CONTINUING MEDICAL EDUCATION

Section Editor: Daniel F.I. Kurtycz, M.D.

The Role of Cytology in Oral Lesions:
A Review of Recent Improvements

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Historically, sensitivity and specificity of oral cytology is poor. Using conventional oral cytology for the diagnosis of cancer and its precursors has not had the success that cytologists had hoped for; however, with improved methodology, oral cytology has enjoyed a resurgence of interest. This renewed interest is partly due to the introduction of a specialized brush that collects a full-thickness epithelial sample and not just superficially sloughed cells, as well as analysis of that sample with computer assistance; in addition, a variety of adjunctive techniques have been introduced to potentially enhance the diagnosis of the cytologic specimens including DNA analysis, immunocytochemistry, molecular analysis, and liquid-based preparations. An increase in sensitivity (>96%) and specificity (>90%) of the oral brush biopsy with computer-assisted diagnosis has been reported for identification of malignant and premalignant lesions. Brush cytology is valuable to prevent misdiagnosing doubtful oral lesions, i.e., those lesions without a definitive etiology, diagnosing large lesions where excision of the entire tissue is not possible or practicable, evaluating patients with recurrent malignancies, and monitoring premalignant lesions. Diagn. Cytopathol. 2012;40:73–83.

Key Words: oral cytology; brush biopsy; DNA ploidy; precursor lesions; cancer

Oral squamous cell carcinoma (OSCC) is associated with high morbidity and mortality, which is due, at least in part, to late detection.[1] Early diagnosis and prevention of OSCC are the best interventions for improving survival and quality of life. Despite the marked advances in therapy of other malignancies, the life expectancy of patients with oral malignancies has not improved for the last 50 years. OSCC is the sixth most common malignancy in the world. It is estimated that 36,540 (25,420 men and 11,120 women) new cases of oral cavity and pharyngeal malignancies will be diagnosed in the United States during 2010, whereas 7,880 (5,430 men and 2,450 women) patients will die of the disease.[2]

It is well known that the majority of OSCC, if not all, develop in precancerous fields characterized by specific genetic alterations. Transepithelial "field mapping biopsies" within widespread lesions are even more essential for cytological evaluation and further investigation.[3] Precancerous and cancerous oral lesions may mimic any number of benign oral lesions appearing as a white or red lesion (leukoplakia, erythroplakia, and erythro/leukoplakia).[4] The malignant potential of these lesions is generally assessed by histopathology based on the presence and the degree of dysplasia in biopsy material, graded as mild, moderate, and severe.[5]

Until now, tissue harvesting by scalpel biopsy and subsequent histological examination have been the gold standard for diagnosing premalignant and malignant oral diseases. Oral biopsy is invasive and involves both psychological implications for the patient and technical difficulties for the health practitioner. When lesions are extensive, the most representative areas must be selected to avoid diagnostic errors. A high inter- and intra-observer variability of histological diagnoses for dysplasia is well documented and has been described by several authors.[6,7] As highlighted in a study of 200 patients with oral leukoplakia, when two scalpel biopsies are performed at different times by different examiners, the agreement rate between them was only 56%.[8] The morphology of low-grade dysplasia is significantly variable, and reproducible diagnosis is difficult. For high-grade dysplastic lesions,

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Received 28 July 2010; Accepted 29 September 2010

DOI 10.1002/dc.21581

Published online 25 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

there are fewer diagnostic problems. It is evident that incisional biopsies of suspicious lesions, which have a limited reproducibility within the whole lesion, may result in a more or less aggressive surgical and/or radio-chemotherapeutic approach.[9]

Identifying additional diagnostic tools would be welcome to improve analysis of any suspicious lesion. The oral cytology technique is simple, nonaggressive, relatively painless, and tolerated well by patients.[10] It can be used for diagnosis and identification of recurrent potentially malignant and malignant lesions.[11]

The basic requirements for a useful diagnostic technique include the following: easy to use, causes minimal patient discomfort, and collects sufficient cells.[12] Ideally, a diagnostic procedure should be neither time-consuming nor complicated and, in addition to high sensitivity, should have the potential for automation. High specificity also avoids false-positives and, therefore, reduces patient anxiety, additional investigations, and even unnecessary treatment. Cytology optimally meets all of these requirements, particularly when it is supplemented by an adequate image-analysis method.[13]

Although conventional cytology was used for evaluating oral lesions as far back as 1963,[14] it has not been widely adopted and has fallen into disrepute in most centers because of poor sensitivity and specificity for identifying dysplasia and malignancy. During the 1980s, a cytobrush was introduced for cervical smears in gynecological lesions. This technique improved the process of spreading cells on to slides, compared with smears obtained by using a wooden spatula, thereby improving the quality of the smears.[15] Additionally, sampling of deeper mucosal layers with minimal invasion was possible, especially, cells from the basal and parabasal layers, where most cervical intraepithelial lesions or squamous intra-epithelial lesions usually develop.[16] The adaptation of the cytobrush for oral cancer diagnoses helped revive major interest in oral cytology. Since then, various studies have been published describing different diagnostic techniques that have improved the sensitivity and specificity of conventional oral cytology.[17–20]

This article reviews the developments in oral cytological diagnosis over the years and critically examines the newer adjunctive techniques that have been used to improve these results.

