

■ **Original article**

The conjunctival impression cytology between the diagnosed cases of dry eye and normal individuals

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Abstract

Background: The dry eye or tear film dysfunction is a common ophthalmic syndrome.

Objective: To compare the results of conjunctival impression cytology between dry eye patients and normal individuals.

Subjects and methods: A case control study including consecutive cases of dry eye syndrome was carried out. Individuals without dry eye were taken as control. Impression of conjunctiva with cellulose acetate filter paper was taken from inferonasal bulbar conjunctiva and was stained with Periodic Acid- Schiff (PAS) and counter-stained with haematoxylin and eosin.

Main outcome measure: goblet cell density.

Results: There was a female preponderance in dry eye disease. Of 114 dry eye cases, 49.2% eyes showed decreased or absent goblet cell density. In 72 normal individuals 73.7% eyes showed normal goblet cell density and 26.3% of eyes showed decreased or absent goblet cells ($p < 0.001$). The tear break-up time (TBUT) test was significantly more likely to be less than 10 seconds in cases as compared to the controls (OR = 19.36, 95% CI = 7.56 – 52.52). Similarly, the goblet cell density was likely to be significantly reduced in cases with dry eye syndrome (OR= 2.25, 95% CI = 1.26 – 4.02, $p = 0.003$).

Conclusion: Goblet cell density significantly reduces in dry eye syndrome. The impression cytology is a useful test for the diagnosis of dry eye syndrome.

Key words: impression cytology, conjunctiva, dry eyes, tear film

Introduction

Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface (i.e. exposed eye surface) and is associated with symptoms of ocular discomfort (National Eye Institute Workshop Report, 2003). The tear film not only keeps the surface of the eye moist but also acts as an important optical medium and prevents from

damages that take place due to trivial trauma to eye. The cause of dry eye must be determined because it helps identify how aggressive the therapy should be. In addition, potential toxic therapies and side effects of unnecessary or ineffective medications can be avoided (Nelson, 1994). Egbert et al (1977) first introduced ocular surface impression cytology into ophthalmic practice. These authors used cellulose acetate filter paper for collection of cells from the conjunctival superficial layer. This method subsequently got modified by several authors (Nelson et al 1983, Nolan et al 1994) for investigation of dry eyes, staging conjunctival squamous metaplasia, diagnosis of vitamin A deficiency, ocular cicatricial pemphigoid limbal stem cell failure, microbiological

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and viral infections and the diagnosis of ocular surface squamous neoplasia.

Impression cytology provides an alternative to conjunctival diagnostic excision biopsy or conjunctival smears made from scrapes taken with a blunt spatula (Dart, 1997). It is known that the cellulose acetate filter paper pressed onto the ocular surface takes off one to three cell layers of the surface epithelium, preserving its morphology and permitting the use of a limited range of histological techniques^{7, 8}. For comparison, conjunctival scraping destroys much of the morphological information and conjunctival biopsy provides information of a relatively small sample of the surface epithelium. Impression cytology is, therefore, the sampling technique of choice to provide the surface epithelium as the target tissue of interest rather than the basal epithelium or basement membrane (Dart, 1997).

This study was carried out to evaluate and compare conjunctival impression cytology results between dry eye patients and normal individuals.

Materials and methods

It was a hospital based case control study done between Jan 1, 2005 – July 1, 2006 using a convenient sampling method including the consecutive diagnosed cases of dry eye syndrome. Age and gender matched normal individual without dry eyes symptoms were selected from the general OPD. An especially designed pro forma was used to collect particulars of the patients, history, clinical examination findings, visual acuity, grading of dry eye and of impression cytology. An informed consent was taken from all the cases before including them in the study. The cases that had undergone surgeries like cataract, glaucoma, pterygium or other surgeries were excluded. External ocular diseases like conjunctivitis, corneal ulcer, episcleritis, and scleritis; and history of use of drugs like steroids until a month prior to the study were the other exclusion criteria.

For diagnosis of dry eyes, the following diagnostic criteria were used.

