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CLINICAL INVESTIGATIONS

Impression Cytology, Tear Film Break Up, and Schirmer Test in Patients With Inactive Trachoma

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Abstract: Schirmer I and tear film break up time (BUT) tests were used to determine cytological changes and conjunctival surface epithelial morphology was investigated using impression cytology in patients with inactive trachoma; patients with mild and severe scarring, and control subjects, were compared. Schirmer I, BUT, and goblet cell populations were significantly lower whereas the grade of squamous metaplasia was significantly higher in patients with inactive trachoma. There was a close correlation between our findings and the clinical severity of trachoma. **Jpn J Ophthalmol 1997;41:305–307** © 1997 Japanese Ophthalmological Society

Key Words: Impression cytology, inactive trachoma, Schirmer I, tear film break up time.

Introduction

Trachoma is the greatest single cause, worldwide, of preventable blindness:[1] 400 million people may be afflicted by this disease and six million are blind.[2] Acute trachoma is characterized by bilateral follicular nonpurulent conjunctivitis. The greatest morbidity occurs, however, when chronic inflammation leads to scarring of the conjunctiva. This scarring may lead to obliteration of the lacrimal and meibomian ductules, and destruction of accessory lacrimal glands and goblet cells with subsequent dry eye, trichiasis, and entropion.[3,4] Mucus production decreases because of goblet cell destruction and is followed by drying of the conjunctiva and eventual keratinization of the epithelial surface.[5,6]

Although damage from trachoma is clinically apparent, the correlation between the degree of scarring and cytological changes has not been well documented. In the present study, we used impression cytology to investigate this correlation in patients with inactive trachoma.

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Patients and Methods

Twenty-four eyes of 16 patients with inactive trachoma, selected from outpatients of our eye clinic, were included in this study. Selection criteria included an absence of signs of active trachoma, and presence of the characteristic conjunctival scars, Herbert's pits and pannus. Subjects were divided into two groups of 14 eyes with mild scarring and no entropion, and 10 eyes with severe scarring, entropion, and trichiasis. In the group with mild scarring, there was no constriction of the cul-de-sac; there was a superior micro-pannus in one eye. In the group with severe scarring, there were varying degrees of pannus involving no more than 1/3 of the cornea (three eyes), and minimal constriction of the cul-de-sac (two eyes). Normal control eyes (32) of 16 subjects without ocular complaints and with normal ocular examinations were also studied.

All subjects had biomicroscopic ophthalmic evaluations for clinical signs of conjunctival cicatrization, follicular reaction, pannus, lid sequelae, corneal lesions, and Herbert's pits. Schirmer I and tear film break up time (BUT) results were obtained for all groups.

A cellulose-acetate filter paper (Sartorious-11107-50-N) was cut into 4 × 5 mm strips with a pointed tip

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at one corner, to facilitate manipulation of the paper. After topical anesthesia with oxybupiprocaine solution, blunt smooth-ended forceps were used to grasp the point and apply the filter paper to the upper central palpebral and bulbar conjunctiva. The paper was carefully peeled off after 3 seconds; cells on the filter were fixed in 96% ethanol for at least 10 minutes. The samples were stained with periodic acid (Schiff) and hematoxylin.[7]

Quantitative studies of conjunctival goblet cells were done with a calibrated grid at ×200 or ×400 magnification. Mean goblet cell densities were reported per square millimeter ± standard deviation (SD); Nelson's[8] grading system was used. Statistical analysis was done using ANOVA, LSD, and the Kolmogorov-Simirnov test.

Results

Average patient age was 45.31 ± 19.87 years (range: 19–71); control group, 53.69 ± 6.95 years (range: 43–72). The groups did not differ significantly in age or sex.

Table 1. Mean Results of Schirmer I, BUT, Goblet Cell Densities and Grades by Compression Cytology for Each Group

Group	x ± SD	F	*P*
Schirmer I			
Control	24.12 ± 5.36		
Mild	13.71 ± 3.77	45.07	<0.001
Severe	9.60 ± 2.63		
BUT			
Control	14.22 ± 2.69		
Mild	12.22 ± 4.18	9.43	<0.001
Severe	9.4 ± 2.37		
MGCD of Palpebral Conjunctiva			
Control	503.88 ± 37.08		
Mild	99.80 ± 76.29	497.39	<0.001
Severe	32.0 ± 46.80		
MGCD of Bulbar Conjunctiva			
Control	418.44 ± 71.56		
Mild	221.93 ± 111.52	30.70	<0.001
Severe	126.60 ± 140.28		
Grades of Palpebral Conjunctiva			
Control	0.06 ± 0.25		
Mild	2.28 ± 0.46	284.55	<0.001
Severe	2.60 ± 0.51		
Grades of Bulbar Conjunctiva			
Control	0.06 ± 0.25		
Mild	1.21 ± 0.89	44.02	<0.001
Severe	2.0 ± 0.94		

MGCD = Mean goblet cell density/mm^2.