Conventional Cytology

The morphology of malignant cells in the sputum from an oropharyngeal carcinoma was first described in the second half of the 19th Century.[21] Cytological diagnosis of malignancies was suggested by Papanicolaou and Traut who introduced new methods for collecting and staining cells in gynecological diagnosis.[22,23] Initially, use of oral cytology was limited to comparative studies of oral and cervical cytology.[24] Montgomery and Haam von[25] used these staining methods to diagnose nasopharyngeal carcinomas and tried to work around the limitations of oral cytology to improve the quality of smears. Further studies explored the application of oral cytology in animal experiments.[26–28] The goal was to collect an adequate number of cells, to sample a large cellular area and to improve the cellular staining. Therefore, new sampling tools and staining techniques were applied (Table I).[29–41] Fluorescent DNA-specific dyes, such as acridine orange, have been used to measure the cellular DNA content.[42,43] Image analysis of nucleolar size and diameter as parameters for malignancy was also studied.[44,45]

Table I. Methodical Modifications in Oral Cytology Collection Devices and Techniques

Year of publication	*Author*	*Methodical modifications*
1951	Gladstone[29]	Improved quantities of obtained cells by use of a "sponge biopsy"
1952	Schneider[30]	Modifications of staining
1960	Cawson[31]	Modifications of staining
1963	King[32]	Use of frosted glass slides
1963	Staats and Goldsby[33]	Comparison of wooden and metal spatula. Recommendation of the metal spatula
1964	Sandler[34]	Removal of keratotic layers with a sharp curette
1981	Dumbach et al.[35]	Smear curettage. Inclusion of deeper cell layers by use of a curette
1999	Sciubba[36]	Oral CDx brush
2001	Remmerbach et al.[37]	Combined conventional with DNA-image-cytometry
2003	Remmerbach et al.[38]	Conventional combined with AgNOR-analysis
2007	Gupta et al.[39]	Toluidine blue with conventional brush
2007	Driemel et al.[40]	Conventional brush combined with hm Tn-C immunocytochemistry
2008	Mehrotra et al.[41]	Conventional brush without computer assistance

The factors that contribute to a false-negative diagnosis in exfoliative cytology include the selection of the site of biopsy, necrosis, crusting of blood, lack of adequate training, and the fact that malignant features of squamous cell carcinoma can subtly resemble dysplasia.[46,47] To reach the cells of the basal and parabasal layers, the atypical keratotic cell layers have to be removed. Even though the sensitivity of cytology was somewhat increased by the modification of new collecting devices, these kind of devices were more invasive, and the essential benefits of cytology vis-à-vis surgical biopsy were lost.

Unlike the cervical Papanicolaou smear, which has become an established adjunct in clinical practice, markedly reducing mortality from cervical cancer, conventional exfoliative cytology in the oral cavity has proven to be of little value because of high false rates, which could

