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Different conjunctival adaptive response in patients with aqueous-deficient and with mucous-deficient dry eyes

L. RIVAS[1], J.S. LÓPEZ-GARCÍA[2], J. MURUBE[1], I. GARCÍA-LOZANO[2]

[1]Ocular Surface Unit, Research Laboratory, Service of Ophthalmology, University Hospital Ramon y Cajal
[2]Service of Ophthalmology, Hospital Central Cruz Roja, Madrid - Spain

PURPOSE. *To describe the different cellular adaptive patterns found in the conjunctival epithelium from patients with aqueous-deficient and mucous-deficient dry eyes.*
METHODS. *The authors studied different conjunctival areas, by impression cytology and by biopsy, 50 eyes with facial nerve paralysis (FNP), 50 eyes with ocular cicatricial pemphigoid (OCP), and 50 eyes from patients with primarily Sjögren syndrome (1SS).*
RESULTS. *Eyes with FNP from the first clinical grade showed a progressive alteration of the nonsecretory cells, with a significant decrease in density goblet cells, generally with a PAS-positive staining. Eyes with OCP, during clinical grades 1 and 2, showed a slow deterioration of the nonsecretory cells; but from clinical grade 3, there was a significant increase of the cellular size and the thickness of the conjunctiva. Goblet cells showed a significant decrease in density from clinical grade 1, generally with a PAS-negative staining. Eyes with 1SS during clinical grades 1 and 2 showed a progressive alteration of the nonsecretory cells, with a significant decrease in density goblet cells, and a PAS-positive staining. From clinical grade 3 appeared a significant increase of nonsecretory cellular size and thickness of conjunctiva, with a significant decrease in goblet cell counts, and a PAS-negative staining.*
CONCLUSIONS. *Patients with FNP (a primarily aqueous-deficient alteration) follow completely the squamous metaplasia process. Patients with OCP (a primarily mucous-deficient syndrome) have a hypertrophy and hyperplasia process along the ocular surface. Patients with 1SS (a primarily aqueous-deficient and mucin-deficient alteration) have a squamous metaplasia process, but from clinical grade 3 also appears a hypertrophy and hyperplasia process. (Eur J Ophthalmol 2007; 17: 160-70)*

KEY WORDS. *Conjunctiva, Goblet cells, Hypertrophy, Hyperplasia, Impression cytology, Squamous metaplasia*

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INTRODUCTION

The evaluation of patients with dry eye syndromes in daily practice is usually performed by clinical data, using a clinical grading system; however, laboratory and histopathologic tests can be helpful in the diagnosis of a dry eye syndrome (1). The epithelium of normal ocular surface is stratified and not keratinized. There are many goblet cells scattered among nonsecretory cells, playing an important role in the ocular surface integrity. Classically, in dry eye syndromes, the normal epithelium suffers a squamous metaplasia, so the conjunctival epithelium is modified and becoming nonsecretory and keratinized (2).

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Clinically, the keratinization of the ocular surface in dry eye syndromes is recognized by slit-lamp biomicroscopic examination, but squamous metaplasia prior to keratinization can only be demonstrated by morphologic techniques (impression cytology and biopsy). As discussed by the majority of authors with regard to the diagnostic efficacy of the most frequent clinical tests for dry eye, mainly in the first phases of the disease where symptoms cannot be clinically detected (3), morphologic techniques could allow an exact structural study of the epithelium and connective tissue, marking it possible to compare pathologic and non-pathologic material and permitting a more accurate diagnosis (2).

Besides a squamous metaplasia process, there are several cellular mechanisms to defend the organ against environmental changes or internal pathological stimuli, in order to take adaptation responses. These other cellular responses are hyperplasia, hypertrophy, atrophy, dystrophic and cellular accumulations (4).

The Madrid triple classification of dry eye (1), a very reported graduation, classifies the different dry eye syndromes according to clinical dry eye severity, histopathologic characteristics, and etiologic type; so, FNP was included in the neuropathic etiologic group and showed primarily aqueous-deficient alterations. OCP was included in the immunopathic etiologic group and was primarily a tear mucous-deficient syndrome. And finally, 1SS was included in the immunopathic etiologic group and was primarily an aqueous and mucous-deficient syndrome (1).

The aim of this article is to describe the involution patterns of ocular surface in different diseases' etiology, by impression cytology and by biopsy, in order to search for specific signs that could help in early detection of the disease, to know better the ocular features of the disease, and to formulate a possible differential mechanism between an aqueous-deficient syndrome and a mucin-deficient syndrome.

MATERIALS AND METHODS

We studied 50 eyes with FNP (14 with grade 2, 16 with grade 3–4, 12 with grade 5, and 8 with grade 6), patient age from 18 to 75 years (mean age, 47 years) of both sexes (56% female and 44% male). We also studied 50 eyes with OCP (10 with grade 1, 13 with grade 2, 20 with grade 3, and 7 with grade 4), patient ages from 20 to 81 years (mean age, 49 years) of both sexes (54% female and 46% male). We also studied 50 eyes with 1SS (14 with grade 1, 15 with grade 2, 15 with grade 3, and 6 with grade 4), patient ages from 19 to 79 years (mean age, 47 years) of both sexes (54% female and 46% male). As controls, we studied 50 normal eyes, patient ages ranging from 17 to 82 years (mean age, 47 years) of both sexes (56% female and 44% male).

