Exfoliative Cytology in Every Day Practice

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Abstract

A general dental practitioner has a pivotal role in the early identification of malignancy. Malignancy is like an uninvited guest, which overstays its welcome. This can perturb even the most experienced clinicians. Thus it is safer to be well prepared mentally and physically to appropriately handle such cases. Chair-side investigations are simple and effective tools for the diagnosis of oral malignancy. Oral Exfoliative cytology is a patient-friendly procedure in this regard. This review broadly covers the significance of oral exfoliative cytology and also concludes with a practical guideline for the clinicians’ benefit.

Keywords: Cell Differentiation, Cytodiagnosis, Oral Cancer

1. INTRODUCTION

Oral cancer is a major concern affecting our country. It is the third most common form of cancer plaguing our country. 1 Globally it is the sixth most common form of cancer. 2 Oral cancer is defined as the cancer of lips, mouth and tongue. This is the definition accepted during the committee meeting held by International Classification of Diseases (ICD) in Geneva, Switzerland. 3 Regardless of the development of science and technology, development of multiple primary tumors and delayed diagnosis of oral lesions seem to guard the prognosis of oral cancer. When the overall five-year rate of survival was analysed over the last five decades, the results were approximately around 50%. 4

Many lesions could be innocuous and asymptomatic, located in areas difficult to examine, inadequate awareness by the clinician to diagnose precancer and cancerous lesions.

It is at this juncture, that the adequate usage and interpretation of simple screening tests for oral cancer and precancer becomes imperative. This paper aims to review the available literature on oral exfoliative cytology and highlights the proper application of the same for early diagnosis of oral lesions. Lingen et al. have comprehensively reviewed the various diagnostic aids (Table 1) 4.

Exfoliative cytology has been a matter of keen interest among the clinicians of the 18th century. However, extensive work supported by accurate diagnostic evidence was possible only in the mid19th century. Among the various clinicians who worked in this regard, George N. Papanicolaou published a research paper in 1941 with his observations in cervical scrapings and washings among female patients. His work received extensive credit due to the meritorious correlation of uterine cervical cancers with vaginal smears.

In due honour to his work, this procedure has been popularised as PAP test and Papnicolaou referred to as the father of exfoliative cytology. Inspired by his work, Zaskin used a similar procedure for early detection of oral cancers. However, it was Montgomery and Von Hamm in 1951 who proved the usage of exfoliative cytology for the diagnosis of oral cancer. 5

Thus oral exfoliative cytology has been globally practiced for over six decades. It has helped in identifying and preventing about 80% of
mortality and 60% of morbidity. Cytology is the study of cells.

It employs a patient-friendly procedure with applications in the early diagnosis of multiple oral lesions accounting for its popularity. Exfoliative cytology is the science of microscopic examination of shed or desquamated cells from the epithelial surface of the mucous membrane. Examination of cells collected by scraping the tissue surface or collected from body fluids such as sputum, saliva, etc are done. Understanding the principle of exfoliation of oral mucosa is quintessential. The cells of oral mucosa synthesizes keratin and contains a protein called cytokeratin. Thus, these cells are called keratinocytes. Keratinocytes are arranged in specific layers called strata, namely: stratum basale, stratum spinosum, stratum granulosum, stratum corneum.

A single keratinocyte migrates from the bottom most layer to the superficial layer through a series of physiologic migration and maturation. During this process it also features morphologic changes called differentiation. Once the keratinocyte reaches the surface, it is cast off/ shed away. This process is called desquamation. These cells have close apposition and connections with adjacent cells through desmosomes. This is a regular and steady process. In the state of malignancy or pre malignancy, the deeper cells, loose the desmosomal attachments and migrate to the superficial layers.