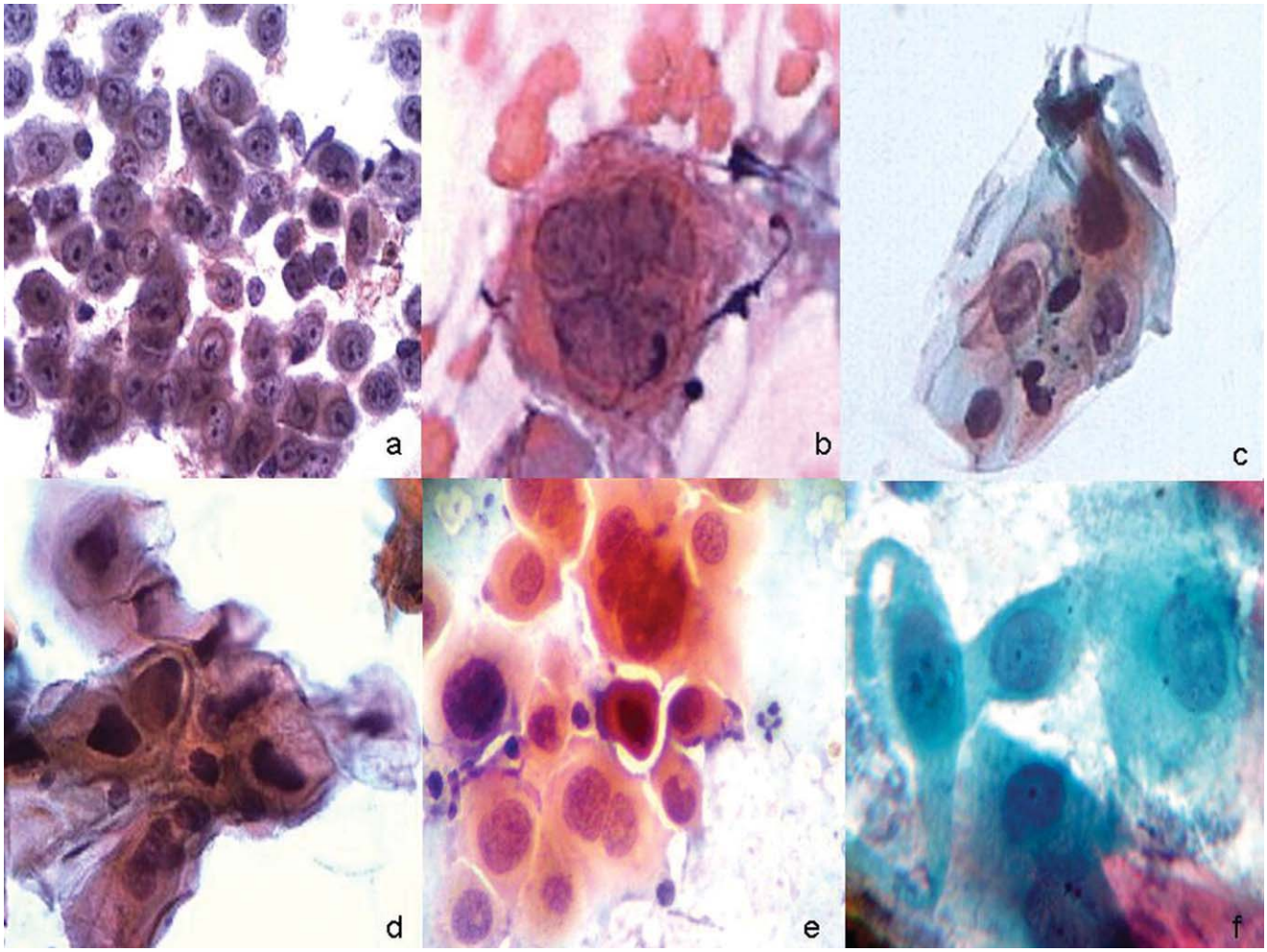

Fig. C-1. a: Pemphigus (Papanicolaou, ×100). **b:** Giant cell in a patient with herpes simplex virus infection (Papanicolaou, ×100). **c:** Postradiation nucleomegaly (Papanicolaou, ×100). **d:** Atypical squamous cells consistent with dysplasia (Papanicolaou, ×100). **e:** Pleomorphism and hyperchromasia in an oral malignancy (Papanicolaou, ×100). **f:** Tadpole cell in oral malignancy (Papanicolaou, ×1,000).

exceed 30%.[48] Oral topography and the size of the oral cavity make it impossible to examine the complete mucosal surface with these devices. Therefore, only visible lesions could be cytologically examined. Furthermore, a clear-cut transformation zone at the squamocolumnar junction does not exist in the oral cavity.[49]

Unlike the cervical epithelium, the oral mucosa is more fibrotic, preventing exfoliation of dysplastic cells to the surface of the epithelium. Therefore, dysplastic and malignant cells could only be obtained by conventional smears if the lesion was a carcinoma that was far advanced or ulcerated.[34] Without loss of minimal invasiveness, it was not possible to access the deeper cell layers of the oral cavity with conventional exfoliative cytology.[50]

Despite these severe limitations, conventional oral cytology was found to be useful for monitoring large lesions and could guide the choice of sites for incisional biopsies.[51] By improving both the instruments that collect cytologic samples and the analysis of those samples, cytology has the potential to fill the "screening gap," which currently challenges the early detection of any epithelial cancer, including oral cancer; cytology can be an effective and noninvasive means of detecting dysplasia and early carcinoma in those patients with benign-appearing clinical lesions who are either asymptomatic or have minor symptoms and, therefore, would not be subjected to scalpel biopsy.

