



Assessment of cytology techniques in oral mucosa of Sjögren's syndrome patients

Valoración de técnicas de citología en mucosa bucal en pacientes con síndrome de Sjögren

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ABSTRACT

The aim of the present research project was to compare two techniques for oral cytology study (exfoliative cytology and impression cytology) in order to assess changes in oral mucosa which might allow non-invasive diagnosis of Sjögren's syndrome cases (SS). **Patients:** 50 patients were selected, patients were paired by age and gender, and had been diagnosed with Sjögren's syndrome according to criteria of the American-European Consensus. Patients were distributed into the following three experimental groups: Healthy control group (C), n = 14, Dry mouth and eyes group without SS, (ME) n = 13, and SS group n = 23. **Material and methods:** A cell harvesting brush (*Cytobrush*) was used for the exfoliative cytology procedure, sliding it along a glass plate and later fixating harvested cells in 95% ethanol. Cellulose acetate paper (Millipore Hawp 304[®]) was used for the impression cytology procedure. The paper was in 1 cm long stripes which were placed on the oral mucosa surface above upper vestibular groove; stripes were immobilized and pressure was applied for three seconds. Papanicolau (PAP) technique was used for dyeing. Morphology and histomorphology were assessed studying the following: cytoplasmic area (CA), nuclear areas (NA) nucleus-cytoplasm relationship (N:C) and amount of cells per square millimeter (mm²). **Results:** Both techniques revealed the following in C: isolated single-layered epithelial cells, basophils, normal central nuclei, 20 to 30 per mm², N:C ratio 1:8. In the eye and mouth group (EM): grouped and folded isolated cells, cytoplasm with eosinophilic predominance increase of cell amount to 40 per mm², nucleus-cytoplasm relationship N/C 1:4. SS patients showed the following: nuclear area with denser chromatin, 400 cells per mm², and 1:2 N/C relationship with respect to mouth and ears and control. Statistically significant differences were observed among groups in all studied characteristics. **Conclusion:** We can infer that impression cytology can be used in systemic and oral lesion's diagnosis in patients afflicted with hyposalivation.

Key words: Oral mucosa, cytological diagnosis, hyposalivation.

Palabras clave: Mucosa bucal, diagnóstico citológico, hiposalivación.

RESUMEN

El objetivo del presente trabajo es comparar dos técnicas de estudio de citología bucal, la citología exfoliativa y de impresión, para evaluar cambios en la mucosa oral que permitan el diagnóstico no invasivo de síndrome de Sjögren (SS). **Pacientes:** Se seleccionaron 50 pacientes apareados por sexo y edad, diagnosticados con síndrome de Sjögren según criterios del Consenso Americano-Europeo. Los pacientes fueron distribuidos en tres grupos experimentales: controles sanos (C), n = 14, boca y ojo secos sin SS (BO), n = 13, y con SS n = 23. **Material y métodos:** Para la citología exfoliativa se utilizó cepillo recolector de células (*Cytobrush*) deslizando a lo largo de la lámina de vidrio, fijándose posteriormente en etanol al 95%. En citologías por impresión se utilizó papel de acetato de celulosa (Millipore Hawp 304[®]), en tiras de un cm de longitud colocadas sobre la superficie de la mucosa bucal sobre el surco vestibular superior inmovilizando el papel y presionando por tres segundos. Se tiñeron con técnica de Papanicolau (PAP). Se valoró la morfología e histomorfometría, estudiando: área citoplasmática (AC), área nuclear (AN), relación núcleo-citoplasma (N:C) y cantidad de células por milímetro cuadrado (mm²). **Resultados:** Ambas técnicas nos permitieron observar en C: células epiteliales pavimentosas aisladas, basófilos, núcleos centrales normales, 20 a 30 por mm², relación N/C 1:8. En el grupo BO: células aisladas, agrupadas y plegadas, con citoplasma a predominio eosinófilo, aumento de la cantidad de células 40 por mm², relación núcleo-citoplasma relación N/C 1:4. En los pacientes SS se observó el área nuclear con cromatina más densa, 400 células por mm², y relación N/C 1:2 en relación a BO y C. Hubo diferencias estadísticamente significativas entre los grupos en todas las características estudiadas. **Conclusión:** Podríamos inferir que la citología por impresión puede ser utilizada en el diagnóstico de lesiones orales y sistémicas en pacientes con hiposalivación.

