

Research Article

Study of conjunctival impression cytology in assessing goblet cell density in dry eyes

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ABSTRACT

Background: Dry eye is a chronic multi-factorial condition characterized by disturbances in the tear film and ocular surface with a decrease in goblet cells which can be assessed by conjunctival impression cytology (CIC) which is as effective as biopsy for diagnostic purposes. It helps establish not only the diagnosis of dry eye but also aids in grading the severity. This is a highly sensitive method to detect pathological changes in the conjunctival surface and confirm the clinical diagnosis. The aim of the study was to determine the goblet cell density by impression cytology, grading the severity of dry eyes and comparing the study with other studies.

Methods: Study was conducted for a period of 2 yrs from August 2011 to July 2013 in 80 patients above 20 yrs age with clinically established diagnosis of dry eye who were subjected to conjunctival imprint cytology and stained with PAS to estimate the goblet cell density.

Results: Out of the 80 cases of dry eye studied 45 were females and 35 were males with a female to male ratio of 1.28: 1 with a slight female preponderance. Age of the patients ranged from 21yrs to 73 yrs. CIC showed positivity in 43 cases (53.75%) and negative in 37 cases (46.25%). 39 cases (90.70%) of positive CIC were above the age of 40 years.

Conclusions: Dry eyes were found to be more common in age group above 40yrs with slight female preponderance. CIC showed decreased goblet cell density with increasing age in clinically diagnosed dry eyes.

Keywords: Dry eye, Impression cytology, Goblet cell density

INTRODUCTION

Dry eye is a chronic, multifactorial condition characterized by disturbances in the tear film and the ocular surface. It can be caused by deficiency of any one or more of the tear film components, or can be a component of systemic diseases, including Sjogren's syndrome, lupus and Stevens-Johnson syndrome. Factors such as wearing contact lens and adverse environmental exposures, windy conditions or visual tasking can exacerbate the symptoms of dry eye. Prevalence of dry eye increases with age. It is estimated that nearly 75% of people over 65yrs will experience dry eye syndrome.¹

Impression cytology provides a qualitative method to evaluate the changes of conjunctival morphology and goblet cell density in dry eyes.² Impression cytology was introduced in ophthalmology by Egbert et al in 1977 which involves taking impression of conjunctiva by cellulose acetate paper and staining the adherent cell layer.³ It is a standard method for study of ocular surface in various conditions such as viral conjunctivitis, keratoconjunctivitis sicca, vitamin A deficiency, chronic renal failure, psoriasis, ocular surface malignancies etc.⁴ But its main advantage has been in the field of dry eye syndrome where there will be depletion of goblet cells. It is as effective as biopsy for diagnosing and grading the severity of dry eye. It helps in grading the severity of dry

eye. This is a highly sensitive method to detect pathological changes in the conjunctival surface and confirm a clinical diagnosis. In the present study, an attempt was made to determine goblet cell density in dry eye patients.

The aim of the study was to determine the goblet cell density by impression cytology in dry eyes and comparing it with the cytology of normal eye, grading the severity of dry eyes and comparing the study with other studies.

METHODS

This is a prospective study conducted in the dept. of Pathology, Andhra Medical College & Regional eye hospital, Visakhapatnam, for a period of 2 years from August 2011 to July 2013. 80 cases of dry eyes and 20 normal eye controls were studied.

Inclusion criteria

Patients equal to and above 20 yrs. presenting with symptoms and signs like burning sensation, sandy & gritty sensation, foreign body sensation, photophobia and heavy lids.

Exclusion criteria

Patients less than 20 yrs. and patients with h/o increased mucoid discharge and watery secretion suggestive of vernal keratoconjunctivitis, alkali burns, trachoma, acute ocular infections, ocular surgery within last 6 months, impaired eye lid function and contact lens users. Written consent of the patient was taken. A complete slit-lamp examination of the lid margins, tear meniscus, conjunctiva, cornea and tear film was done. Tests to diagnose dry eye were performed which included tear break up time (TBUT), ocular surface staining, and Schirmer's test. Those who had at least two tests positive out of the three were labelled as having dry eye.