2. ARMAMENTARIUM (Refer Figure 1)

1. One to two glass slides
2. Glass marking pencil/slide labels
3. Instrument to collect the oral swab
4. Fixative
5. Personnel protection equipment
6. Material for carrying & transportation of the sample

![Figure 1: Armamentarium required for conventional exfoliative cytology sample collection](image)

3. PROCEDURE

1. Before beginning the procedure record the patient’s case history and follow the guidelines for the indications of this procedure (listed below). Explain the procedure to the patient briefly and identify the site of sample collection
2. Record the site of collection in the case history sheet. If multiple samples are required, explain the same in writing and label it as site A, B and so on. Using the glass marking pencil/slide labels, write the patient details on the glass slide. If multiple sites are collected, label A, B….of each site on a different glass slide
3. Wipe any extra saliva or debris using gauze roll
4. Using a swab stick, vigorously scrape in the area of interest
5. Carefully spread the collected swab on the labelled side of the glass slide until a thin white-film is seen.
6. Fix the cells using a suitable fixative
7. Send the sample to the pathology laboratory for microscopic evaluation.
8. Enter all relevant details in the case history sheet.

4. STAINING:

The staining technique employed is a multichromatic staining procedure developed by George Papanicoloau. It is a reliable technique
which conventionally contains five dyes in three solutions. The advantage of this technique lies in its selection of the processing solutions. It clearly demarcates cell and nuclear boundaries and features. Hues of different colours of yellow, orange, pink, green and blue are seen. These colours indicate cells from different layers of the oral mucosa. Though conventional staining procedure is commonly followed, recently developed kits which provide rapid results are gaining popularity. (Refer Figure 2)

Cellular features represents the biological activity of the cell. Frost et al stated that the fundamental biological activity is best reflected in the nucleus and functional activity in the cytoplasm.

Features of dyplasia such as nuclear hyperchromasia, increased nuclear to cytoplasmic ratio, nuclear pleomorphism, irregular nuclear membrane, nuclear clumping and irregular distribution of chromatin, mitosis should be examined.

Class II (Atypical Cytology): Indicates the presence of minor atypia due to inflammation. No evidence of malignancy.

Class III (Intermediate Cytology): Cells display wider atypia suggestive of severe dysplasia, Carcinoma In Situ/cancer. Repeat is advised after treating. If it remains unchanged, biopsy is recommended.

Class IV (Suggestive of Cancer): Shows a few epithelial cells with malignant changes. Few cells show borderline characteristics. Biopsy is mandatory.

Class V (Positive for Cancer): Cells show characteristic features of malignancy. Biopsy is mandatory.

6. CHARACTERISTICS THAT RAISE THE SUSPICION OF MALIGNANCY IN AN EXISTING LESION

1. Erythroplasia: Red or speckled red appearance of the lesion.
2. Leukoplakia: White-grey appearance of the lesion.
3. Ulceration: Ulcers on the surface
4. Duration: Persistence of lesion more than 2 weeks
5. Growth rate: Signs of speedy growth
6. Bleeding: Frequent bleeding on gentle manipulation
7. Indurations: lesion and surrounding tissue is hard
8. Fixation: To adjacent structures/ base

5. INTERPRETATION OF THE REPORT

Papanicoloau Cytologic Classification: The original classification given by Papanicoloau has been modified to classify oral lesions. It is categorized into five, as follows:

Class I (Normal Cytology): Indicates only normal cells are observed.

Class II (Atypical Cytology): Indicates the presence of minor atypia due to inflammation. No evidence of malignancy.

Class III (Intermediate Cytology): Cells display wider atypia suggestive of severe dysplasia, Carcinoma In Situ/cancer. Repeat is advised after treating. If it remains unchanged, biopsy is recommended.

Class IV (Suggestive of Cancer): Shows a few epithelial cells with malignant changes. Few cells show borderline characteristics. Biopsy is mandatory.

