

Virtual Database of Exfoliative Cytology in Mouth Injuries

MORAES, Maiara de, ARANTES, Silvio Batista, VIANNA, Leonora Maciel de Souza, GUERRA, Eliete Neves da Silva, MELO, Nilce Santos de. Virtual Database of Exfoliative Cytology in Mouth Injuries. *Oral Sci.*, Jan/Apr. 2010, vol.2, no.1, p. 17-22.

ABSTRACT - Exfoliative cytology is a basic low-cost procedure, helpful in the diagnosis of mouth lesions. Although effective as a diagnosis tool in healthcare areas, the technique is less common among dentistry professionals. The aim of this study was to present the results of the use of exfoliative cytology at a Stomatology Clinic through the aid of a virtual database. During a two-year period, around 1,300 clinical exams were carried out at the Stomatology Clinic of University Hospital of Brasília. Material was collected from a variety of lesions, and was fixed and processed in accordance with the standards of the anatomopathology laboratory using Papanicolaou, Gomori-Grocott colors, among others. The slides were photographed and the images were captured under Zeiss (Germany) microscopy to construct a digital file. With the digital images, the website <http://www.unb.br/fs/citovirtual/> was created, with unrestricted access. The creation of the virtual oral cytology database is an efficient way of calling the attention of technicians to this mouth injury diagnosis tool. The aim was to make exfoliative cytology more popular among dental surgeons, by placing images onto the internet, and creating a virtual database to help diagnose oral diseases through the exfoliative cytology.

KEYWORDS - Exfoliative cytology, oral mucosa, diagnostics, brush.

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Received April 1, 2009
Accepted June 11, 2009

Introduction

Exfoliative cytology is the study of exfoliated or desquamated tissue cells. Since the 19th century, the collection, identification and differentiation of exfoliated cells have been used as a diagnosis instrument. With the studies of Papanicolaou, this method was used for the detection of uterine cancer, and cytological examination became the method of excellence to evaluate the degree of alteration of the cervical squamous epithelium (1). Since the oral mucosa are recovered by squamous epithelium, similar to uterine cervix, cellular alterations observed in these cases can be similar, justifying the use of cytological examination as diagnostic tool in dentistry (1, 2).

Despite tissue similarity, exfoliative cytology does not have the same impact on dentistry as it does on gynecological medicine. However, with the development of new techniques, such as molecular biology, immunohistochemistry and cytometry flux, the precision of cytological examinations increased, rekindling the interest for exfoliative cytology (3).

The advantages obtained from exfoliative cytology are mainly the high specificity, high sensitivity, low cost, quickness, easiness of execution, dispensing of anesthesia and less discomfort for the patient (4, 5).

The recognition of the technique as a

useful tool for the diagnosis of oral injuries, especially oral carcinomas (6, 7, 8, 9), justifying the creation of a virtual exfoliative cytology database. The inputs for this new pedagogical resource can be found on the internet, allowing access to consultation, the method and contributing towards the learning of oral pathology and cytology.

Material and Methods

The patients who were evaluated the University of Brasilia Stomatology clinic over the period of two years comprised the sample. After agreeing to the research and signing Term of Free and Informed consent, with the approval of Research Ethics Committee of the School of Medicine of the University of Brasilia (CEP - FM 015/2004), the patients were submitted to oral examinations. If necessary, in the presence of oral lesions the patients were submitted to cytological examinations or surgical biopsy for diagnosis. The samples of oral lesions were collected from the most varied oral injuries, including leukoplakias, malignant neoplasia, and inflammatory, fungal, infectious and glandular diseases.

To perform the cytology we used cytobrush type brushes (Industrial Commercial Kolplast of Brazil Ltda, São Paulo, SP, Brazil). The material was collected by scraping vigorously the oral injuries of the patients, which was later spread onto clean and dry glass plates, and fixed immediately in alcohol 95%. The plates contained the identification of the patient and a brief clinical summary. Two samples of each injury were collected. The blades were conditioned in appropriate containers and prepared according to the Anatomopatologia Laboratory routine of the University Hospital of Brasilia where they were bleached by Papanicolaou. In the event of clinical doubt of

fungi lesions special coloration was used, such as Gomori-Grocott.