Each group of patients was graded according to a specific ocular clinical grading system. The ocular clinical classification was divided in four progressive pathologic grades and one normal:

- Grade 0 - Normal subjects, without symptoms and signs of dryness.
- Grade 1 - Symptoms of dryness (dryness sensation, itching, sand sensation) only under overexposure, which in normal people would not produce disturbances.
- Grade 2 - Symptoms in normal daily life, but no clinical signs.
- Grade 3 - Symptoms of grade 2 plus reversible clinical signs (redness, corneal staining, keratitis punctata, filaments), observed at first sight or under fentobiomicroscopy.
- Grade 4 - Grade 3 plus irreversible signs (corneal scars, corneal vascularization, lower fornix/lacunar sulci retraction).
- Grade 5 - Grade 4 plus corneal keratinization and visual deficiency.

The clinical evaluation of FNP has been described in the grading system developed by House and Brackmann and Moody-Antonio and House (5, 6):

- Grade 1 - Has a normal facial function.
- Grade 2 - Shows a mild dysfunction (slight weakness is noted on close inspection. The patients may have a slight synkinesis. A normal symmetry and tone is noted at rest. The forehead motion is moderate to good, complete eye closure is achieved with minimal effort, and slight mouth asymmetry is noted).
- Grade 3 - Shows a moderate dysfunction (an obvious but not disfiguring difference is noted between both the sides. A noticeable but not severe synkinesis, contracture, or hemifacial spasm is present. A normal symmetry and tone is noted at rest. The forehead movement is slight to moderate, complete eye closure is achieved with effort, and a slightly weak mouth movement is noted with maximum effort).

• Grade 4 - Shows a moderately severe dysfunction (an obvious weakness and/or disfiguring asymmetry is noted. Asymmetry and tone are normal at rest. No forehead motion is observed. Eye closure is incomplete, and an asymmetric mouth is noted with maximal effort).
• Grade 5 - Shows a severe dysfunction (only a barely perceptible motion is noted. An asymmetry is noted at rest. No forehead motion is observed. The eye closure is incomplete and mouth movement is only slight).
• Grade 6 - Grade 6 shows a total paralysis (gross asymmetry is noted, and no movement is noted). Was defined as complete facial paralysis; all the other grades are defined as incomplete.
An incomplete facial paralysis denoted an anatomically, and to some degree functionally, intact nerve. The degree of facial nerve function should be noted in the chart at the initial visit.
Foster and Ahmed et al (7, 8) proposed a clinical classification for grading patients with OCP, with four pathologic states and one normal group: grade 0 was normal, grade 1 had cicatrization and subconjunctival fibrosis, grade 2 showed shrinkage of the fornix, grade 3 had formation of symblepharon, and grade 4 had ankyloblepharon.
The clinical diagnosis of 1SS was made according to the criteria proposed by Vitali et al in 1996 (9). Patients must present alterations in four or more of the following groups: ocular symptoms, oral symptoms, ocular signs, histopathology in minor salivary glands, objective evidence of salivary glands involvement, and presence of autoantibodies in serum. The clinical grading system was based on the number of inflammatory foci in biopsies of minor salivary glands. One focus was defined by an accumulation of 50 or more lymphocytes in 4 mm^2 of glandular tissue.
The clinical evaluation of FNP has been described as a function of the lachrymal gland activity and the health of the ocular surface (Tab. I) (10, 11).
Dryness effect on the ocular surface, by impression

TABLE I - THE CLINICAL EVALUATION OF FNP

Clinical tests	Normal	Mild dryness	Moderate dryness	Severe dryness
Schirmer test, mm/min	>10/5	8 to 10/5	5 to 7/5	<4/5
BUT, s	>12	9 to 12	4 to 8	<4
Rose bengal vital staining	<4 crosses/9	5 to 4 crosses/9	7 to 6 crosses/9	>7 crosses/9
Osmolarity, mOsm/L	<310	310 to 324	325 to 339	>340