Procedure of conjunctival impression cytology (CIC)

This test was conducted using cellulose acetate strips having a pore diameter of 0.45 µm with the patient in supine position, a drop of 4% lignocaine was instilled into the eye. Millipore cellulose acetate filter strip was cut into 3x10mm size pieces with a diagonal edge and after inserting a wire speculum the rough surface of the filter paper was applied onto the inferior-nasal bulbar conjunctiva with the help of a blunt, smooth edged forceps. A smooth glass rod was used to press the paper gently. The strip was then removed with a peeling motion after 2-3 seconds. The filter papers were fixed in a fixative solution containing absolute alcohol + glacial acetic acid + 40% formaldehyde in a ratio of 20:1:1 respectively and then stained with Periodic acid Schiff's stain and counter stained with haematoxylin. The filter paper was then mounted and examined under

microscope and graded according to standard criteria proposed by Nelson.⁵

Grading of squamous metaplasia as proposed by Nelson³

Grade 0

The epithelial cells are small, round with eosinophilic cytoplasm, nuclei are large, basophilic with a nuclear cytoplasmic ratio of 1:2 and goblet cells are abundant, plump and oval.

Grade 1

The epithelial cells are slightly larger, more polygonal have eosinophilic cytoplasm, nuclei are smaller with a nuclear cytoplasmic ratio of 1:3 and goblet cells are decreased in number.

Grade 2

The epithelial cells are larger, polygonal, occasionally multinucleated with variably staining cytoplasm, nuclei are smaller with nuclear cytoplasmic ratio of 1:4 to 1:5 goblet cells are markedly decreased in number.

Grade 3

The epithelial cells are larger and polygonal with basophilic cytoplasm, nuclei are small, pyknotic or completely absent. The goblet cells are totally absent.

Interpretation

The eyes having grade 2 or grade 3 changes were considered positive for dry eye. Eyes with grade 0 or grade 1 change were considered negative.

RESULTS

A total of 80 dry eye patients who were positive for at least two of the three clinical diagnostic tests and 20 normal eye patients who were negative for all the three clinical diagnostic tests were taken for study and subjected to conjunctival impression cytology. Age of the patients ranged from 20-65 years and their mean age in dry eye patient was 51.85 years and that of normal subject 42.55 years. Out of 80 dry eye patients 43 patients (53.75%) showed positivity for dry eye on CIC, 37 (46.25%) were negative and all the 20 normal eye subjects were negative. 39 cases (90.70%) of positive CIC were above the age of 40 years. Among the total numbers of clinically diagnosed dry eye patients, females were 45 (56.25%) and males were 35 (43.75%) and female male ratio was 1.28: 1 with a slight female preponderance. In our present dry eye study, female cases were more than male cases.

The proportionate positivity for CIC in patients less than 40 years in clinically diagnosed dry eyes was 9.30% (n=4), and patients more than 40 years was 90.70% (n=39) which was statistically significant, which showed that the goblet cell density decreased with increasing age in clinically diagnosed dry eyes. Out of the 45 females of clinically diagnosed dry eyes, conjunctival impression cytology showed positivity in 23 cases (51.11%) of which 20 cases (86.96%) of positive CIC were above the

age of 40. The proportionate positivity for CIC in female patients above the age of 50 years was 61.9% as compared to female patients below the age of 50 years which was 38.1%, indicating decreased goblet cell density in postmenopausal women. Out of the 35 male cases 20 (57.14%) were CIC positive, of which 19 (95%) were above the age of 40 yrs. and 15 (42.86%) were negative. All the 20 normal subjects who were taken as controls showed negative CIC.

Table 1: Grading of CIC in dry eyes in relation to age and sex.

Age (years)	Dry eyes		Grade 0		Grade 1		Grade 2		Grade 3	
	F	M	F	M	F	M	F	M	F	M
21 – 30	0	0	0	0	0	0	0	0	0	0
31 – 40	10	4	3	2	4	1	3	1	0	0
41 – 50	14	12	2	0	5	6	7	6	0	0
51 – 60	16	10	3	1	3	3	10	6	0	0
>60 yrs	5	9	0	1	2	1	2	7	1	0
Total	45	35	8	4	14	11	22	20	1	0
Total cases of dry eyes on CIC(42+1)=43							42		1	

Table 2: Comparison of the results of present study with other studies.

