in absolute alcohol for two or three changes for the removal of water. Smears left in rinses for long will lose too much stain. Alternative to 100% ethanol are 100% isopropanol and 100% denatured alcohol. Rectified spirit affects the cytoplasmic staining and hence is not recommended.
- e. *Clearing:* Cells are not transparent while the smear is in the staining or alcohol solutions. During clearing, alcohol is being replaced with Xylene, which is also miscible in mounting medium. Xylene has a refractive index as that of glass and mounting medium and it prevents cellular distortion.
- f. *Mounting of slide:* The mounting media must be miscible with the clearing agent to prevent fading of the stains. Practice is essential to achieve well-mounted slides, free of air bubbles and artifacts. A minimum of mounting medium should be used. Too much mounting medium interferes with microscopic detail, making the cell film appear hazy or milky when examined under the high power objective. If the mounting medium

and cover slip are applied too slowly, a common artifact appears as a brown refractile pigment like substance on the surface of the cell when xylene evaporates. If this artifact occurs, the slide must be soaked in xylene, absolute alcohol and 95% alcohol, rinsed in running tap water and restained in OG and EA. A possible means of preventing the “brown artifact” is to coverslip slide behind a transparent chemical splash shield set at the front edge of the fume hood. The shield diverts air around the local workspace and reduces the rate of xylene evaporation. The usual size of the coverslip for a cervical smear is 22x30mm. If the smear spread is beyond the coverslip area, ideally use another small coverslip or put a drop of DPX and spread evenly with the same coverslip without affecting the focus.

**Papanicolaou Staining Procedure**

1	90% Ethanol (fixation)	15 minutes(mt)
2	80% Ethanol	2 mt
3	60% Ethanol	2 mt
4	Distilled water	5 dips
5	Distilled water	5 dips
6	Haematoxylin stain	2 mt.
7	0.05% HCl solution	2 mt.
8	Running tap water	10 mt.

	(Bluing)	
9	60 % Ethanol	2 mt.
10	80% Ethanol	2 mt.
11	80% Ethanol	2 mt.
12	95% Ethanol	2 mt.
13	OG-6 stain	2 mt.
14	95% Etanol	2 mt.
15	95% Etanol	2 mt.
16	95% Etanol	2 mt.
17	EA-36 Stain	2 mt.
18	95% Etanol	2 mt.
19	95% Etanol	2 mt.
20	95% Etanol	2 mt.
21	95% Etanol	2 mt.
22	Absolute Ethanol	2 mt.
23	Absolute Ethanol	2 mt.
24	Absolute Ethanol	2 mt.
25	Absolute Ethanol+ Xylene (1:1)	2 mt.
26	Xylene	5 mt.
27	Xylene	5 mt.
28	Xylene	Till clear
29	Mounting in D.P.X	

**Rapid Papanicolaou Staining**

The purpose is to save staining time and money by combining Orange G and Eosin Azure and reducing the number of rinses. This procedure needs to be done only for emergency situations and not for routine use.

**Haematoxylin and Eosin (H&E) Staining Method**

Some laboratories use routine H&E stain for non-gynecological smears. The benefits of using Papanicolaou stains are clear definition of nuclear details and differential counter staining giving

cytoplasmic transparency. H&E stain does not satisfy these criteria and hence unacceptable for cervical smears.

### **May-Grunwald-Giemsa (Mgg) Staining Method**

Many laboratories use MGG (Romanowski type stain) staining method for cytological diagnosis of non – gynaecological specimens in addition to Pap and H&E stains. Combination of all these stains increases the efficiency of microscopical interpretations. MGG stain is performed in air –dried aspirates or fluids. Stock solutions of May-Grunwald Reagent and Giemsa Stain are available commercially.

### **Labelling of Slides**

After the slides have been cleaned, they are ready for labelling. Place a small square label on the edge of the slide on the same side as the cover slip. Use water proof ink and record the institution, the number, the year, the nature of specimen etc. on it.

### **Filing the Slides**

The slides must be protected from breakage, light, moisture and dust. After microscopical interpretation, the slides must be filed in slide filing cabinets in serial order, in numbered slots. They are kept for a minimum of 5 years and are retrieved when necessary.