FNP = Facial nerve paralysis; BUT = Tear break up time

TABLE II - THE GRADING SYSTEM OF SQUAMOUS METAPLASIA BY IMPRESSION CITOLOGY

Grade		Goblet cells/mm^2	Cytoplasm staining	Non- secretory cellular size, μm^2	Nucleus–cytoplasm ratio	Nuclear changes
0	Conjunctiva	>400	Eosinophilic	200–300	1:2–1:3	No
	Cornea	–	Eosinophilic	300–350	1:3	No
1	Conjunctiva	400 to 300	Eosinophilic	200–350	1:3–1:4	No
	Cornea	–	Eosinophilic	300–400	1:4	No
2	Conjunctiva	350 to 200	Eosinophilic	350–500	1:5	Binucleated cells
	Cornea	–	Eosinophilic	500–1000	1:8	Binucleated cells
3	Conjunctiva	200 to 50	Metachromatic	500–900	1:10	Binucleated and pyknotic cells
	Cornea	–	Metachromatic	1000–1800	1:15	Binucleated and pyknotic cells
4	Conjunctiva	50 to 10	Basophilic	900–1600	1:20	Pyknotic cells
	Cornea	–	Basophilic	1800–2500	1:20	Pyknotic cells
5	Conjunctiva	<10	Keratinized	>1600	1:30	Anucleated cells
	Cornea	–	Keratinized	>2500	1:30	Anucleated cells

cytology, was graded according to the classification for squamous metaplasia given by Murube and Rivas in 2003 (12), with five pathologic grades and one normal. The grading system of squamous metaplasia has the following cytologic features: morphologic nuclear changes, nucleus-cytoplasm (N/C) ratios, cytoplasm metachromatic changes, cytoplasmic keratinization, and goblet cells densities (Tab. II).

Dryness effect on the ocular surface, by biopsy, was also graded according to the classification for squamous metaplasia given by Murube and Rivas in 2003 (1), with five pathologic grades and one normal (Tab. III).

Besides the squamous metaplasia, we have studied the hyperplasia, the hypertrophy, and the atrophy of each eye. Hypertrophy occurs due to an increase in cell size rather than division with the increase of the size of an organ or a tissue; this is a mechanism to defend the organ against environmental changes or internal pathologic stimuli, in order to take adaptation responses. By contrast, hyperplasia (or hypergenesis) is a general term for an increase in the number of the cells of an organ or tissue causing it to increase in size. It may be due to any number of causes including (but not limited to) increased demand, chronic inflammatory response, hormonal dysfunctions, or neoplasia. Atrophy is the partial or complete wasting away or decrease in size of a body organ, tissue, or part owing to disease, injury, or lack of use, involving apoptosis on a cellular level (4).

With the patient's consent, the impression cytology and biopsy were obtained from all the eyes included in the study. Impression cytology specimens were taken after topical anesthesia with a drop of double anesthetic (Colircusí®, Alcon Cusí, SA. Barcelona, Spain).

TABLE III - CLASSIFICATION FOR SQUAMOUS METAPLASIA BY BIOPSY

Grade	Goblet cells/mm^2	Tear mucous layer	Non- secretory cellular size, µm^2	Nucleus–cytoplasm ratio	Nuclear alterations
0	>100	Very high	110	1:3	No
1	100 to 50	Slight reduction of the height	180	1:5	No
2	50 to 25	Narrow and complete	220	1:7	Binucleated
3	25 to 5	Narrow and incomplete	380	1:10	Binucleated
4	< 5	Narrow and discontinuous	500	1:18	Pyknotic
5	—	No	620	1:30	Pyknotic or absent

TABLE IV - NUMBER (%) OF EYES WITH ALTERED TEAR TEST

Disease		Test			
Clinical grade	**Patients**	**Schirmer**	**Rose bengal**	**BUT**	**Osmolarity**
Control (grade 0)	50	0 (0)	0 (0)	0 (0)	0 (0)
FNP grade 2	14	3 (21.5)	3 (21.5)	1 (7.1)	1 (7.1)
FNP grades 3–4	16	3 (18.8)	3 (18.8)	4 (25.0)	3 (18.8)
FNP grade 5	12	7 (53.3)	8 (66.7)	6 (50.0)	5 (41.7)
FNP grade 6	8	7 (87.5)	7 (62.5)	5 (38.5)	7 (87.5)
OCP grade 1	10	0 (0)	1 (10.0)	1 (10.0)	1 (10.0)
OCP grade 2	13	1 (7.7)	3 (23.1)	3 (23.1)	2 (15.4)
OCP grade 3	20	3 (15.0)	9 (45.0)	10 (50.0)	4 (20.0)
OCP grade 4	7	2 (28.6)	5 (71.4)	4 (57.1)	4 (57.1)
1SS grade 1	14	2 (14.3)	2 (14.3)	3 (21.4)	2 (14.3)
1SS grade 2	15	2 (13.3)	7(46.7)	5 (33.3)	4 (26.7)
1SS grade 3	15	5 (33.3)	8 (53.3)	7 (46.7)	7 (46.7)
1SS grade 4	6	4 (66.7)	4 (66.7)	4 (66.7)	5 (83.3)

BUT = Break-up time; FNP = Facial nerve paralysis; OCP = Ocular cicatricial pemphigoid; 1SS = Primarily Sjögren syndrome

Strips of cellulose acetate filter paper (Millipore HAWP304) were applied on the upper bulbar, on the interpalpebral, on the lower bulbar, and on the lower palpebral conjunctiva. The filter material was fixed in 96% ethanol, stained with PAS and hematoxylin, dehydrated in ascending grades of ethanol, then xylene, and permanently mounted. Conjunctival biopsy samples, after topical anesthesia with double anesthetic (Colircusí®), were obtained with a volume approximately of 2 mm^3 from the central lower bulbar area. The material was fixed by immersion in 1.5% glutaraldehyde buffered with cacodylate to pH 7.3–7.4, and subsequently postfixed with 1% osmium tetroxide. The tissue was dehydrated in acetone and embedded in araldite resin (Durcupan, Ciba-Geigy). Afterwards, sections were stained with uranyl acetate and lead citrate.