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INTRODUCTION

Sjögren's syndrome is an autoimmune, chronic exocrinopathy, of slow progression and unknown etiology. It is characterized by dryness in the mucosa, mainly oral mucosa (xerostomia) and ocular mucosa (xerophthalmia) although it can frequently elicit symptoms due to nasal, dermal or vaginal dryness. Even though in most patients the disease can be located in exocrine glands (glandular manifestations),^{1,2} due to its systemic characteristics, it frequently exhibits extra-glandular manifestations.³ Approach for SS diagnosis can be difficult, controversial and late. Diagnosis encompasses two different objectives: evaluation of systemic, salivary and ocular components and determination of whether it represents a primary or secondary disease. Some diagnostic criteria are not accurate and patients can be erroneously evaluated especially at initial stages of the disease.⁴ Within evaluation criteria the following is assessed: dryness of eye and mouth, antibody determination and biopsy of minor salivary glands.^{2,5}

Some studies incorporate image-supported diagnoses, nevertheless they are non-specific, thus, classification criteria grant diagnostic value to determination of anti Ro and anti La antibodies, which can be found in other autoimmune diseases.⁵

Biopsy of labial minor salivary glands is an invasive technique that can leave sequels in the oral mucosa.⁵

Exfoliative cytology is a simple, non-invasive procedure to study epithelial cells in the mucosae, this technique has been used to diagnose certain types of oral lesions, most of them related to viral and fungal diseases.⁶

The term impression cytology was first introduced by Egbert et al in 1977,^{7,8} it was first used in conjunctiva mucosa. Later on, several authors have conducted modifications according to their needs, describing the use of this technique to study mucus purity on conjunctiva surface⁹ as well as changes in nuclear chromatin of epithelial cells in Sjögren's syndrome.

The conventional method is the most frequently used, this could be due to scarcity of studies on impression cytology to examine oral mucosa.

The aim of the present project was to compare two cytology techniques: exfoliative and impression cytology in a study of oral mucosa of patients afflicted with sicca and Sjögren's syndrome, which might allow diagnosis with a non-invasive technique.

MATERIALS AND METHODS

Patients

For the present study, 50 patients were selected (p) from the Rheumatology Clinic of the Allende Sanatorium

and Cordoba Hospital. Patients were consecutively admitted and treated between January 2010 and January 2013. Patients were distributed into the following experimental groups: group 1, clinically healthy patients, control group[®], n = 14, group 2 patients with dry mouth and eyes not afflicted by Sjögren's syndrome (ME), n = 13, group 3: patients afflicted with primary Sjögren's syndrome, (pSS), n = 23.

Consensus criteria of the European-American Rheumatology group were used to establish SS diagnosis.³

Exclusion criteria were the following: patients undergoing courses of chemotherapy or radiotherapy in the craniofacial region, patients with neoplasms in the head and neck region, patients with psychiatric disorders, patients with metabolic compromise or ingesting anti-hypertensive drugs, psychopharmaceutical drugs, Hepatitis C and B, HIV, sarcoidosis, IgG4- related diseases.

Methods

One single operator performed all sample taking for exfoliative cytology technique. This procedure was conducted with a cytological harvesting brush (*Cytobrush*), sliding it along the laboratory slide. It was later fixated in 95% ethanol. Samples for impression cytology were achieved with cellulose acetate paper (Millipore, Hawp 304) of 45 µm thickness, cut into 1 cm stripes. Stripes were placed on the surface of the oral mucosa, on the upper vestibular groove and were kept in place for five seconds while pressure was being applied. Samples were placed in 96° alcohol for fixation and later dyeing.

Samples of the upper vestibular groove were thus obtained. Papanicolaou technique (PAP) was used to dye all samples. Morphological assessment was conducted encompassing the following: predominant cell type, nuclei, cell grouping and folding as well as histomorphometry.

Within the context of histomorphometric study the following was assessed: cytoplasmatic area (CA), nuclear area (NA), nucleus/cytoplasm relationship (N/C) and amount of cells per mm². Images were obtained with optical microscopy; histomorphometric analysis was performed with Image Pro Plus 4.1. program.