The diagnosis was established and the patients treated according to their therapeutic needs. The prepared slides were selected and photographed under an optic microscope (Zeiss, Germany) at the Anatomopatologia Laboratory of the University Hospital of Brasilia and, then made available on the internet as a exfoliative cytology atlas of mouth injuries. All cases were digitalized in different sizes and filed in accordance with the type of cellular alteration found.

Results

Over the period of two years, approximately one thousand and three hundred (1300) patients were evaluated at the Stomatology Clinic of the University of Brasilia. A total of 44 oral lesion samples submitted to exfoliative cytology and confirmed by biopsies were selected to evaluate diagnostic validity. The cytological examination was done for several oral lesions, such as fungal infections, malignant neoplasias and glandular diseases.

The oral smears were processed according to routine, stained and analyzed under optical microscope by a pathologist (LV). The selection criterion of the slides was the presence of a typical morphological characteristic of injuries that could identify them.

The capture of the images through optic microscopy allowed the register of the morphologic data and the compiling of the virtual database. The images are available at <http://www.unb.br/fs/citovirtual/>, as a source of information for students and professionals of health. Images and explicative texts are provided that explain the technique and describe the main characteristics of the exfoliated cells (Figure1).



Figure 1. Citovirtual Home Page, www.unb.br/fs/citovirtual

The main cytologic results found and photographed show that the microscopic analysis was efficient for the diagnosis of illnesses such as paracoccidioidomycosis (Figure 2).

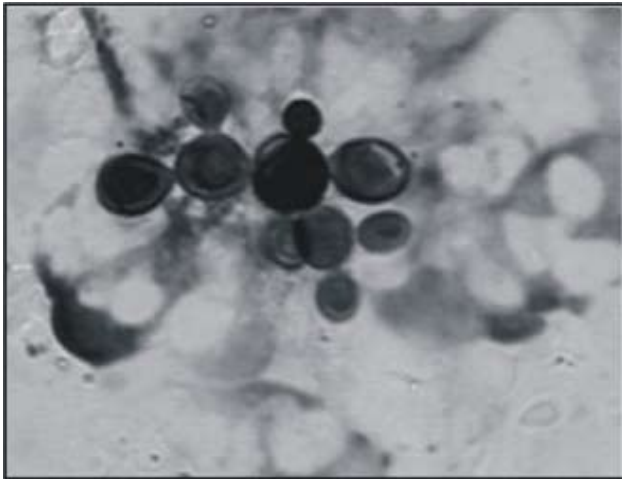


Figure 2. Multiple budding of fungi *P. brasiliensis*, refractile wall. Gomori-Grocot, 100x.

In the results, it may be seen that 10 of the 44 patients who underwent biopsies and traditional exfoliative cytology diagnoses were positive for the presence of carcinomas and 10 were correctly diagnosed by exfoliative cytology - a sensitivity of 100%. Furthermore, 34 patients had negative diagnoses for the presence of carcinomas and all were correctly identified by exfoliative cytology - a specificity of 100%. The characteristics of malignancy may be found in all samples of clinically suspicious malignant lesions (Figure 3).

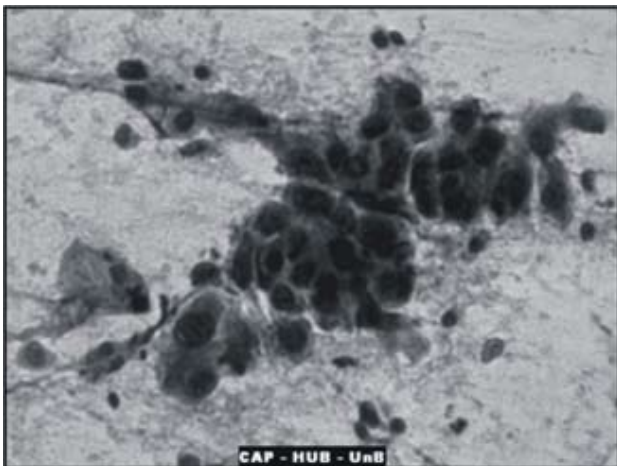


Figure 3. Cells with variations in the size and forms and alteration of the nucleus/cytoplasm ratio, nuclear hiperchromasy, macronucleolus, characteristic of malignancy. Papanicolaou, 20x.