The quantitative studies of conjunctival goblet cells were carried out using a calibrated grid at original magnifications of 40x. The mean goblet cell density was obtained by counting 10 random areas for each specimen. The densities were reported per square mm with standard deviation for each area. The mean cellular size of the goblet cells was obtained by the cellular diameters.

Total cellular, cytoplasmic, and nuclear size of goblet cells and non-secretory epithelial cells from conjunctiva were measured by image planimetry Pro/Media Cybernetics with a CDA system based on IPP-Plus software. The samples were examined with a Nikon Ophtiphot-2 microscope, attached to a Microflex DX photograph and an EVI-1011P color video camera. The software is designed specifical-

TABLE V - GOBLET CELL DENSITIES IN DIFFERENT AREAS OF THE CONJUNCTIVA IN FNP, OCP, 1SS, AND CONTROLS (number of cells per mm^2 ± SD)

	Conjunctival area						
Clinical grade of disease	**Upper bulbar**	**p**	**Interpalpebral**	**p**	**Lower bulbar**	**p**	**Lower palpebral**
Controls	647±58	NS	615±76	NS	708±78	NS	847±89
FNP grade 2	362±51	NS	347±55	†	421±63	NS	443±54
p	*		*		*		*
FNP grades 3–4	265±35	NS	253±39	†	307±45	NS	322±40
p	*		*		*		*
FNP grade 5	102±20	NS	94±24	†	182±31	NS	196±28
P	*		*		*		*
FNP grade 6	30±2	NS	32±3	†	57±5	NS	54±3
P	*		*		*		*
OCP grade 1	155±38	NS	149±32	NS	176±40	NS	185±53
p	*		*		*		*
OCP grade 2	66±12	NS	57±11	NS	62±14	NS	74±27
p	*		*		*		*
OCP grade 3	13±8	NS	11±7	NS	15±10	NS	23±14
p	*		*		*		*
OCP grade 4	0	NS	0	NS	0	NS	0
p	*		*		*		*
1SS grade 1	345±43	NS	338±45	NS	372±39	NS	380±40
p	*		*		*		*
1SS grade 2	254±53	NS	240±30	NS	275±26	NS	278±32
p	*		*		*		*
1SS grade 3	58±45	NS	60±28	NS	62±11	NS	70±25
p	*		*		*		*
1SS grade 4	11±9	NS	10±7	NS	18±6	NS	16±10
p	*		*		*		*

First clinical grade in each disease is compared with controls.

* = Sstatistical differences with regard to the preceding clinical grade in each conjunctival area.

† = Statistical differences with regard to the immediately topographical location within the same clinical grade.

FNP = Facial nerve paralysis; OCP = Ocular cicatricial pemphigoid, 1SS = Primarily Sjögren's syndrome, NS= No statistical differences

ly for measuring the appearance of the cells or two-dimensional features. Areas of cytoplasm or nucleus are included in the limit of the membrane. With a pointer connected to the planimetry, morphometric characteristics were performed over photographs of 10x15 cm, prepared at a nominal magnification of 40x. The pixels circumscribed in the delineation of the nucleus and cellular peripheries were transferred to square microns, and successive cycles of mathematic morphology operations gave the size or shape. Analysis of the relation between area of the cell nucleus and the surrounding cell cytoplasm (N:C ratio) was made.

The statistical analysis was performed with a statistical package (SPSS 10.5 for Windows). An unpaired two-tailed Student test and analysis of variance (ANOVA) were used to compare independent groups. Significant statistical difference was obtained with a probability higher than 95% (confidence interval, $p<0.05$).

RESULTS

The number of altered eyes studied with tear clinical tests and laboratory tests is shown in Table IV.

In eyes from controls, by impression cytology, the non-secretory epithelial cells of the normal conjunctiva were small (mean size around 245 μm^2) and perfectly joined. The cytoplasm was eosinophilic and the nucleus was round. The N:C ratio was 1:2. The epithelium was made up for five layers (Tabs. V and VI, and Fig. 1). There

TABLE VI - THE DIAMETER OF GOBLET CELLS IN DIFFERENT AREAS OF THE CONJUNCTIVA IN FNP, OCP, 1SS, AND CONTROLS (number of cells per mm^2 ± SD)