The present study was approved by the ethics committee of the Allende Sanatorium (CIEIS) under the guidelines of the International Medical Association; patient's informed consent was obtained.

Statistical analysis was conducted with «t» Student test for independent data, p < 0.05 value was established for statistical significance.

RESULTS

Patients' average age was 50 years, 86% of them were female.

Both techniques allowed us to observe in C regular-shaped isolated one-layered epithelial cells, basophils, normal shaped and sized, central and picnotic nuclei (*Figure 1A*); in ME group folded and grouped isolated cells with predominantly eosinophilic cytoplasm and lacking nucleus alterations were observed (*Figure 2B*); in SS patients, smaller sized cytoplasmatic area and increased nucleus area with denser chromatin was observed (*Figure 3C*).

Histophormometric study of both techniques revealed the following: in group C a cell amount reaching 40 per mm² area, in correspondence to C p<0.01, nucleus-cytoplasm relationship N/C 1:4, and in SS patients a N/C 1:2 relationship of 400 cells per mm², with statistically significant increase p < 0.001 with respect to ME and C (*Figures 4 and 5*).

DISCUSSION

The procedure of exfoliative cytology of the oral mucosa is increasingly becoming more important in the early diagnosis of different local and systemic conditions, it is considered a procedure for the procurement of cell samples that can later be analyzed though sophisticated diagnostic techniques such as cytomorphometry, AND cytometry and molecular analyses.¹⁰

Use of sophisticated computer programs has changed the scenario and rendered result interpretation more reliable.

The technique of exfoliative cytology for cells of the mouth is non-aggressive, simple and expedite; it is therefore well accepted by patients and suitable for routine application in population screening programs for early analysis of suspicious lesions as well as for pre- and post-monitoring of oral lesion treatments.¹⁰⁻¹²

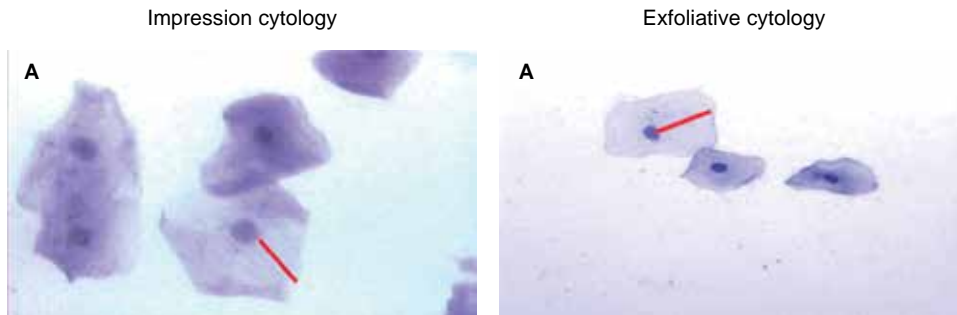


Figure 1.

A) Patient relation (N/C) 1: 8 image 200 x, scale = 100 microns.

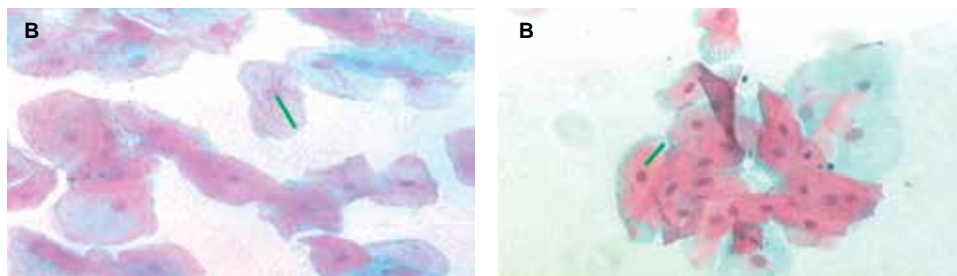


Figure 2.

B) Patient relation (N/C) 1: 4 image 200 x, scale = 100 microns.