With respect to the presence of other lesions, such as inflammatory or infectious by fungi or viruses, all patients were submitted to traditional biopsies and cytological examination. The inflammation present in clinical oral lesions caused by HPV was considered suspicious in oral smears, but not conclusive (Figures 4 and 5). Only 23 lesions of these were correctly diagnosed by exfoliative cytology.



Figure 4. Koilocytosis: clear well-defined perinuclear halo, with edges and dense core cytoplasmic volume increased, with irregular and coarse chromatin. Papanicolaou, 1000x.

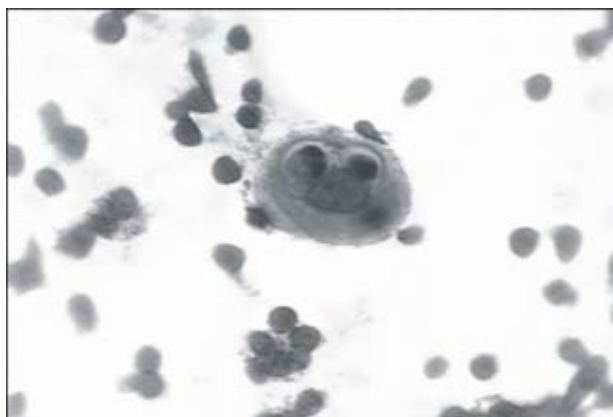


Figure 5. Koilocytosis and giant multinucleated cells, but with clear ill-defined perinuclear halo, and without significant nuclear changes. Papanicolaou, 1000x.

- a sensitivity of 57.5%. Among patients considered positive by exfoliative cytology, 100% actually had the disease, i.e., a positive predictive value equal to one. On the other hand, a negative predictive value cannot be determined because no data were computed for negative lesions. The accuracy of the method was 57.5%, based on the assessment of all correct results, which in this case were only positive values.

To show these results, the images and the texts were categorized to provide the user knowledge of the methodology, aspects of normality, the main cellular alterations and the illness diagnosed by exfoliative cytology. The images and texts are available at <http://www.unb.br/fs/citovirtual/>. The site, developed at the laboratory of computer science of the

School of Health Sciences, provides space for contacts and visitors, with downloads allowed for didactic purposes. Users have partial or total access to the virtual database, which may be used as a new pedagogical tool for education and research.

Discussion

Anamneses and physical examinations allowed a complete evaluation regarding the appearance and the evolution of the illness, and a diagnosis of the oral injuries. The complementary examinations (laboratory, radiology or biopsy) comprise the second pillar that supports the stomatology, contributing to diagnose oral injuries. Among complementary examinations, the traditional biopsy is the surgical procedure

through which tissues are obtained for histopathological evaluation. However, in some cases, when biopsy is not indicated, exfoliative cytology may be used (11). In recent years, exfoliative cytology is being used to assist the early diagnosis of pre-malignant and malignant injuries. The use of the technique may contribute to the pre-surgical diagnosis of oral lesions, facilitating the forecast and actual treatment of the patient (12).

The background of the use of exfoliative cytology in Dentistry indicates the possibility of the technique to recognize malignancy and, in many cases, to detect the type of injury. This serves to clarify the role of the technique as an alternative (2, 4).

In specific situations, as in the suspected clinical cases of paracoccidioidomycosis, the diagnostic confirmation of the cytologic findings is possible. The fungi in spherical form and classical budding shapes are clearly evidenced in the Gomori-Grocott colored slides. The visualization of fungi in the cytologic scraping is sufficient to diagnose and prescribe treatment (Almeida *et al* (2003); Araújo *et al* (2003) and Cardoso *et al* (2001)).

