Usefulness of Oral Exfoliative Cytology in Dental Practice

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ABSTRACT
Early detection of oral cancers is not easy, because oral precancerous lesions and early oral cancers can mimic many benign conditions in the mouth, leading to delays in diagnosis and treatment. There is a need to emphasize the early diagnosis of oral cancers in order to reduce the unacceptably high morbidity and mortality. Oral exfoliative cytology can be a powerful tool for early detection of malignant and premalignant lesions as well as for some viral and fungal infections. The use of oral exfoliative cytology in clinical practice declined due to the subjective nature of its interpretation and because there may be only a small number of abnormal cells identifiable in a smear. The more recent application of quantitative techniques, together with advances in immunocytochemistry, has refined the potential role of cytology, stimulating a reappraisal of its value in the diagnosis of oral cancer. The limitations of the method should be thoroughly understood and appreciated by the dental clinician. The aim of the publication is to encourage maximum participation of dental professionals in early detection and control of oral cancer by means of early diagnosis through use of cytological smear.

Keywords: Exfoliative cytology, Oral cancer, Precancerous lesions, PAP stain.

INTRODUCTION
Early detection of a premalignant or cancerous oral lesion promises to improve the survival and the morbidity of patients suffering from these conditions. Cytological study of oral cells is a non-aggressive technique that is well accepted by the patient, and is therefore an attractive option for the early diagnosis of oral cancer, including epithelial atypia and squamous cell carcinoma. However its usage has been limited so far due to poor sensitivity and specificity in diagnosing oral malignancies. In 1967, editorial in the journal of ADA explains that oral exfoliative cytology must be a part of every oral examination in which dentists detect even the least suspicious lesion. 9.2% of dentists in practice have ever done an oral exfoliative cytology smear because of lack of knowledge & lack of infrastructure. Program to teach the technic of oral cytology to large number of dentists have already been initiated in New York, Chicago and several other Cities in the Western countries. Oral exfoliative as a diagnostic tool has been around for more than 40 years. 60% of oral cancers are well advanced by the time of diagnosis and 80% of deaths could be prevented by earlier recognition (1).

Exfoliative cytology is the 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. Continuous exfoliation of epithelial cells is a part of physiological turnover. Deeper cells which are strongly adhered in normal conditions become loose in the case of malignancy and tend to exfoliate or shed along with superficial cells (2).

A NOTE FROM HISTORY
Exfoliative cytology is not a new science. It has a long history dating back to the nineteenth century. In the 1920s, aspiration and exfoliative cytology were introduced. Johannes Müller (1801-1858), a pathologist in Berlin was the first, in 1838, to show
cancer cells as they appeared in the microscope on scrapings from the cut surface of surgically excised tumors. In 1843, cancer cells were shown by Walsh on scrapings from a uterine cervical cancer and cytologic preparations were made from a fistulous parotid tumor believed to be malignant. A year later, colored prints of tumor cells of breast carcinoma, sarcoma of the mandible, and soft tissue sarcomas of the leg were published. Lebert (1813-1878), a French pathologist, collected specimens for cytologic examination from effusions, tracheobronchial secretion, and urine. Paget (1814-1879) prepared smears from needle aspirate of a breast carcinoma in 1853. Malignant cells were diagnosed in urine in 1856 and in 1869 cancer cells were recovered from material released from urethra. The 1920s were momentous years in diagnostic cytology. The first monograph of clinical cytology was published in Spain. James Ewing (1866-1943) introduced aspiration cytology in New York City and Aurel Babes (1886-1961), of Rumania, and George Papanicolaou (1883-1962), of New York City, published papers on detection of uterine cervical cancers by examination of vaginal smears. In 1941, George Papanicolaou started using what is called today as PAP test as a routine procedure for early detection. Zaskin was the first person to have reported the use of exfoliative cytology in oral cavity. Montgomery and Von Himm in 1951 used exfoliative cytology for the diagnosis of oral cancer (3-5).

The reliability of the different instruments used in oral exfoliative cytology has been reviewed in different studies (6). Scrapings of oral mucosa can be obtained by using cytobrush, wooden spatula, cotton tip applicators, vigorous saline rinse, forceful aspiration of cells from the surface, aspiration of resting saliva from the floor. Pros and cons of each method was discussed by Henry Sandler (7).