### **Discussion**

As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated or abraded from the surface of the oral mucosa. The superficial epithelial cells do contain nuclei and alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes.<sup>3</sup>

Exfoliative cytology is based on epithelial physiology. A normal epithelium is exposed to regular exfoliation, namely the loss of cell surface, and the thickness of the epithelium is constant. Under normal conditions, epithelial cells are strongly held in place. The presence of benign disease or the occurrence of malignant epithelial formation causes the cells to lose their cohesive force and results in exfoliation. Loss of cohesion between the cells enables the collection of the exfoliated cells for microscopic examination.<sup>8</sup>

The basic defect of any cell alteration begins at the molecular level triggering a series of reactions and thereby affecting the entire cell system and consequently its morphology. The general biological activities is reflected best in nucleus and

functional activity is reflected in cytoplasm.<sup>9</sup>

Oral exfoliative cytology is a simple, non-invasive, and painless method that involves microscopic analysis of cells collected from the surface of the oral mucosa. However, this method had been abandoned because of problems such as inadequate tissue samples, technical errors and the incorrect interpretation of findings. Today, with advanced imaging techniques, computerized systems and the use of quantitative techniques to verify the reliability of cytomorphometric analysis, this method is gaining in popularity once again.<sup>8</sup>

Oral exfoliative cytology is particularly valuable for mass screening purposes. It has been used in the detection of Oral Squamous Cell Carcinoma and has been shown to have a sensitivity of 94%, specificity of 100% and an accuracy of 95%.<sup>10</sup>

Advantage of exfoliative cytology included that it is a painless, bloodless non-invasive, quick and simple procedure, suitable in patients with systemic disease who are contraindicated for biopsy, guards against false negative biopsy, post biopsy complications can be eliminated.<sup>9</sup>

Correct and adequate sampling of cells is mandatory for an accurate diagnosis with

cytomorphometric analysis. Numerous instruments were used including metal and wooden tongue spatulas, cotton sticks and brush biopsy instruments. Although there are studies reporting that metal and wooden tongue spatulas are sufficient for cytologic sampling, brush biopsy was shown to enable sampling of cells from all layers of epithelium thus giving the opportunity to detect early precancerous changes in contrast to traditional oral exfoliative cytology that was found to yield unreliable result and high false negative rates.<sup>11</sup>

The ideal instrument used for making a good cytological smear should be easy to use in any location, cause minimum trauma and provide an adequate and representative number of epithelial cells. It has been shown that a brush is an adequate instrument due to its ease in sampling and to the quality of the oral cytologic sample. Brush biopsy is a simple, relatively inexpensive, highly sensitive, risk free method of screening for cancer and serve as an aid to the clinical examination.<sup>12</sup>

Cytobrush is a convenient instrument capable of sampling less accessible oral sites. Cytobrush pulls together an adequate number of cells and allows uniform dispersion of cells on a microslide which facilitates an accurate cytopathologic diagnosis.<sup>13</sup>

Papanicolaou technique and its modifications have been used to study premalignant and malignant oral lesions. Advantage of PAP staining lies in the fact that 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 bi or multinucleated cells. Then again like some other techniques of good standing we do have a stability of stain over long periods, stability of colour and of course the better reproducibility or results.<sup>9, 14</sup>

Diagnostic and prognostic techniques are continually being developed and refined to detect cancer in its early stages. It is believed that the detection of oral cancer tumors when they are small provides an opportunity for less invasive treatment, thus improving the patient's quality of life and contributing to a better prognosis.<sup>15</sup>

Cytomorphology is the most widely used method of oral exfoliative cytology, and assesses parameters such as Cellular Diameter (CD), Nuclear Diameter (ND), Nuclear Area (NA), Cytoplasmic Area (CA), NA/CA ratio, nuclear shape, nuclear membrane continuity, optical density and nuclear texture. These parameters, especially NA, CA and NA/CA ratio, have been shown to provide meaningful results in the diagnosis of oral lesions.<sup>8</sup>

## Conclusion

Early diagnosis of oral cancer is difficult, due to the asymptomatic nature and benign appearance of these lesions on initial presentation. The major advantage of exfoliative cytology is the noninvasive character of the technique, which allow a simple and pain free collection of intact cells from different layers in the epithelium for microscopical examination and quantitative evaluation. The use of this technique may improve the diagnostic reliability of exfoliative cytology in the management of oral malignancy.

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