1. Symptoms of dry eye like burning sensation, foreign body sensation, photophobia, heaviness of eyes etc

OR

2. Signs of dry eye like presence of mucin debris, superficial punctate keratopathy (SPKs), corneal

filaments and corneal abrasion

OR

3. Schirmer's test value of less than 10 mm in 5 minutes

OR

4. Tear break-up time (TBUT) of less than 10 seconds

Before taking the conjunctival impression for cytological evaluation, a limited physical examination was done that included a slit-lamp evaluation for every case, measurement of tear secretion using the standard Schirmer's test and tear film stability using the TBUT in accordance with the standards set by the International Ophthalmology Clinic (IOC), 1994 (Nelson, 1994; David et al 1994).

Materials used were as follows.

1. Cellulose acetate filter paper
2. Absolute methyl alcohol
3. Haematoxylin
4. Periodic acid
5. Schiff reagent
6. Water, distilled
7. Xylene
8. Staining dishes
9. Slide tray
10. Glass slides
11. Cover slip

Specimen collection procedure

1. After instilling a drop of 4% xylocaine for topical anesthesia to each eye, excessive tear fluids were wiped out. One minute of time was given for anesthesia to work.
2. The filter paper was applied to the inferionasal bulbar conjunctiva using a pair of smooth and flat-ended forceps. Whenever there was an unwanted tearing excessively moistening the filter paper, it was discarded and another one was applied after wiping out the tears.
3. The filter paper was placed onto the ocular surface by holding with the forceps tips at each of the four corners of the paper and gently smoothed against the ocular surface.
4. The filter paper was removed by picking up its tip with the same forceps following a 'peeling' maneuver from the ocular surface.
5. The filter paper was placed on the glass slide with albumin paste to transfer the specimen to the slide.
6. The filter paper was then removed from the slide.

7. The slide was labeled and serially numbered
8. The slide was kept at room temperature.
9. The slides were stained with Periodic acid-Schiff (PAS) and counter stained with haematoxylin and eosin.

Each mounted slide was examined under microscope with 10 x high power field (HPF). The cells were localized first then examined with 40 x HPF magnification. At least 10 HPF were examined for goblet cells and epithelial cells. Grading of cytology was done according to Nelson's grading system (Nelson et al 1983).

Nelson's Grading system of conjunctival impression cytology

Grade 0: The epithelial cells are small and round. The nuclei are large. The goblet cells are abundant, plump and oval.

Grade 1: The epithelial cells are slightly larger and more polygonal. The nuclei are smaller. The goblet cells are decreased in number; however they still maintain their plump and oval shape.

Grade 2: The epithelial cells are larger, more polygonal and occasionally multinucleated. The nuclei are small. The goblet cells are markedly decreased in number and are smaller with well-defined cellular borders.

Grade 3: The epithelial cells are large and more polygonal with the nuclei small and pyknotic. The goblet cells are completely absent.

Grade 0 and 1 were regarded as normal whereas grade 2 and 3 as abnormal cytology.

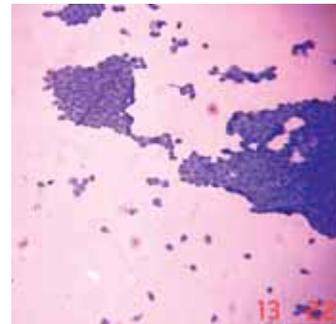
Data processing and analysis

The data were entered into the computer database for statistical analysis. The SPSS 11.0.1 version was used for this purpose. Fisher's Exact test was employed to test for the statistical significance of the results of conjunctival impression cytology.

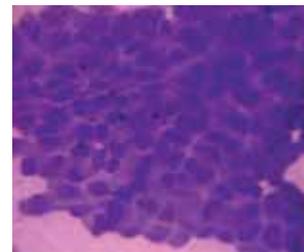
Results

A total of 205 samples from cases of dry eye were collected. The 19 (9.2%) slides were unreadable leaving behind 186 readable ones. This was probably because of the default of Schiff's reagent. Of 186 slides, 114 were dry eye cases and 72 were normal individuals. Out of 114 dry eye cases 58 (50.8%) slides showed normal cytology and 56 (49.2%)

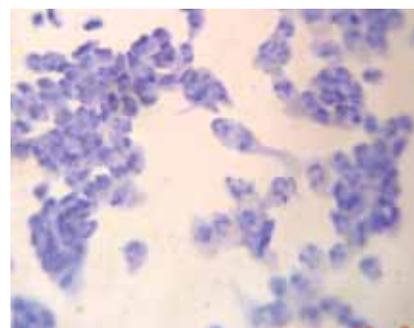
showed abnormal cytology. Out of 72 normal individuals 53 (73.7%) had normal cytology and 19 (26.3%) had abnormal cytology.