Brush biopsy (Oral CDX) is a simple, relatively inexpensive, high sensitive, risk-free method of screening for cancer and serves as an aid to the clinical examination. The improved accuracy is attributed to the ease in obtaining full transepithelial cellular samples and the evaluation of smears with an image analysis system that has been adapted specifically to detect oral epithelial abnormalities by some workers. Full-thickness is essential if histomorphological, evaluation of the collected cells is to yield representative findings. For example, many dysplastic lesions are first identified in the basal epithelial layers, and the diagnostic histomorphological findings may be lost as the cells mature and parakeratin and keratin are produced. To the classical applications of the oral cytologic studies, such as oral candidiasis, others have been added, such as studying the epithelial infection due to Epstein-Barr virus in oral lesions of hairy leukoplakia, widening its possibilities. The importance of brush biopsy has been recently emphasized in a multicenter study where nearly 5% of clinically benign-appearing mucosal lesions were sampled by this technique and later confirmed by typical scalpel biopsy to represent dysplastic epithelial changes or invasive cancer (8,9).

Despite the improvements in the methods used for collecting oral cytological material this methodology still presents problems in diagnosing oral cancer. Problems are mainly due to the existence of false negatives obtained as a result of a non representative sample as well as the subjectivity of the cytologic evaluation.

**TECHNIQUE**

The supplies needed for oral cytology are 1-2 glass slide, Swab stick, icecream stick or cytobrush and Spray cyte or alcohol as a fixative (Figure 1). Prior to doing the smear, explain to the patient the purpose of technique. Write the patient's name, date and anatomic location of smear on one side of glass slide with sticker or diamond marker. With a gauze remove any excess saliva in the area that will be smeared. Vigorously scrape and rotate the cytobrush or swab stick (Figure 2). Spread the cell onto the glass slide- white film like layer on the glass slide should be seen (Figure 3). Spray the surface of the glass slide with spray cyte which acts as a fixative. Alcohol (95%) can also be used as a fixative. Send the fixed smear to the pathologist’s laboratory for interpretation. PAP is the stain of choice (Figure 4). The smear is immediately fixed with a cytological spray fixative or in an alcohol-ether dip. Fixation or preservation is one of the most important steps in the procedure. Drying of the cells prior to fixa-
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IMPORTANCE OF PAP

Papanicolaou stain (also Papanicolaou’s stain and Pap stain) is a multichromatic staining cytological technique developed by George Papanikolaou, the father of cytopathology. Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells. Pap staining is a very reliable technique. The entire procedure is known as Pap smear. The classic form of Pap stain involves five dyes in three solutions.

- A nuclear stain, haematoxylin, is used to stain cell nuclei. The unmordanted haematoxylin 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. It stains 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, eg. 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.
- 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. 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 (10).

Nuclear hyperchromasia, increased nuclear to cytoplasmic ratio, anisonucleosis and nuclear pleomorphism, irregularities of nuclear membrane, nuclear crowding, nuclear moulding, clumping and irregular distribution of chromatin are the changes that is seen during malignancy. Nuclear membrane has pointed spicules, razor sharp angles and sharp angle infoldings. Nucleoli in cancer are usually enlarged and numerous in number but also show pointed spicules and sharp angles. Mitosis is also abnormal in cancer as in the case of nucleoli. Radiation and chemoradiation changes mainly include micro nucleation which must be considered in post radiation settings (2).

INDICATIONS

- Mucosal lesions that appear clinically innocuous and otherwise would not be biopsied.
- 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

- Provides identification of few specific tissue changes other than cancer.
- Does not give any notion of extent of invasion.
- Cannot identify degree of differentiation of malignancy.
- Reliability of technique is a limitation.

ADVANTAGES

- Post biopsy complications can be eliminated.
- Useful for mass screening.
- Has potential for early detection of malignant lesions.
- Useful for repeated follow up examination to indicate appropriate site for biopsy of diffuse lesion.

DISADVANTAGES

- Relatively less information than histological slides.
- 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.

APPLICATIONS

- Early detection and control of oral cancer.
- Microbial diseases and dermatological lesion.
- Assessment of nutritional iron deficiency.
- Forensic dentistry.
- Assessment of potential oral candidiasis and viral infections.
- Study of conditions like Diabetes Mellitus, smoking, alcoholism, pregnancy, ageing.
- To determine sex chromatin accuracy.
- Marked cyclic fluctuations in keratinisation during normal menstrual cycle.
- Alterations of nuclear size from anaemic patients have been reported.
- Similar changes are reported in patient with tropical sprue.
- Used effectively as research tool for evaluation of experimentally induced CA in hamster cheek pouches and in healing of gingivectomy wound.
- Predicting the cellular response of a tumour to irradiation.
- Cytological finding in pemphigus vulgaris.
- Evaluation of some hereditary disease, for toxic reaction subsequent to cancer.