	Conjunctival area						
Clinical grade of disease	**Upper bulbar**	**p**	**Interpalpebral**	**p**	**Lower bulbar**	**p**	**Lower palpebral**
Controls	19.8±1.3	NS	20.1±2.2	NS	19.6±1.8	NS	20.6±2.0
FNP grade 2	19.3±1.9	NS	19.6±2.3	†	19.2±2.9	NS	19.6±2.5
p	*		*		*		*
FNP grades 3–4	18.2±2.1	NS	18.0±1.7	†	18.3±2.5	NS	18.5±2.7
p	*		*		*		*
FNP grade 5	17.4±3.5	NS	17.3±2.9	†	17.9±3.0	NS	18.1±3.4
p	*		*		*		*
FNP grade 6	16.8±4.7	NS	16.7±4.6	†	16.6±3.9	NS	16.8±4.2
p	*		*		*		*
OCP grade 1	21.7±4.6	NS	22.0±4.2	NS	22.8±4.3	NS	21.4±3.8
p	*		*		*		*
OCP grade 2	26.6±15.1	NS	26.7±16.8	NS	27.6±15.5	NS	27.0±14.7
p	*		*		*		*
OCP grade 3	40.2±25.4	NS	39.6±22.5	NS	42.0±20.3	NS	40.6±20.5
p	*		*		*		*
OCP grade 4	—	NS	—	NS	—	NS	—
p	*		*		*		*
1SS grade 1	18.9±2.3	NS	19.0±2.1	NS	19.7±2.9	NS	19.9±2.6
p	*		*		*		*
1SS grade 2	19.3±2.0	NS	19.4±2.9	NS	21.7±3.2	NS	21.6±2.7
p	*		*		*		*
1SS grade 3	21.4±8.1	NS	23.8±8.7	NS	24.2±9.5	NS	24.9±9.1
p	*		*		*		*
1SS grade 4	21.5±9.9	NS	21.2±9.1	NS	22.4±10.2	NS	22.5±9.7
p	*		*		*		*

First clinical grade in each disease is compared with controls.

* = Statistical differences with regard to the preceding clinical grade in each conjunctival area.

† = Statistical differences with regard to the immediately topographical location within the same clinical grade.

FNP = Facial nerve paralysis; OCP = Ocular cicatricial pemphigoid, 1SS = Primarily Sjögren's syndrome, NS= No statistical differences

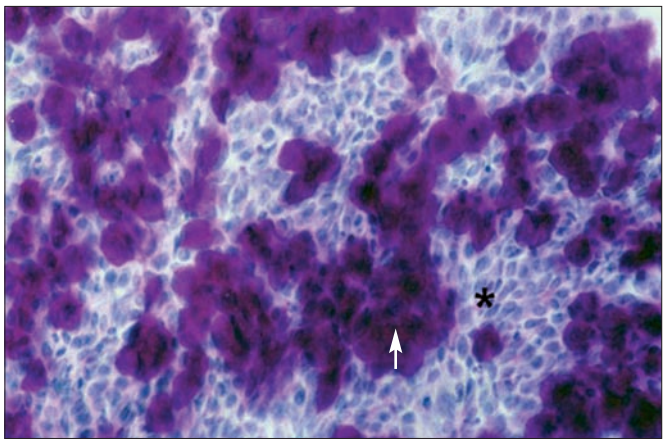

Fig. 1 - *Impression cytology on the lower bulbar conjunctiva in a control subject. Non-secretory cells are small and joined (*). There are a high number of goblet cells with a similar cellular size (↑). PAS–hematoxylin staining. Original magnification x40.*

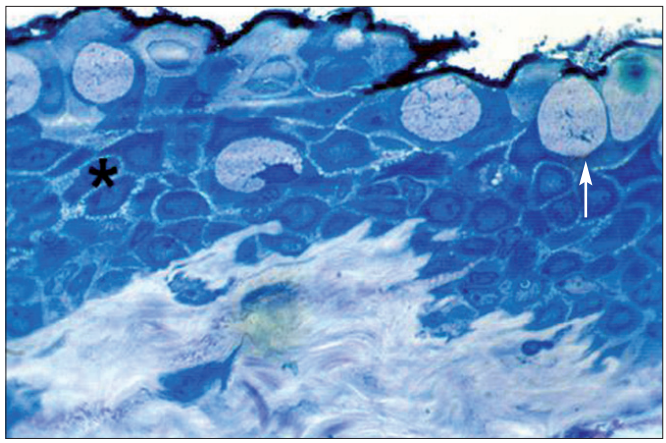

Fig. 2 - *Biopsy on the lower bulbar conjunctiva in a control subject. Non-secretory cells are small and joined (*). There are a high number of goblet cells (↑). Conjunctiva has five cellular layers. Richardson blue staining. Original magnification x40.*