Modern Cytological Techniques

The brush proved to be a more convenient instrument to the examiner compared with the wooden spatula when dealing with oral lesions.[12,49] Furthermore, the brush has been used to diagnose other oral diseases like oral candidiasis, epithelial infection due to Epstein-Barr virus in oral lesions of hairy leukoplakia, pemphigus (Fig. C-1a), Herpes simplex virus (HSV) (Fig. C-1b), and radiation response (Fig. C-1c).[52–54]

The importance of brush biopsy in evaluating benign-looking oral lesions that would have been "watched" and not tested has been emphasized in a multicentre study where nearly 5% of such lesions which were sampled by using brush biopsy, analyzed by image analysis, and later confirmed by using scalpel biopsy to represent dysplastic epithelial changes (Fig. C-1d) or invasive cancer (Figs. C-1e and f).[36] Earlier studies from the author's group have also demonstrated the ability of brush cytology without the use of automated screening, albeit with reduced sensitivity, to uncover precancers and cancers among lesions that were not clinically suspicious, avoiding a delay in diagnosis.[41]

If a malignancy covers a large area, it is important to carefully select the most appropriate site of the scalpel biopsy. A report emphasized the value of brush biopsy in the follow-up of large oral lesions covering a wide area.[11] Combined use of toluidine blue staining and brush cytology has also been attempted to help localize the right site for brushing a lesion. This combination was found to be highly sensitive and moderately specific for malignant lesions (false-negative rate of 6%) but less sensitive for the premalignant lesions with a sensitivity of 89% and a specificity of 92%.[39]

Remmerbach et al.[38] propounded both the usefulness of cytology for diagnosing suspect lesions and the routine application of the AgNOR (nucleolar organizer regions) analysis to determine the nucleolar activity of oral cancer.

Liquid-Based Cytology

Liquid-based cytology (LBC) is a specimen filtration method that was originally developed to provide a near-monolayer of superficial cervical cells that could be more easily inspected by a cytotechnologist or a simple computer examining Papanicolaou smears. Application of LBC on oral smears collected by using a cytobrush has been claimed to show significant improvement in cell distribution and smear thickness, leading to easier identification of abnormal cells.[55] The thin-layer preparation permits less time consuming manual analysis, akin to cervical liquid-based preparations. Reports of combined use of an invasive dermatological curette and the LBC on oral carcinomas showed a sensitivity of 95.1% and a specificity of 99.0%.[56]

Another study showed that the liquid-based preparations resulted in higher specimen resolution and better cytological morphology for pemphigus vulgaris, squamous cell carcinomas, HSV lesions, and fungal infections. For HSV lesions, in particular, the observation of the cytopathological features indicative of viral infections (binucleated and multinucleated cells) has been claimed to greatly improve with the liquid-based technique.[51]

Kujan et al.[57] showed that immunocytochemical staining of oral cells based on LBC slides is also feasible. All the stained slides in their study were consistent and presented high-quality immunoreactivity for fragile histidine triad gene (*FHIT*). The human *FHIT* gene is a tumor suppressor gene and was identified at 3p14.2. Likewise, human papillomavirus (HPV) detection in oral LBC samples was also found to be reliable.

The low sensitivity of conventional oral cytology for dysplasia is not due to how the specimen is prepared but rather because, unlike the uterine cervix, there is a relative blockade by keratin on oral suspicious lesions, which substantially prevent abnormal cells from being present on the epithelial surface. More specifically, leukoplakia represents the most common oral precancerous lesion and clinically has a distinct white appearance because of a thickening keratin layer. This keratin layer, often quite thick, prevents abnormal cells from reaching the superficial layer rendering conventional oral cytology ineffective for identifying dysplasia and evaluating suspicious white oral lesions. Therefore, a conventional oral cytology specimen tests only the superficial layer and not the entire thickness of the epithelium, which is required to rule out the presence of oral dysplasia or carcinoma, and the specimen will be deficient no matter how it is prepared, conventional or LBC. Furthermore, LBC was designed for superficial cytology samples and not biopsies and leads to the destruction of epithelial fragments and the three-dimensional presentation collected by a brush biopsy (microbiopsies), which may prevent proper microscopic evaluation of the biopsy.[50] With these limitations, it is likely that LBC for diagnosing oral lesions is of minimal value.

Automated Analytical Methods

Cytomorphometry. OralCDx (OraCDx Laboratories, Suffern, NY) is a computer-assisted method for the analysis of cellular samples collected by using a patented brush (Fig. C-2a). This technique was designed to evaluate any oral epithelial abnormality without an obvious etiology for dysplasia or cancer.[58] The improvement over conventional cytology is due to the brush, which collects a complete transepithelial sample, including the basal layer, and the analysis of that sample with computer assistance (Figs. C-2b and c). Divani et al.,[46] in a recent study, reported that a full-thickness sampling is essential. "It is well known that atypical cells of the squamous epithelium are first recognized in the basal cell layer. This can be the only layer that contains abnormal cells, so the correct diagnosis finally may be lost." The oral brush technique overcomes this difficulty because it harvests cells from all layers of the epithelium without any topical or local anesthetic.