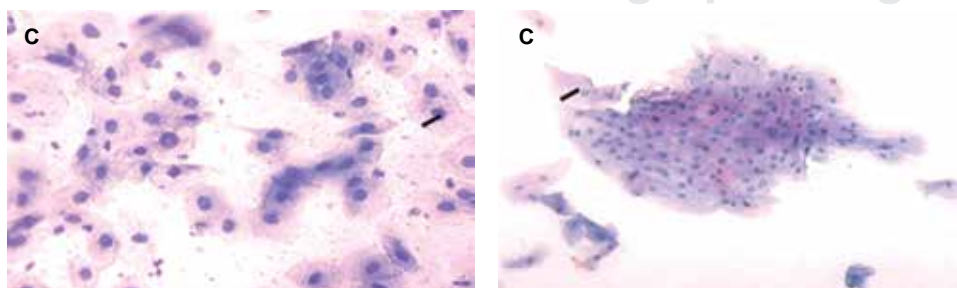


Figure 3.

C) Patient relation (N/C) 1: 2 image 200 x, scale = 100 microns.

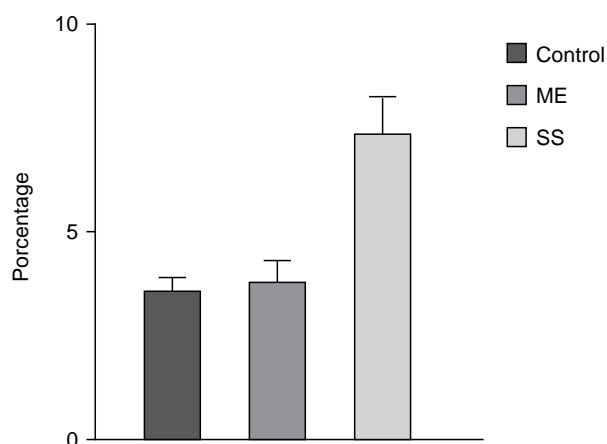


Figure 4. Nucleus/cytoplasm relationship in different experimental groups $p < 0.01$.

Impression cytology used in conjunctiva of the eye has enabled to easily recognize squamous metaplasia in severe and moderate dry eyes found in SS patients, thus contributing to clinical diagnosis confirmation and disease follow-up.¹³⁻¹⁶ It is worth mentioning that there is no present evidence supporting use of this procedure in the mouth.

Both cytology techniques allowed us to identify similar parameters, such as type, color size and amount of cells. In the case of autoimmune diseases such as SS, although both techniques were efficient to establish diagnosis, perception of cell grouping was better characterized in impression cytology preparations.

Our study established comparison of both techniques, exfoliative and impression cytology, measuring fit of the sample and diagnosis coincidence in the aforementioned groups.

Sample analysis revealed that cytologies processed with the impression technique exhibited certain advantages, since the thin and uniform distribution of cell material allowed more accurate observation of nuclei and cells' shape, moreover, cell superposition was decreased and presence of polymorphonuclear cells was observed.

In histomorphometric studies, impression cytology also exhibited advantages over conventional techniques, because it allowed for a better observation of studied parameters such as changes in nucleus-cytoplasm relationship of different experimental groups.

In the present study, results associated to biochemical and clinical observations suggest that SS can cause morphological and functional alterations in epithelial oral cells which can

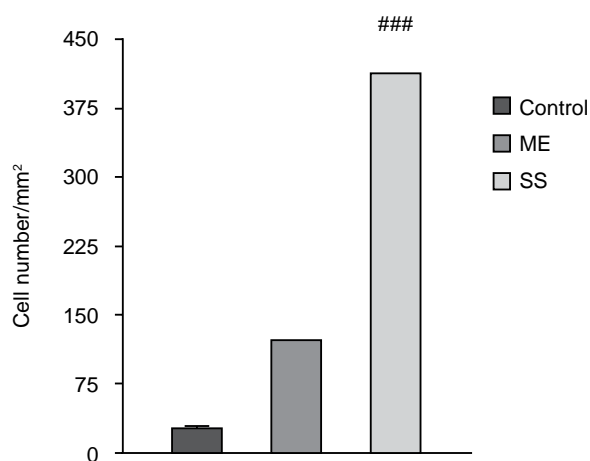


Figure 5. Number of cells in different experimental groups $p < 0.01$.

be detected through microscopic analysis and cytometry using cytology techniques.

CONCLUSION

Preliminary data of histological description evidenced greater detail when using impression technique as compared to the exfoliative technique. From this we might infer that impression cytology can be used in the diagnosis of oral and systemic lesions in patients afflicted with hyposalivation.

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