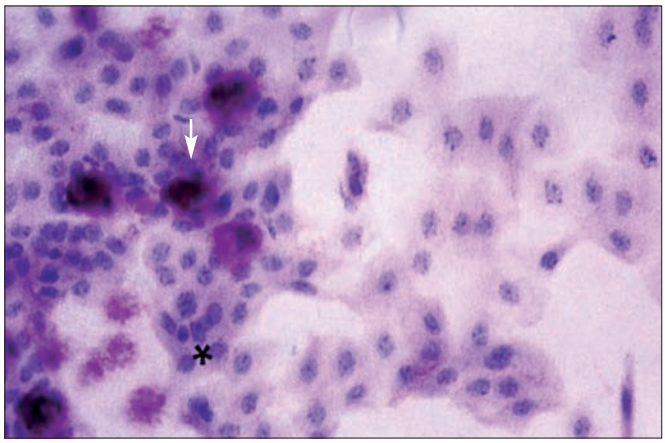

Fig. 3 - *Impression cytology on the lower bulbar conjunctiva in a patient with clinical grade 5 of facial nerve paralysis. Non-secretory cells have a high size and are mildly separated (*). There is a mild number of goblet cells with similar cellular size and plenty of mucin (↑). PAS–hematoxylin staining. Original magnification x40.*

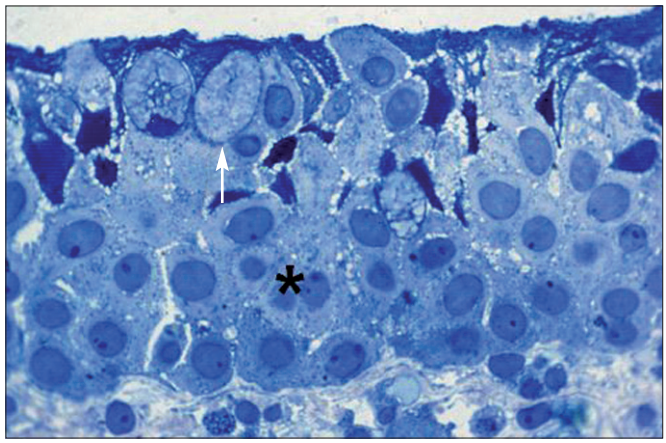

Fig. 4 - *Biopsy on the lower bulbar conjunctiva in a patient with clinical grade 5 of facial nerve paralysis. Non-secretory cells have a high size and are mildly separated (*). There is a mild number of goblet cells (↑). Conjunctiva has five cellular layers. Richardson blue staining. Original magnification x40.*

were abundant goblet cells, more than 400 cells/mm^2, with an average size around 20 microns, and a PAS-positive cytoplasm. By biopsy, non-secretory conjunctival cells were small and joined, forming an epithelium of five layers, with goblet cells present in an abundant number (Fig. 2).

Eyes with FNP, a pure and primarily aqueous-deficient alteration, by impression cytology, according to increase the clinical severity; the non-secretory epithelial cells had a significant increase in cellular size and in cellular separation from the first clinical grade. Goblet cells also had a significant decrease in their density and a progressive decrease in the mean cellular size with regard to the control group. Mucin contents are positive, except in clinical grade 4 (Tabs. V and VI, and Fig. 3). By biopsy, conjunctival non-secretory cells were larger and more separated with regard to controls, forming an epithelium with five layers (Fig. 4), and a moderate number of goblet cells.

Eyes with OCP, a pure and primarily mucous-deficient syndrome, by impression cytology, according to increase the clinical severity; the non-secretory epithelial

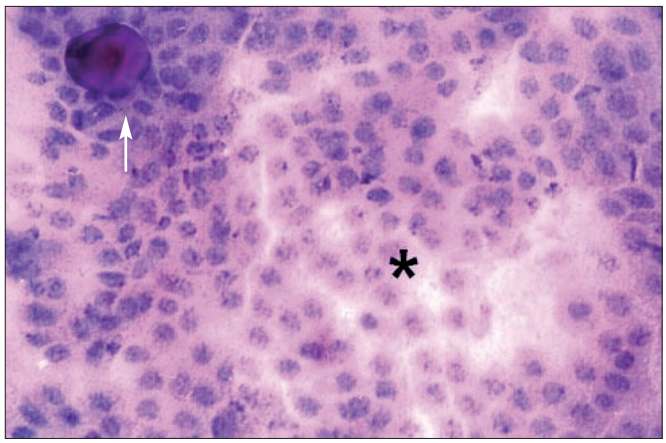

Fig. 5 - *Impression cytology on the lower bulbar conjunctiva in a patient with clinical grade 3 of ocular cicatricial pemphigoid. Non-secretory cells have a mild size and are moderately separated (*). There is one goblet cell with a very high cellular size and empty of mucin (↑). PAS–hematoxylin staining. Original magnification x40.*