The computer analyzes the scanned digital microscopic image of the collected cells using a specialized neural network-based image processing system specifically

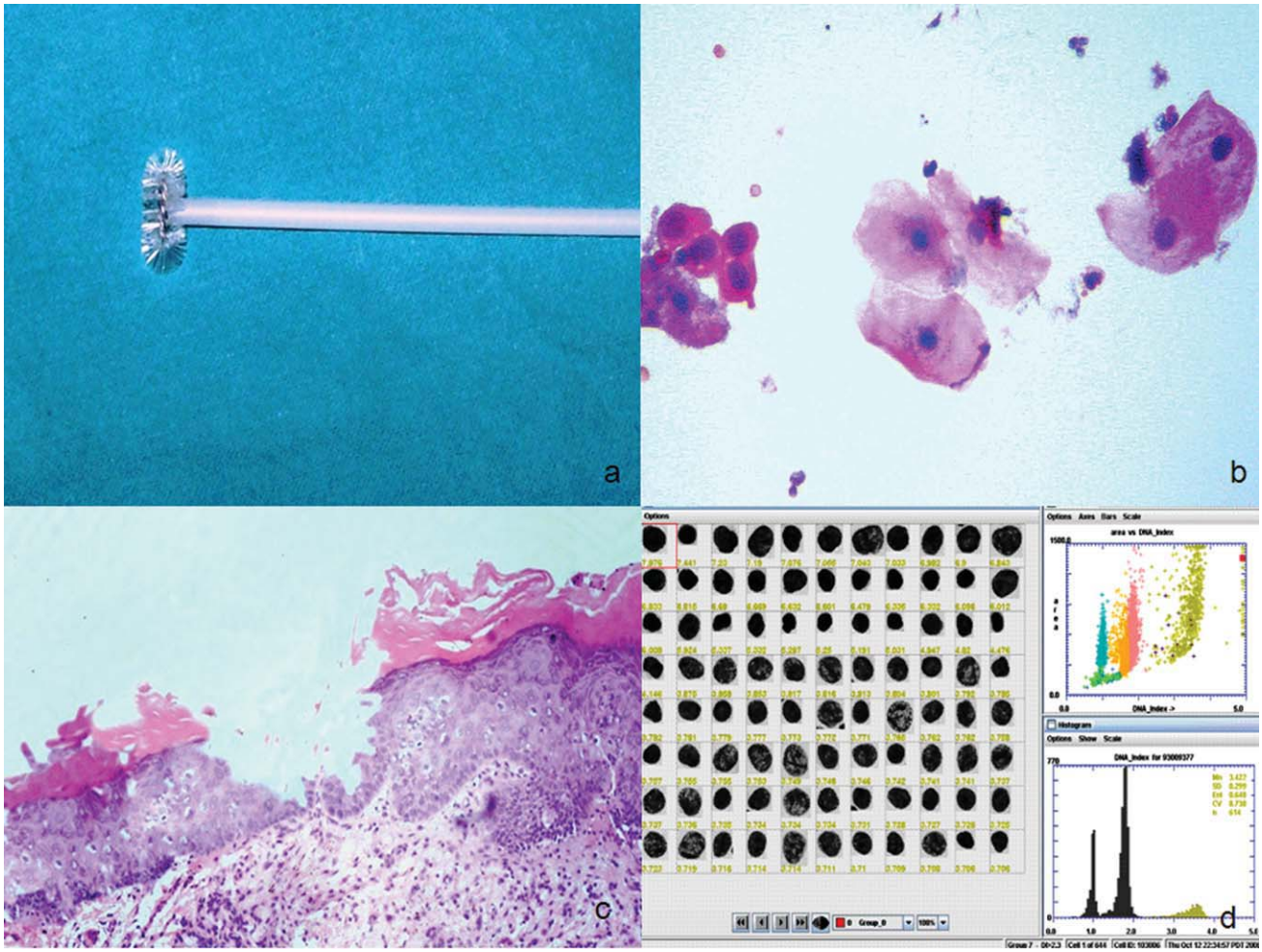

Fig. C-2. a: Special brush for oral brush biopsy. **b:** Cytology specimen showing sampling of all the three layers: superficial, intermediate, and basal (H&E, ×400). **c:** Scalpel specimen showing prior brush indentation demonstrating full-thickness sampling (H&E, ×40). **d:** DNA histogram.

designed to detect oral precancerous and cancerous cells. Criteria of atypia include cellular keratinization and morphological deviations. Speight reviewed the various criteria for grading dysplasia as well as the rate of progression of different grades of dysplasia to malignancy.[59] The analytical results and representative examples are presented to a cytopathologist who makes the final diagnosis and suggests follow-up to the clinical practitioner such as clinical close observation, repeat brush biopsy, surgical biopsy, etc.

