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Evaluation of Ocular Surface Changes Using Impression Cytology in Acne Vulgaris Patients

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Abstract: Purpose: Acne vulgaris is a disease with no known ocular involvement and is most commonly seen in the younger population. The aim of this study was to examine the ocular surfaces of acne vulgaris patients with no ocular signs and symptoms by impression cytology.

Methods: The study groups included 74 eyes of 37 acne vulgaris patients and 28 eyes of 14 healthy control subjects from the same age group. Schirmer I test, the tear break-up time test and the conjunctival impression cytology were performed in each patient.

Results: We observed no difference between

the tear break-up time and the Schirmer's test values between the two groups. However, there was a statistically significant difference between the two groups in terms of impression cytology results ($p=0.0001$).

Conclusion: It was determined that impression cytology properties were altered in patients with acne vulgaris although there was no ocular involvement. Consequently, we suggest that acne vulgaris patients require more detailed ophthalmologic evaluation

Key Words: Acne vulgaris, impression cytology, tear function tests

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Introduction

Acne vulgaris is a disease of pilosebaceous follicles characterized by comedones, papules, pustules, nodules and formation of cysts, mostly encountered between 12 and 25 years of age. At these ages, the dermal lesions may have physical and psychological effects.

The primary defect in acne is the formation of a keratinous plug in the infundibulum of the hair follicle. Two major factors in the formation of keratinous plugs are androgenic stimulation of sebaceous glands and colonization of follicles by *Propionibacterium acnes*. The occurrence of acne secretion at puberty explains the usual onset of acne at that period of age. Much evidence suggests that liberation of free acids by the metabolic activity of propionibacterium acnes is the basic factor in acne vulgaris. The pathology in the development of acne lesions may progress as abnormal keratinization that causes the obstruction of the follicle, increase of sebum production in the obstructed follicle, increase of microorganisms such as propionibacterium acnes in this media and formation of inflammation (1-4).

Impression cytology is a non-invasive, easy-to-perform method that lies between clinical and histological examination, and it depends on collecting a layer of cells from the conjunctival surface. Egbert et al. introduced this technique in 1977 (5). The Schirmer test is used to assess the tear volume and at the same time its flow velocity (6). Break-up time (BUT) is a qualitative test that assesses the tear film layer (6).

This study was designed to analyse the ocular surface and tear function tests in patients with acne vulgaris since this disease is known to have no ocular involvement, it affects younger population, it has no proven etiology and its treatment modalities are still under investigation (7-9). As far as we are aware, this is the first study in which ocular surface changes in acne vulgaris patients are examined by impression cytology.

Materials and Methods

This study includes 74 eyes of 37 nodulo-cystic acne vulgaris patients with no ocular complaint, findings or treatment, and 28 eyes of 14 healthy control subjects

from the same age group. None of the acne vulgaris patients underwent treatment for acne vulgaris before or during this study. The mean age was 21.40 years (range: 16-36y) in acne vulgaris patients and 20.56 years (range: 16-30y) in the control group.

All patients underwent the Schirmer I test, BUT examination and conjunctival impression cytology following routine ophthalmologic examination. The Schirmer 1 test which involves measuring the wetting of standard Schirmer test strips placed on unanesthetized eyes for 5 minutes in a dark room. A wetting of at least 15 mm was accepted as normal. BUT was the period in seconds which the first dark break was observed after blinking in an eye into which 2% fluorescein had been dropped. BUT longer than 10 seconds was accepted as normal (6).

Conjunctival impression cytology was carried out by placement of a triangular-shaped (3x3x4 mm) GSWP cellulose acetate filter paper on the inferior nasal conjunctiva, 2 mm away from the limbus after instillation of 0.4% benoxinate hydrochloride (Novesin). When the paper was in total contact with the conjunctiva, it was lifted and fixated in 95% ethyl alcohol. The samples were dyed by periodic acid Schiff (PAS) and Papanicolau, and then were examined under light microscope. Results were graded according to Nelson (10):

Grade-0 (normal): Epithelial cells small and round, nucleus/cytoplasm (N/C) ratio around 1/2. Goblet cells numerous, ovaloid with PAS (+) cytoplasm.

Grade I (slightly abnormal): Epithelial cells slightly larger and polygonal, N/C ratio 1/3. Goblet cells lower in number but well formed, ovaloid, and with PAS (+) cytoplasm.

Grade II (abnormal): Epithelial cells larger and polygonal, N/C ratio between 1/4 and 1/5. Goblet cells significantly lower in number, weaker and with PAS (+) cytoplasm.

Grade III (significantly abnormal): Epithelial cells larger and polygonal, their nuclei are small, pycnotic, N/C ratio 1/6, goblet cells not present. Statistical analysis was carried out with the X² test.