Class V (Positive for Cancer): Cells show characteristic features of malignancy. Biopsy is mandatory.
7. INDICATIONS

1. Oral lesions which are clinically occult
2. Patient with systemic complications and biopsy is contraindicated
3. Lesions which require multiple biopsies
4. A lesion appearing red or white or a combination of both
5. Periodic monitoring of patients after treatment
6. To monitor patients after radiotherapy
7. To rule out non-specific inflammatory/reactive lesions
8. To specifically identify oral candidiasis

8. ADVANTAGES

1. Non-invasive, simple, fast, economically feasible, bloodless procedure
2. Multiple sites and multiple swabs can be made without causing discomfort to the patient
3. Most simple chair-side investigation which requires minimum armamentarium
4. Good tool for the early detection of malignancy
5. Complications following the procedure are minimal
6. Aids in the identification of representative biopsy site
7. Easy to use in screening procedure for community-based studies

9. DISADVANTAGES

1. False negatives are common
2. Benefits only superficial lesions of keratinized mucosa. Deeper lesions cannot be assessed
3. Histopathological grading of carcinoma cannot be reported. This mandates a biopsy
4. Highly technique sensitive. Selection of representative site, adequacy of sample, spread of the smear, laboratory processing and interpretation of the cytological features are all rate determining steps
5. Because of the above mentioned drawbacks, it is an adjunct diagnostic aid

10. APPLICATIONS

1. Early diagnosis of oral cancer (like Oral squamous cell carcinoma) and pre cancer (like leukoplakia)
2. Assessment of microbial lesions like Candidiasis, Herpes, Paracoccidioidomycosis
3. Assessment of skin lesions with oral manifestations such as Pemphigus
4. Identification of sex in forensic investigations
5. Study of systemic illnesses such as Diabetes Mellitus & nutritional deficiencies such as anemia
6. To study aging changes

11. RECENT ADVANCES AND APPLICATIONS IN ORAL EXFOLIATIVE CYTOLOGY

Conventional procedure and processing of oral Exfoliative cytology samples has a few setbacks, such as cell folding, lack of background clarity, uneven cell thickness, etc. These precluded the genesis of liquid-based cytology. This technique overcame the limitations of the conventional procedure. It allowed better study of cytology through uniform cellular thickness and distribution besides providing the facility to archive the samples.

The procedure is however technique-sensitive and requires a well-established infrastructure. This requires the patient to provide an oral rinse and expectorant. The sample is then fixed, centrifuged, filtered and then the smear is
prepared and analysed. Specific computer software’s have been developed to improve the efficiency of this technique. 15, 16

Recent development in image analysis of the cells have led to the following methods: 17
a. Cytomorphometry: Quantitative assessment of cell and nucleus to identify shift to malignancy
b. Automated Systems: Developed in countries such as Great Britain and Japan which quantitatively and qualitatively studies the nucleus and cytoplasm with the aid of specific computer soft wares
c. DNA Image cytometry (DIC): Stoichiometric method to assess the ploidy status of DNA using Feulgen stain. Specifically focuses on nuclear changes
d. Molecular Analysis: Keratin markers,18 Polymerase Chain reaction, In-situ hybridisation, Nano diagnostics have been used in combination with conventional methods

Several notable studies have been conducted employing the above mentioned techniques. Ogden et al studied epithelial keratin markers in malignant and non-malignant oral mucosae. They concluded that this was an efficient method for the early detection of oral cancer.18 Biocompatibility of recent dental restorative materials can be assessed by the simple procedure of exfoliation. A recent cohort-study conclude that composite restorative material was superior in biocompatibility. 19 Shaila M et al analysed oral expectorate using Cytomorphometric method and concluded that it was a relatively easier method to screen oral cancer. 20 Hegde et al conducted a research study to compare the efficacy of Centrifuged liquid-based cytology over conventional Exfoliative cytology in the oral cavity. Nuclear and cytologic features were distinctly identified due to clarity in the background. This method also showed lesser false-negatives. 21 Qualitative and quantitative analysis of nuclear and cytologic features were noticed in a case-control study among Type II Diabetes Mellitus patients. The oral smear was studied under confocal laser scanning microscope. The researchers concluded that the early changes can forewarn the clinician about the impending plasma glucose levels of the patient. 22 The average size of cells in a buccal smear using conventional Exfoliative cytology was analysis radiovisiography for age estimation in a group of population. The results demonstrated the accuracy of age with cytology as potential tool for forensic investigations. 23

12. CONCLUSION

The general dental practitioner has a vital role in the early diagnosis of oral precancer and cancer. Theoretical knowledge and practical application of skills is the need of the hour. As it is better to be forewarned than to be forearmed.

REFERENCES

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