In every study in which a lesion was simultaneously tested with both a brush biopsy and scalpel biopsy, OralCDx has been shown to have a sensitivity and specificity well over 90%.[36,60] These studies demonstrate that the brush biopsy is at least as sensitive as the scalpel biopsy in ruling out the presence of dysplasia and cancer. Given the fact that for a large oral lesion, the brush samples a greater surface area than an incisional punch biopsy, the brush biopsy may, in fact, have greater sensitivity in identifying dysplasia and cancer in such cases. The use of the oral brush biopsy as a highly accurate method of detecting precancers and cancers is incorporated into the United States National Cancer Institute's Physician Data Query, better known as PDQ. The PDQ only references articles where brush biopsies and scalpel biopsies were performed at the same time because these are the only studies that can accurately demonstrate the sensitivity and specificity of OralCDx.[61]

When a brush biopsy is "positive," cytopathologic findings pathognomonic for dysplasia or carcinoma are present in the sample. When the brush biopsy is "negative" (>90% of all cases), the test is as definitive as a negative scalpel biopsy. Positive predictive values of an "atypical" OralCDx was reported as 42% by Kosicki et al.,[62] 35% by Scheifele et al.,[60] 44% by Poate et al.,[63] 38% by Svirsky et al.,[64] and 30% by Sciubba et al.[36] These high

values are significantly greater than other well-accepted, life-saving tests, including the Papanicolaou smear (7–20%) and mammogram (2.6–16%).

HPV and Oral Cytology

The role of HPV infection in the etiopathogenesis of oral potentially malignant and malignant lesions has been extensively studied in recent years. Several epidemiologic studies, including studies form the author's group, have reported on the involvement of HPV in the initiation and progression of oral neoplasia.[65,66] HPV (specifically HPV16) is now recognized to play a role in the pathogenesis of a subset of oral cavity carcinomas, particularly those that arise from the lingual and palatine tonsils within the oropharynx as well as the base of the tongue.[67] The author has recently reported on comparison of the polymerase chain reaction and Hybrid capture techniques in oral lesions.[68] The detection of HPV in the oral mucosa may also be accomplished by cytology, and the cytologic findings are characterized by koilocytosis, perinuclear cytoplasmic haloes, nuclear dysplasia, atypical immature metaplasia, and binucleation. Adjunctive techniques of identifying HPV that have been used includes in situ hybridization and immunohistochemistry.[69]

DNA Analysis

The ploidy status of oral cells is important to predict the lesions that may undergo malignant change. After staining with Feulgen dye, the cytological samples are compared with a reference group of 300 normal epithelial cells of the oral mucosa. Remmerbach et al.[37] reported on 251 cytological diagnoses obtained from exfoliative smears of 181 patients from macroscopically suspicious lesions of the oral mucosa and from clinically seemingly benign oral lesions, which were excised for establishing histological diagnoses. These were compared with histological and/or clinical follow-ups of the respective patients. Additionally, nuclear DNA-contents were measured after Feulgen restaining using a TV image analysis system. The sensitivity of the cytological diagnosis when used in addition to DNA image cytometry on oral smears was found to be 98%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 99%. Schimming et al.[70] evaluated the correlation between DNA distribution and the clinicopathological characteristics of OSCC and found a higher metastasis rate, lower patient survival rate, and noted that nondiploid tumors presented in significantly more advanced stages. DNA image cytometry measures DNA ploidy to determine the malignant potential of cells. A computer-assisted analysis has been developed to identify deviations of cellular DNA content (a typical DNA histogram in dysplasia is shown in Fig. C2d) (Oral Advance, Vancouver, BC). Using this technique, an increase in sensitivity and specificity of oral cytology to 100% has been reported.[71,72] Data on accuracy in high-risk oral neoplasia are, however, limited. Furthermore, because Oral Advance uses a soft cytobrush, it is unlikely to collect basal cells.

Semi-automated multimodal cell analysis (MMCA) is another novel technique for the early detection of cancer for cases with a limited number of suspicious cells.[13] Application of Feulgen staining, DNA image cytometry, and finally silver-nitrate staining for argyrophilic nucleolar organizing regions (AgNORs) analysis were combined in this analysis to identify early malignant transformation. The MMCA approach is based on the sequential application of multiple staining of identical, slide-based cells and repeated relocalization and measurements of their diagnostic features, resulting in multiparametric features of individual cells. Data integration of the variously stained cells was found to increase diagnostic accuracy. The implementation of MMCA also enables fully automatic, adaptive image preprocessing, including registration of multimodal images and segmentation of cell nuclei. This technique is claimed to improve the specificity of cytologic screening without compromising sensitivity. In this way, false-positive results can be decreased, thereby obviating unnecessary histologic biopsies.