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chemotherapy and for identification of oral microorganisms

- One study reported the finding of giant cell compatible with tuberculosis in an oral cytology specimen, which led to confirmation of that diagnosis by biopsy (10-12).

**RECENT ADVANCES IN APPLICATIONS**

Exfoliative cytology is useful as an additional tool to aid in the diagnosis of diabetes mellitus. The morphologic changes in the oral epithelial cells of diabetic subjects in a study by Albeit et al. showed nuclear enlargement, binucleation, karyorrhexis and polymorphonuclear leukocyte infiltration. In this study, cytomorphometric findings revealed an increase in NA of diabetic subjects. Meanwhile, there was no significant difference in CA between two groups. C/N ratio was found to be lower in diabetic group (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 peri-implantitis. The concentration of titanium was higher in the peri-implantitis group compared to the group without peri-implantitis (14).

A study was done to assess keratin profiles from smears of malignant and contralateral normal oral mucosa as part of the development of a screening procedure for oral cancer based on exfoliative cytology. Smears were taken from oral cancers (confirmed by biopsy) and from the contralateral site of 20 patients. Using a panel of antikeratin antibodies, the keratins expressed by these cells were identified using a standard immunocytochemical technique (Vectastain) and assessed on a 3 point scale. Individual keratins can be identified in smears from oral cancers. The identification of simple epithelial keratins seems to be the best keratin markers associated with malignancy. Their detection within smears from oral lesions could be valuable in the early diagnosis of oral cancer (15).

In another study to evaluate the usefulness of exfoliative cytology as a diagnostic tool for patients with clinical symptoms of desquamative gingivitis was carried out. The cytologic findings showed diffuse or collective Tzanck cells. Thus by using the cytologic technique may occasionally be of some value as a minimally invasive screening tool (16).

A study revealed that oral mucosa of burning mouth syndrome patients exhibited significant cytomorphometric changes in the oral epithelial cells. These changes probably are associated with epithelial atrophy and a deregulated maturation process that may contribute to the oral symptoms of pain and discomfort in BMS. While the classic oral cytologic evaluation is labour intensive and requires a high degree of expertise for identifying and evaluating cells with suspicious morphology the analysis of molecular alterations is objective and tries to identify specific genetic anomalies. The possibility of extracting RNA from cells obtained by scraping has recently been demonstrated emphasizing its usefulness in the early diagnosis of oral premalignant and cancerous lesions (17).

Nowadays malignancy is considered as a process caused by the accumulation of multiple genetic alterations, which affect the cell cycle as well as normal cell differentiation. Several authors have studied and in some cases demonstrated the potential clinical application of oral cytology for detecting point mutations in p53 as a specific neoplastic marker in OSCC.

Nunes et al. performed a microsatellite analysis of cells sampled from the oral cavity of oral and oropharyngeal cancer patients by exfoliative cytology and by mouthwash, finding LOH in 84% of samples, though with differences depending on tumour stage (18). These authors suggested that techniques of this type might be useful for early diagnosis and for patient monitoring. In another study, Spafford et al. identified genetic alterations (LOH or MI) in all of the malignant lesions of the oral cavity included in their sample (19).

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 (20). The validity of oral cytology for analyzing the number of keratinised cells and the nucleolar activity (AgNORs) in smoking patients has recently been demonstrated (21).

**CONCLUSION**

Diagnostic aids in the evaluation of oral mucosal lesions can serve an important role by identifying lesions that need to be biopsied in spite of a “benign” appearance. Exfoliative cytology, as well as vital staining, may aid in this goal. This has implications regarding undergraduate and postdoctoral education.

Oral cancer can be best detected by the dentists than by any other health profession. Forward-looking practitioners and dentists should gain more information regarding its detection. Dentists, who persist in attitudes comprising only the “hard tissue” outlook lack new information about “soft tissue” areas.

Practitioners must have a value and attitude intervening between research and adoption of technique.

Lack of success is contributed to lack of knowledge, no wonder that this economical and feasible branch is degenerated to such extent. The rationale for oral cytology is developed and documented, and interpretations of the results are explained.
Advantages and disadvantages of this technique are given and indications and contraindications for this diagnostic adjunct are discussed. A simple method involving the use of inexpensive equipment in the office is suggested.

REFERENCES