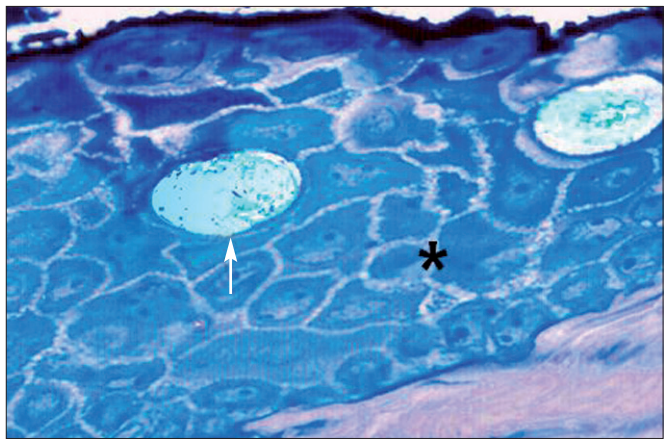

Fig. 6 - *Biopsy on the lower bulbar conjunctiva in a patient with clinical grade 3 of ocular cicatricial pemphigoid. Non-secretory cells have a mild size and are moderately separated (*). There are two goblet cells with a very high cellular size and empty of mucin (↑). Conjunctiva has six cellular layers. Richardson blue staining. Original magnification x40.*

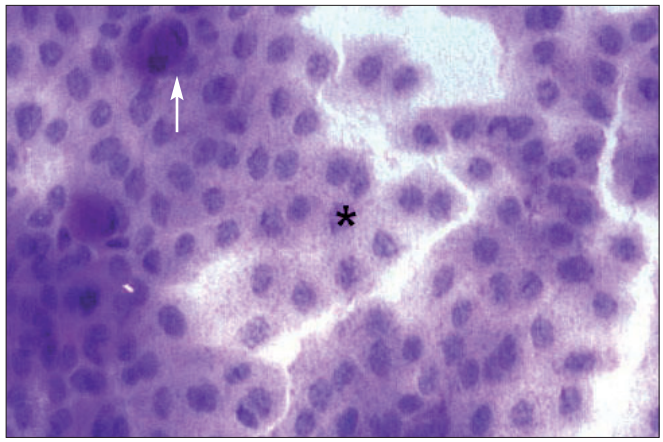

Fig. 7 - *Impression cytology on the lower bulbar conjunctiva in a patient with clinical grade 3 of primarily Sjögren syndrome. Non-secretory cells have a moderate size and are more moderately separated than grade 1 (*). There are very few goblet cells with small quantity of mucin (↑). PAS–hematoxylin staining. Original magnification x40.*

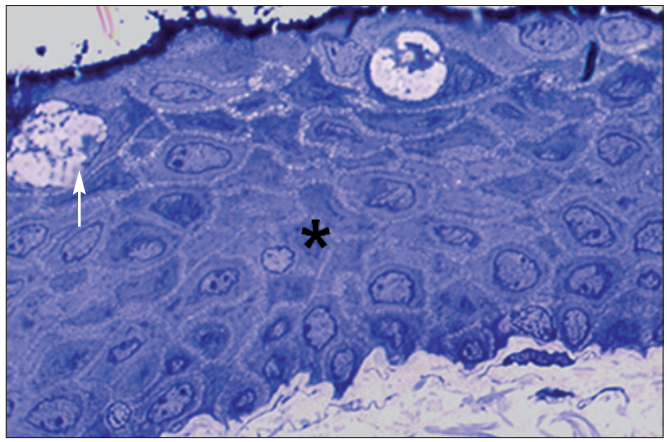

Fig. 8 - *Biopsy on the lower bulbar conjunctiva in a patient with clinical grade 3 of primarily Sjögren syndrome. Non-secretory cells have a moderate size and are more moderately separated than grade 1 (*). There are two goblet cells with small quantity of mucin (↑). Conjunctiva has seven cellular layers. Richardson blue staining. Original magnification x40.*

cells had no significant differences in cellular size and in cellular separation until clinical grade 3, where began a significantly increased size with regard to the control group. Goblet cells had a significant decrease in their density and a significant increase in the average size from the first clinical grade with regard to the control group. Mucin contents are only positive in clinical grades 1 and 2, and from clinical grade 3 usually appeared a PAS-negative cytoplasm (Tabs. V and VI, and Fig. 5). By biopsy, conjunctival non-secretory cells were larger and more separated with regard to controls, but smaller than in patients with FNP, forming a hyperplastic epithelium with more than six layers (Fig. 6), and goblet cells were usually altered and empty.

Eyes with 1SS, a primarily aqueous-deficient and mucin-deficient alteration, by impression cytology, according to increase the clinical severity; the non-secretory epithelial cells had a significant increase in cellular size and in cellular separation from the first clini-

cal grade with regard to the control group. Goblet cells had a significant decrease in their density and a progressive decrease in the mean size from the first clinical grade with regard to the control group. Mucin contents are positive, except in clinical grade 4, where always appeared a PAS-negative staining (Tabs. V and VI, and Fig. 7). By biopsy, non-secretory cells were larger and more separated with regard to controls and to patients with OCP, forming a hyperplastic epithelium with similar number of layers than patients with OCP (Fig. 8). Goblet cells were moderately altered, more than in patients with FNP, but less than in patients with OCP.