Spectral Cytopathology

Spectral cytopathology (SCP) is a novel approach for diagnostic differentiation of disease in individual exfoliated cells. SCP is carried out by collecting information on each cell's biochemical composition via an infrared microspectral measurement, followed by multivariate data analysis. Deviations from a cell's natural composition produce specific spectral patterns that are exclusive to the cause of the deviation or disease. These unique spectral patterns are reproducible and can be identified and used via multivariate statistical methods to detect cells compromised at the molecular level by dysplasia, neoplasia, or viral infection. In a recent proof-of-concept study, a benchmark for the sensitivity of SCP was established by classifying healthy oral squamous cells according to their anatomical origin in the oral cavity.[73] The biochemical signatures of disease found are reproducible throughout the majority of cells from each sample. These signatures are used in SCP rather than the distribution of stains and cellular or nuclear morphology for diagnosis. SCP is based on automated instrumentation and unsupervised software, and it is claimed to constitute a diagnostic workup of medical samples devoid of bias and inconsistency.

Molecular Analyses

Various molecular markers used to identify dysplasia and the development of malignancies have been recently reviewed by the author.[20] In a recent study, melanoma-

associated antigen A (MAGE-A) was applied to brush biopsy material in a 49-year-old male patient with a persistent-suspicious looking leukoplakia using real-time reverse transcription-polymerase chain reaction. Early invasive carcinoma was identified because of significant MAGE-A3 and A4 expression pattern.[74] MAGE-A3 and A4 encoding genes are usually expressed in various tumor types, whereas they are genetically silent in all normal tissues except testis, placenta, and fetal tissues. Ries et al.[75] had earlier showed MAGE-A3 and A4 encoding genes to be overexpressed in more than 93% in OSCC. These authors contend that the detection of these markers, which are highly specific to cellular malignancy, makes them potential markers for early diagnosis and prognosis, and even a target for immunotherapy.

Hirshberg et al.[76] combined cytogenetic fluorescence in situ hybridization and cytomorphometric analysis and reported increased specificity in predicting the nature of suspicious oral lesions. Driemel et al.[77] have studied the gamma 2-chain of the extracellular matrix protein Laminin-5 (ln-5) in oral brush biopsises. Laminins function as heterotrimeric, noncollagenous glycoproteins composed of combinations of five known alpha, four beta, and three gamma chains, with each chain type representing a different subfamily of proteins. The protein encoded by this gene belongs to the alpha subfamily of laminin chains and is a major component of basement membranes. Laminin-5 is a representative of proteins associated with the process of tumor invasion and metastasis. Laminin-5 is a key protein of the epithelial adhesion complex, which, among other functions, solidly connects the oral mucosa with the underlying stroma.[78] Together with embryonal isoforms of fibronectin and tenascin-C, these are the components of the invasion pathway.[79] With regard to the identification of pre-invasive epithelial cells, Sordat et al.[80] studied the cytoplasmic ln-5 staining in colorectal neoplasms. In preneoplasia of the cervix uteri, ln-5 is an indicator of severe dysplasia and potentially invasive cells.[81,82] High-molecular-weight tenascin-C (extracellular matrix glycoproteins) has also been studied by Driemel et al. for the identification of oral cancer. Tenascin-C isoform has antiadhesive properties. One mechanism to explain this may come from its ability to bind to the extracellular matrix glycoprotein fibronectin and block fibronectin's interactions with specific syndecans. The expression of tenascin-C in the stroma of certain tumors is associated with a poor prognosis. Both these proteins are said to have a key function in the cascade of invasion and metastasis of OSCC and are highly over-expressed.[40] The sensitivity of 93–95% of this adjunctive method is said to lower the rate of false-negatives by using oral brush biopsy. These researchers also analyzed oral brush biopsies of normal, inflammatory, hyperproliferative, and malignant lesions with Protein-Chip arrays (SELDI) and identified two specific proteins (S100A8 and S100A9) that might be useful for the monitoring of oral lesions.[83]

Tumor-acquired alterations in DNA methylation include both genome-wide hypomethylation and locus-specific hypermethylation. Promoter hypermethylation often coincides with loss of heterozygosity at the same loci, and, together, these events can result in loss of function of the gene in tumor cells. Carvalho et al.[84] described the analysis of aberrant promoter hypermethylation in exfoliated mucosal cells acquired by brushing of the oral cavity surface followed by vigorous mouth washing. This method is not strictly brush cytology of a lesion, but it could easily be performed on cells obtained on brush cytology. Also, the evaluation of DNA and protein expression in oral mucosal cytological specimen might be used as prognostic factors for squamous cell carcinomas or to evaluate the effects of EGFR tyrosine kinase inhibitors, which could lead to new treatment strategies.[85,86]