Results

While the results of Schirmer I and BUT tests were within normal limits in all 74 eyes of the acne vulgaris patients and all 28 eyes of the 14 healthy control subjects (Table 1), the results of the impression cytology differed between the two groups. In acne vulgaris patients, impression cytology was grade 0 in 25 eyes (33.8%), grade I in 34 eyes (45.9%), and grade II in 15 eyes (20.3%). In the control group, impression cytology was grade 0 in 22 eyes (78.6%) and grade I in 6 eyes (21.4%). The difference between the groups was statistically significant (p=0.0001) (Table 2).

	Schirmer (mm.) Mean±SD	BUT (sn) Mean±SD
Acne vulgaris patients	25.4±5	13.1±4
Healthy control Individuals	22.8±3	15.3±6

Table 1. Schirmer 1 test and BUT values of acne vulgaris patients and control subjects.

Chi-square test, p>0.05

	Grade 0	Grade 1	Grade 2	Total
Acne vulgaris patients	(25) 33.8%	(34) 45.9%	(15) 20.3%	(74) 100%
Healthy control subjects	(22) 78.6%	(6) 21.4%	(0) 0%	(28) 100%
Total	(47) 46.1%	(40) 39.2%	(15) 14.7%	(102) 100%

Table 2. Comparison of impression cytology results in acne vulgaris patients and healthy control subjects.

Chi-square test, p=0.0001

Discussion

Impression cytology of the conjunctiva is an important diagnostic tool in investigating ocular surface disorders such as xerophthalmia, keratoconjunctivitis sicca, ocular pemphigoid, vernal conjunctivitis and Stevens-Johnson syndrome (11-14).

Since the cell density and characteristics of the conjunctival surface may differ according to localization (15), and the changes in ocular surface disorders are first observed in bulbar then palpebral conjunctiva (16), in the present study, all samples were taken from the inferior nasal conjunctiva.

The normal conjunctival epithelium is multi-layered and non-keratinized. Its keratinized form is called squamous metaplasia. Metaplastic changes are in the form of a decrease in goblet cell number, an increase in the nucleocytoplasmic ratio (N/C) of surface epithelium cells, widening, and eventual keratinization (17, 18).

Conjunctival keratinization may be seen in various clinical forms such as exposure and drying in ectropion and lagophthalmus, scar formation after trachoma and Stevens-Johnson syndrome, topical drug administration in normal patients, lid and globe tumors and real squamous metaplasia seen in superior limbic keratoconjunctivitis (18). There were no patients in our study group who did not present any of these clinical forms. Rivas et al. suggested that contact of the epithelium with air and exposure to environmental factors might explain the transformation of the cells in this region (16). They added that changes might be observed in the limbal stem cells, conjunctival epithelium and stroma after chronic exposure to ultraviolet light (19). In an impression cytology study where the conjunctiva in the pterygium region was compared with a conjunctiva away from the pterygium a significant difference was detected, whereas impression cytologic examinations revealed different degrees of squamous metaplasia in the whole interpalpebral region (20).

In our study, although the control group was made up of healthy individuals similar ages who had been exposed to the same environmental factors, the fact that grade II metaplasia was not observed in healthy subjects while it was observed in 20.3% of acne vulgaris patients may exclude the possibility that the results might have been affected by environmental factors and ultraviolet

exposure. Blepharoconjunctivitis and particularly *Staphylococcus aureus* blepharitis are common in acne vulgaris (4). It has been suggested that the inflammatory changes in the tissue may be responsible for the formation of squamous metaplasia (21). However, Nelson reported that he never encountered grade II and grade III changes in blepharitis (11). None of our acne vulgaris patients had a history or complaint of a blepharoconjunctivitis. Maumenee suggests that conjunctival keratinization may even be encountered when the tear film is normal (18). Erda et al. have reported that Schirmer I and BUT tests may reveal normal results in spite of the formation of metaplasia in conjunctiva (22). The normal values of Schirmer I and BUT tests obtained from our acne vulgaris patients with grade II metaplasia correlate well with these findings. Another interesting condition in which the tear quantity and quality are normal despite the formation of a real metaplasia in conjunctiva is vitamin A deficiency (18). Wolbach has demonstrated that vitamin A is essential for the normal differentiation and survival of the mucosal epithelium, and that its deficiency causes a metaplasia of mucosal epithelium to keratinized squamous epithelium. This process is observed diffusely in bulbar conjunctiva (23). Ocular surface investigations with impression cytology in Down syndrome patients have revealed a significantly higher rate of squamous metaplasia than in normal individuals. This difference is thought to arise from a metabolic change in elements such as vitamin A (24).

In light of these findings, the squamous metaplasia detected in our acne vulgaris patients might have resulted from the multi-factorial mechanisms responsible from the general ethio-pathogenesis of the disease. Moreover, other possible mechanisms that have been suggested for the development of metaplastic changes in other disorders might also have played a role here.

Consequently, although no ocular pathology has yet been reported to be associated with the disorder, the results of our study suggest the presence of squamous metaplasia in the bulbar conjunctiva of acne vulgaris patients. We suggest that more detailed ophthalmologic examinations are required for acne vulgaris patients and that more detailed studies should be conducted to explain the mechanism of squamous metaplasia formation in these patients.

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