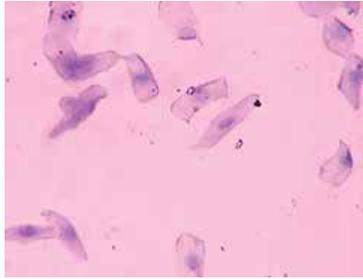
Photograph 1: Grade 0 (Nelson's classification) conjunctival impression cytology from normal individual examined under 40 X high power field magnification



Photograph 2: Grade 1 (Nelson's classification) conjunctival impression cytology from normal individual examined under 40 X high power field magnification.



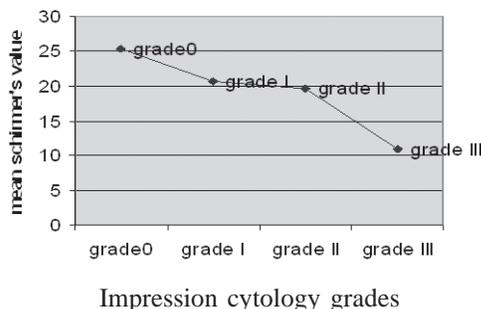
Photograph 3: Grade 2 (Nelson's classification) conjunctival impression cytology from patients of grade III dry eye (Murube del clinical classification) examined under 40 X high power field magnification.



Photograph 4: Grade 3 (Nelson's classification) conjunctival impression cytology from patients of grade IV dry eye (Murube del clinical classification) examined under 40 X high power field magnification.

The age of the patients ranged from 13 years to 74 years in dry eye cases and from 14 years to 80 years in normal individuals. A maximum number of cases was within the range of 21-30 years comprising 30.7 % followed by the group of 10 - 20 years (22.8 %). In normal individuals group also maximum number of patients was within the age range of 21 -30 years (29.2 %) followed by the range of 41 - 50 years (22.3 %). Out of 114 dry eye cases, there were 75 female and 39 male. Out of 72 normal individuals, 37 were female and 35 were male. The male to female ratio was 1 : 2 in dry eye cases and roughly 1 : 1 in normal individuals. The mean age of male was 29.6 and 33.4 years in dry eye cases and normal individuals respectively. The mean age of female was 34.4 years and 40.7 years in dry eye cases and normal individuals respectively.

Fig 1: The Schirmer's test value and conjunctival cytology of subjects with dry eye and normal individuals



The decreasing Schirmer's value is evident with an increasing trend of severity of cytology.

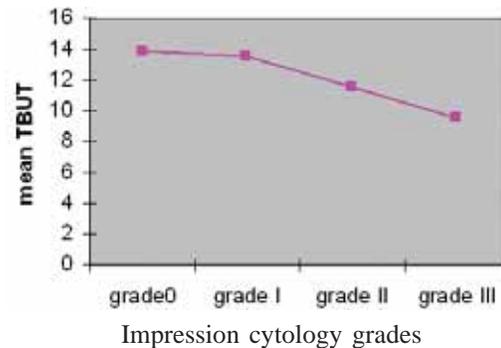
Table 1

The Schirmer's values and conjunctival cytology in dry eye cases and normal individuals

Schirmer's value in mm in 5 minutes	Dry eyes (n = 228)		Normal eyes (n = 144)	
	Normal cytology	Abnormal cytology	Normal cytology	Abnormal cytology
< 5	18 (7.8 %)	49 (21.5 %)	0	3 (2.1 %)
5-10	5 (2.2 %)	16 (7 %)	0	5 (3.5 %)
>10	93 (40.8 %)	47 (20.6 %)	106 (73.6 %)	30 (20.8 %)
Total	116 (50.8 %)	112 (49.2 %)	106 (73.6 %)	38 (26.4 %)

The table 1 shows that in dry eyes with the Schirmer's value of less than 10 mm in 5 minutes 28.5% had an abnormal cytology and only 10% had a normal cytology, whereas, only 8 normal eyes with abnormal cytology had the Schirmer's value of less than 10 mm in 5 minutes.