The inflammatory alterations in the exfoliated epithelial cells were identified by the presence of round, birefringent cells (clear and perinuclear halo), cytoplasmic vacuolization, giant multinucleate cells, cytoplasmic metacromasy, and infiltrated polymorphonuclear neutrophils, in the colored Papanicolau samples. Malignancy can be identified in cells with variations in size and forms, and alteration in the nucleus/cytoplasm ratio, nuclear hyperchromasy, macronucleolus and mitotic values (2).

Although the inflammatory aspects predominate, there are certain elements that are associated or not with the clinical findings, which allows greater precision in the cytological diagnosis: the visualization of fungi in the Paracoccidioidomycosis; the atypical nuclear and cytoplasmic alterations of the squamous cells that appear in the espinocelular carcinoma; and the identification of the tubular formations or myxoid elements in the glandular injuries are all elements that allow diagnostic conclusions (2, 4, 5, 10, 14).

The diagnostic confirmation of oral cancer through cytology is also favored by the fact that the majority of malignant oral lesions are detected in advanced stages, when injuries are ulcerate, exophytic and vegetating, which facilitates the scraping of cells that are modified and subject to analysis.

In the hyperkeratosis injuries, that make the scraping of deep cells difficult; in the bones or in soft tissue injuries found coated by the mucosa; in the tumors or pseudo-tumors injuries which do not allow a precise cytological conclusion; or even in cases where there is a precise cytological conclusion of neoplasia, there always exists indication of biopsy to determine or confirm the diagnosis.

The availability of information on the internet promotes a revolution in the educational methodologies, and the construction of a virtual database of cytological images of oral lesions, along with a detailed description of the technique will bring benefits to practically all areas of health studies. Since no other atlas of oral cytology was found on the internet, a comparison between access and use standards was not possible. We expect that this study and its availability on the internet may contribute to disseminate exfoliative cytology for mouth injuries, and stimulate future research (15).

Conclusions

The images of cytological diagnoses of oral injuries made available on the internet are important to spread the practice of exfoliative cytology, especially among dentists who usually do not have access to the technique, or are unaware of its importance in fast and precise diagnoses.

Despite the limitations regarding the effective use of the site by patients, this study supplies indications of the positive impact of the examinations. Moreover, the online technical knowledge provided will allow dentists, laboratory technicians and students an ample use of the method. The creation of this virtual database facilitates the adoption of new means of teaching and inserting cytology in the educational and research programs of Pathology and Stomatology institutes.

Acknowledgements

I would like to thank the team of pathologists of the Pathology Laboratory of the HUB; the dentistry laboratory technician, Maria da Glória Silva; and to the computer sciences technician of the Health Sciences Computer Science Department, Julio Cesar Cabral.

Resumo

MORAES, Maiara de, ARANTES, Silvio Batista, VIANNA, Leonora Maciel de Souza, GUERRA, Eliete Neves da Silva, MELO, Nilce Santos de. Banco de Dados Digital de Citologia Esfoliativa em Afecções Bucais. *Oral Sci.*, Jan/Apr. 2010, vol.2, no.1, p. 17-22.

A citologia esfoliativa é um procedimento simples e de baixo custo, auxiliar no diagnóstico de lesões bucais. Embora eficaz como instrumento de diagnóstico em outras áreas da saúde, a técnica é pouco difundida

entre os profissionais de odontologia. O objetivo desse trabalho é de apresentar os resultados obtidos com o uso da citologia esfoliativa na Clínica de Estomatologia do Hospital Universitário de Brasília. No período de dois anos foram realizados aproximadamente 1300 exames clínicos, dentre os quais 44 citologias esfoliativas acompanhadas de biópsia cirúrgica, para comparação da validade diagnóstica. A captura das imagens foi feita em um microscópio Zeiss (Alemanha) valorizando-se os sinais característicos ou patognomônicos das diversas lesões. Com as imagens digitalizadas foi construído um website <http://www.unb.br/fs/citovirtual/>, de acesso público, amplo e irrestrito. A disponibilização das imagens na rede mundial de computadores, criando um banco de dados digital sobre o diagnóstico citológico das afecções bucais, visa divulgar a citologia esfoliativa entre cirurgiões-dentistas.

Palavras-chave - Citologia esfoliativa, mucosa oral, diagnóstico.

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