Nanochip-Based Systems

In a recent pilot study, Weigum et al.[87] used a single nano-bio-chip platform for molecular and morphologic analysis in oral exfoliative cytology to enhance the role and utility of oral cytology in clinical diagnostics. The integration of the nano-bio-chip sensor system for concurrent and quantitative analysis of cellular biomarkers and cytomorphology has been studied in 41 patients and 11 controls for multifunctional cytoanalysis. They found six parameters to be significantly altered in OSCC cytospecimens versus healthy mucosa, including (a) nuclear area, (b) nuclear diameter, (c) cellular area, (d) cellular diameter, (e) nuclear-to-cytoplasmic ratio, and (f) EGFR biomarker immunolabeling. The nuclear area, nuclear diameter, nuclear-to-cytoplasmic ratio, and EGFR expression were also found to be significantly altered in oral lesions with diagnosed dysplasia, supporting the use of these markers as diagnostic indicators of early cancer development and premalignancy. These findings are in line with earlier reports by Ramaesh and Ratanatunga,[45] identifying significant changes in cellular and nuclear morphology and EGFR expression associated with oral tumorigenesis.

Salivary Diagnostics

Oral epithelial cells routinely shed and can be detected in saliva and oral rinses, making cytologic and molecular analysis of this fluid attractive for oral cancer screening. The percentage of apoptotic cells has been measured in cytological studies of the saliva of patients treated for OSCC and could be useful for monitoring reactions to chemotherapy.[88] Several small studies have shown differences in DNA and RNA expression patterns and microsatellite motifs between groups of oral cancer patients and normal control subjects.[89,90]

Conclusion

Conventional cytology has a historically poor sensitivity that can be improved upon. The introduction of advanced cytologic methods, including brush biopsy, computer-assisted analysis of brush biopsy samples, as well as molecular and immunohistochemical techniques have made cytology an invaluable tool in evaluating oral lesions. Brush biopsy is particularly useful to evaluate any oral lesion without an obvious etiology such as infection or trauma and to prevent misdiagnosis of dysplasia and cancer among lesions primarily diagnosed as "benign" by clinical inspection. Furthermore, brush biopsy is especially useful when a lesion is large, when multiple lesions are present, or when patients refuse scalpel biopsy. This is a relatively inexpensive, simple, noninvasive, risk-free technique, proven to be highly accurate and well accepted by patients. As such, its widespread use may result in identifying precancers and early cancers at stages when they can be most easily treated.

MCQs on the article

1. A major problem with oral cytology samples is?
   a. The normal flora of the oropharynx
   b. Enzymatic activity of salivary secretions
   c. The extensive surface area to be sampled
   d. The wider squamocolumnar transformation zone

Answer: c

2. Oral cytology is useful for detecting (1 or more)
   a. Candida
   b. Pemphigus
   c. Dysplasia
   d. All of the above

Answer: d

3. In this article, DNA estimation of ploidy was done by
   a. Image analysis
   b. Flow cytometry
   c. Gene sequencing
   d. Thin layer chromatography?

Answer: a

4. The ThinPrep® method is basically (choose the best answer)
   a. A density gradient procedure
   b. A centrifugation procedure
   c. A filtration procedure
   d. A sequencing procedure

Answer: c

5. MAGE stands for:
   a. Malignancy associated antigen
   b. Mast cell associated antigen
   c. Melanoma associated antigen
   d. Metastasis associated antigen

Answer: c

6. FHIT is a suppressor gene whose normal product is lost in squamous cancer. The intials stand for:
   a. Fastidious histamine triad
   b. Fast hit triad
   c. Fragile histamine triad
   d. Fragile histidine triad

Answer: d

7. One of the following techniques are not utilized for HPV detection in oral lesions:
   a. In-situ hybridization
   b. Immunohistochemistry
   c. Polymerase chain reaction
   d. Enzyme linked immunoassay

Answer: d

8. HPV is considered an etiologic factor most closely linked with malignancy of the:
   a. Palatine tonsils
   b. Lower lip
   c. Buccal mucosa
   d. Gingiva

Answer: a

9. Oral bush biopsy is indicated for evaluating:
   a. Hemangioma
   b. Erythroplakia
   c. Lipoma
   d. Nevus

Answer: b

10. The positive predictive value for oral brush biopsy diagnosed as "atypical" ranges from:
    a. 5–20%
    b. 20–30%
    c. 30–45%
    d. 60–75%

Answer: c

Acknowledgments

The author acknowledges help of Dr. Deborah J. Caroll, MD, for providing Figures C-1a–d, Dr. Alexei Doudkin for Figure C-2d, and Dr. Sanjay Mishra and Ms. Shruti Pandya, Research Scholars of the Department of Pathology in proofing the manuscript and for Figures C-2a and c. He also thanks Prof. Mamta Singh of the Department of Pathology for constant encouragement and support.

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