Fig 2: The TBUT value and conjunctival cytology of dry eye and normal individuals



The figure 2 shows that the patients having the TBUT value more than 10 sec had a normal cytology while the decreasing level of TBUT was associated with increasing severity of conjunctival impression cytology.

Table 2
Mean TBUT value and conjunctival cytology

TBUT in seconds	Dry eyes (n = 228)		Normal eyes (n = 144)	
	Normal	Abnormal	Normal	Abnormal
<5	9 (4.0 %)	11(4.8 %)	0	0
5-10	41(17.9 %)	62 (27.2 %)	1(0.7 %)	6 (4.2 %)
>10	66 (28.9 %)	39 (17.2 %)	105 (72.9 %)	32 (22.2 %)
Total	116 (50.8 %)	112 (49.2 %)	106 (73.6 %)	38 (26.4 %)
Odds ratio*	19.36	95% CI*	7.56	- 52.52

*For TBUT values < 10 sec and >10 sec.

The table 2 shows that among the subjects of dry eyes with the TBUT less than 10 sec, 32.0 % had an abnormal cytology, whereas those having more than 10 sec, 17.2 % had an abnormal cytology. The majority of normal eyes (95.1 %) had TBUT more than 10 sec, among which 72.9 % had a normal cytology and only 22.2 % had an abnormal cytology.

Discussion

Among the 186 subjects, 114 were dry eye patients and 72 were normal individuals who did not have any symptoms of dry eyes. In this study, 53 % of cases of dry eyes were found to have normal cytology and 46.9 % had an abnormal cytology i.e. decreased goblet cell density ($p < 0.001$). In the normal individuals group 75.7 % were found to have a normal cytology with normal goblet cell density and only 24.3% had an abnormal cytology ($p < 0.001$).

This study is comparable to the study done by Murube & Rivas (2003), which showed a significant difference in goblet cell densities from conjunctiva of clinically normal eyes and those with dry eyes. They found that the density of goblet cells significantly decreased in cases of dry eyes in comparison to normal eyes.

Another similar study done by Nelson et al (1963) also demonstrated a decrease in goblet cell density in dry eye patients as compared to normal individuals. They found that 85.0 % of normal individuals (n =

Table 3
Murube del clinical classification of dry eye and conjunctival cytology

Murube del clinical classification of dry eye	Grading	Conjunctival impression cytology		Total	P - value		
		Normal	Abnormal				
Grade I		27	7	34	0.0005		
		11.8 %	3.0 %	14.9 %			
	Grade II		55	51		106	0.93
			24.0 %	22.6 %		46.6 %	
		Grade III		34		46	
	15.0 %		20.1 %	35.1 %			
Grade IV		0	4	4	0.05		
		-	1.8 %	1.8 %			
Grade V		0	4	4	0.05		
		-	1.8 %	1.8 %			
Total		116	112	228	0.0005		
Odds ratio*		2.25	95% CI*	1.26 - 4.02			

*For grades I & II vs III – V (**P value = 0.003**)

The table 3 shows division of the dry eye cases into five clinical subgroups according to the Murube del classification. In grade I, 27 (22.6 %) eyes had a normal cytology out of 34 eyes. In grade II, 55 (46.2 %) eyes had a normal cytology out of 106 eyes. In grade IV and V, 4 eyes out of 4 had an abnormal cytology in each group.

20) including the patients with blepharo-conjunctivitis had Grade 0 and 15% of them Grade 1 conjunctival cytology ($p < 0.001$). Among the dry eye patients (n = 46), 60.8% had a Grade 3 conjunctival cytology ($p < 0.001$).

We classified the dry eyes cases into 5 clinical grades according to Murube del classification (1977) and found an association of severity of dry eyes and decreased goblet cell density.

Conclusion

The goblet cell density is significantly reduced in cases with dry eyes as compared to normal individuals. As the dry eye can be a diagnostic dilemma many a times we can use this test in our daily practice to solve this problem.



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