DISCUSSION

Based on subjective symptoms, tear clinical tests, characteristics of meibomian glands, and the presence of mucin strands, patients can be categorized as aqueous-deficient dry eyes, non-aqueous dry eyes, and normal (13). Although there is a cycle between aqueous-deficient and mucin-deficient alterations, the physiologic and morphologic characteristics are different in dry eye syndromes according to their etiology (1, 12). Differences in tear osmolarity and lachrymal proteins can also be found between patients with aqueous-deficient syndromes and mucous-deficient ones, because the ocular surface has a particular pattern depending on its needs, including the goblet cells, thus the cellular division in the conjunctiva is minimal in patients with mucous-deficient syndromes, and their cellular response to increase the production of mucin was to increase the cellular volume with the remaining goblet cells (11, 14).

In general, dry eye disorders were accompanied by squamous metaplasia in the whole ocular surface, whether caused by lipid abnormality, aqueous deficiency, or mucous alterations, showing a significant loss of mucin production by a decrease in the goblet cell density. Thus, the severity of the ocular disease was related to the degree of squamous metaplasia (12, 15). This pathologic process, often reversible, denotes the change of one type of adult cell to another and usually represents an adaptation of the eye and other parts of the organism subdued to abnormal environmental changes or to new functional demands. Although many tissues may develop metaplasia, it is most prominent in dry epithelium. The squamous metaplasia was the result of a primarily conjunctival disease, in which the goblet cell differentiation and mucin production were decreased (10, 15). In this study, impression cytology and biopsy showed complementary results, never contradictory.

Aqueous-deficient syndromes suffer less intense internal cellular injury than mucous-deficient syndromes, because the cause of their alterations was generally in a dysfunction of the lachrymal gland, as the FNP that produces a degeneration of the lachrymal gland, without inflammatory attack, affecting only the aqueous layer of the tear film (6, 17), while the cause of mucous-deficient syndromes coming from the bloodstream, as the OCP that is an inflammatory syndrome, involving severely the ocular and oral mucous (8, 16). Finally, 1SS is an autoimmune disorder mainly characterized by attack of cytoplasmic transport proteins in cells of exocrine glands, and was located between the other studied diseases, because 1SS has both tear layer deficiencies, which could help us understand the adaptation response of the ocular surface to dry eye syndromes (14, 18); thus the different etiologies that caused FNP, OCP, and 1SS could explain the morphologic changes obtained in conjunctiva of the studied eyes.

All these results made us suspect that eyes of patients with FNP, a pure and primarily aqueous-deficient alteration, followed completely the squamous metaplasia process, as well in non-secretory epithelial cells as in goblet cells, in each area and in each clinical grade according to the grading system described by Murube and Rivas in 2003 (1, 12).

Patients with OCP, a pure and primarily mucous-deficient syndrome, did not show a characteristic squamous metaplasia process, consisting of a progressive increase of the cellular size in non-secretory cells with a continuous decrease in the density and mean cellular size of goblet cells according to increase the clinical severity. In opposition to FNP, this kind of syndrome had an increase of the cellular size in non-secretory cells only in clinical grade 3, whereas goblet cells undergo severe alterations (density, mean cellular size, and mucin contents). Searching a pathologic model into the group of cellular responses, we have found, in opposition to FNP, that morphologic characteristics of non-secretory epithelial cells and goblet cells described another adaptive process

called hypertrophy. The slightly increased number of epithelial layers in the conjunctiva of these patients also confirms the existence of a hyperplasia process. The last studied group, patients with 1SS, a primarily aqueous and mucous-deficient syndrome, showed in the three first clinical grades similar characteristics to FNP, following a squamous metaplasia process; but from clinical grade 3, the ocular surface of these patients had similar alterations than in patients with OCP, appearing as strong hypertrophy and a slight hyperplasia process.

Although hypertrophy of goblet cells was described in other altered human organs, this is the first time that a dry eye syndrome is described as a hypertrophy alteration. Hypertrophy is a frequent sign of change, leads to a continuous stimulation of goblet cells, which provokes increased cellular work in response to physiologic needs or diseased conditions, usually followed by a process of hyperplasia following a hypertrophy process (4). As well as in squamous metaplasia, hypertrophy and hyperplasia regress when the stimuli are withdrawn. In the same way, we have found that eyes with mucous-deficient syndromes had both pathologic processes.

In conclusion, non-secretory epithelial cells and goblet cells, by impression cytology and by biopsy, might indicate the nature and severity of ocular disease in dry eye syndromes, being an important parameter, directly related to ocular surface health. We suggest that goblet cells and non-secretory epithelial cells help to make a differential diagnosis between an aqueous-deficient syndrome and a mucin-deficient syndrome; patients with primarily aqueous-deficient dry eye follow a squamous metaplasia process and patients with primarily mucous-deficient dry eye had hypertrophy followed by a hyperplasia process. Finally, patients with both tear film deficient dry eyes have squamous metaplasia, hypertrophy, and hyperplasia.

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Reprint requests to:
Luis Rivas, MD
Ophthalmology Service
University Hospital Ramon y Cajal
Ctra. de Colmenar, km. 9
28034-Madrid, Spain
dr.rivas.oftalmologia@infonegocio.com
lrivas.hrc@salud.madrid.org

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