Table 2. Double Comparisons of Mean Schirmer I, BUT, Goblet Cell Densities and Grades

Groups	Least SD	Differences Between Means	*P*
Schirmer I			
Control–Mild	2.99	10.41	<0.05
Control–Severe	3.36	4.52	<0.05
Mild–Severe	3.94	4.11	<0.05
BUT			
Control–Mild	1.96	2.0	<0.05
Control–Severe	2.22	4.82	<0.05
Mild–Severe	2.62	2.82	<0.05
MGCD of Palpebral Conjunctiva			
Control–Mild	32.92	404.08	<0.05
Control–Severe	37.22	471.88	<0.05
Mild–Severe	43.60	67.80	<0.05
MGCD of Bulbar Conjunctiva			
Control–Mild	6.43	196.51	<0.05
Control–Severe	7.29	291.84	<0.05
Mild–Severe	8.52	95.33	<0.05
Grades of Palpebral Conjunctiva			
Control–Mild	0.22	2.21	<0.05
Control–Severe	0.25	2.53	<0.05
Mild–Severe	0.31	0.32	<0.05
Grades of Bulbar Conjunctiva			
Control–Mild	0.39	1.15	<0.05
Control–Severe	0.42	1.94	<0.05
Mild–Severe	0.52	0.79	<0.05

Mean Schirmer I and BUT results are shown in Table 1. Double comparisons found significant differences between groups ($P < 0.05$; Table 2). Schirmer I and BUT results were significantly lower in patients with inactive trachoma than in the control group, correlating with the severity of trachoma. Mean goblet cell densities are shown in Table 1; double compari-

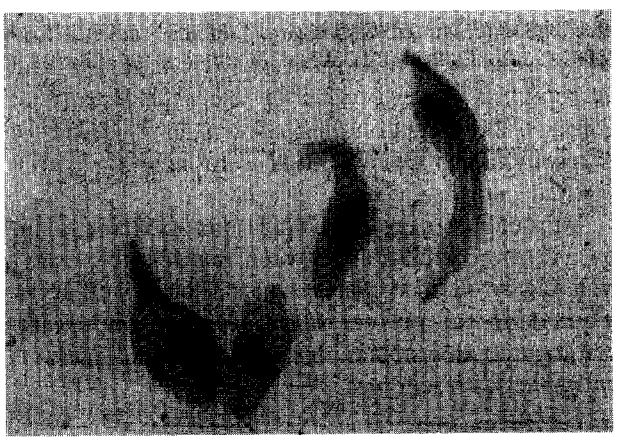

Figure 1. Snake-like chromatin changes in conjunctival epithelial cells (PAS-Hematoxylin stain; original magnification ×400).

sons found significant differences in the groups ($P < 0.05$; Table 2). Goblet cell densities in both the palpebral and bulbar conjunctiva were significantly lower in patients than in the control group. Patients with mild scarring of the conjunctiva had a greater number of cells than patients with severe scarring.

Mean impression cytology grades of the groups are shown in Table 1; double comparisons found significant differences ($P < 0.05$; Table 2), with higher grades found in the control group, correlating with the severity of trachoma. In four eyes (16.66%) of patients with severe scarring, there were "snake-like" chromatin changes in conjunctival epithelial cells (Figure 1).

Discussion

Schirmer I and BUT results were significantly lower in patients with trachoma than in the control group, again correlating with clinical severity. Scarring of the lacrimal ductules and a decrease in the number of goblet cells are responsible for the "dry eye" of patients with inactive trachoma.[3,4]

A study by Blodi et al[9] examined the relationship of clinically inactive trachoma to the impression cytology of the conjunctival surface. They reported that patients with mild scarring of the conjunctiva had a greater number of goblet cells than those with severe scarring, as we found in this study.

The normal conjunctiva is nonkeratinized stratified epithelium. The pathological transition to a nonsecretory keratinized epithelium, termed *squamous metaplasia*, involves a process of abnormal epithelial differentiation.[10] Squamous metaplasia has been identified in several ocular surface disorders including trachoma.[11–18] Tseng et al[19] reported that inflammation and lack of blood supply were major factors in the development of squamous metaplasia. The mean grade of squamous metaplasia was significantly higher in patients with trachoma than in the control group, in both the palpebral and bulbar conjunctiva, correlating with the severity of the disease.

The snake-like chromatin changes in conjunctival epithelial cells were first described by Marner[20] in patients with keratoconjunctivitis sicca. These changes were found in 16.66% of the patients in our study, but their significance is not yet clear.

This study has shown a close correlation between the clinically apparent severity of trachoma and a decrease in Schirmer I and BUT results; and between goblet cell populations and an increase in the grade of squamous metaplasia. We believe that impression cytology is a noninvasive, painless, and efficient technique for evaluating goblet cell population and conjunctival epithelial morphology in chronic trachoma and may be useful in monitoring conjunctival surface changes during topical therapy.

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