Studies	No of study patients		Age group	Sex distribution for dry eye		Sex distribution for normal eyes		Dry eye pts. positive for CIC	Normal eye pts. positive for CIC
	Dry eye	Normal eye		M	F	M	F		
Present study	80	20	More than 20 yrs.	35 (43.75%)	45 (56.2%)	10	10	43 (53.7%)	0
Shrestha E et al study ⁷	114	72	13-80 yrs.	39 (34.2%)	75 (65.8%)	35	37	56 (49.2%)	17 (23.6%)
P Varma et al study ¹⁴	20	20	12-65 yrs.	-	-	-	-	4 (20.0%)	0
M Reddy et al study ⁶	30	10	5-45 yrs.	15	15	-	-	23 (76.6%)	0
SS Gadkari et al study ¹⁵	-	30	20-60 yrs.	-	-	17	13	-	0

DISCUSSION

Dry eye is a chronic, multifactorial condition characterized by disturbances in the tear film and the ocular surface, prevalence of which increases with age. It is estimated that nearly 75% of people over 65yrs. will experience dry eye syndrome.⁵ Dry eye disease is one of the most common reasons for patients to visit an eye care professional. Impression cytology is used to evaluate the conjunctival changes that occur in dry eye disease. This is a highly sensitive method to detect pathologic changes in the conjunctival surface and confirm a clinical diagnosis. As early as 1991, Reddy M and associates did a

comparative study of conjunctival biopsy versus impression cytology to study the histopathological changes occurring in conjunctiva in dry eye states and found that impression cytology was as effective as biopsy for diagnostic purposes.⁶

Cases of dry eye positive for CIC in our study were 43 in number with a percentage of 53.7%. Study of Shrestha E et al showed 49.2% positive which is very close to our result.⁷ Of these 39 cases (90.70%) were above the age of 40 years and 4 cases (9.30%) were below the age of 40 year. Dry eye is more common with increasing age because of the decreased tear production due to depletion

of hormones and dysfunction of Meibomian glands.^{8,9} There will be a decrease in reflex secretion of tears with age, particularly after 40 years of age.¹⁰ Ageing can also be associated with decreased tear volume and flow, increased osmolality decreased tear film stability and alteration in the composition of tear film lipids.^{8,11,12} All these factors may be responsible for the increased number of dry eyes after 40 years.

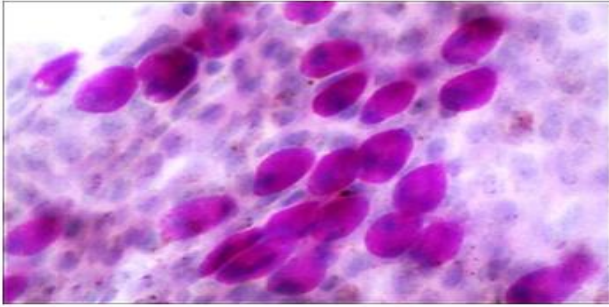


Figure 1: Microscopic picture showing Grade 0 (CIC negative for dry eye): The epithelial cells are small, round with eosinophilic cytoplasm. The nuclei are large, basophilic with a nucleocytoplasmic ratio 1:2. The goblet cells are abundant, plump and oval (PAS, 400X).

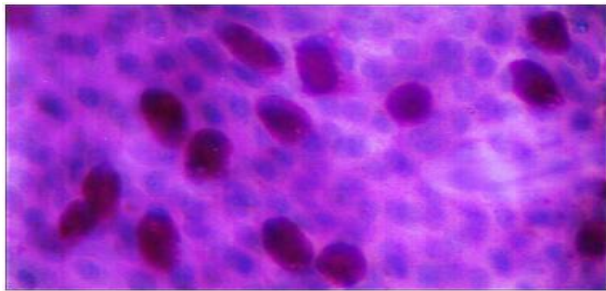


Figure 2: Microscopic picture shows Grade 1: The epithelial cells are slightly larger and more polygonal and have eosinophilic cytoplasm. The nuclei are smaller with a nucleocytoplasmic ratio of 1:3. The goblet cells are decreased in number (PAS, 400X).

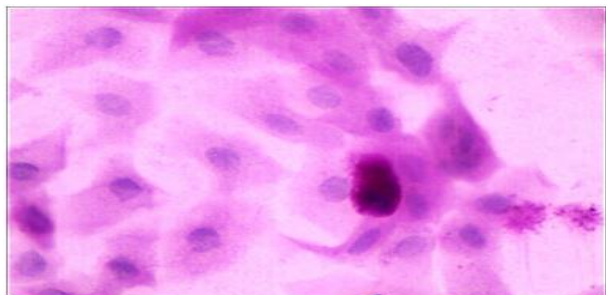


Figure 3: Microscopic picture shows Grade 2: The epithelial cells are larger and polygonal. The nuclei are smaller with nucleocytoplasmic ratio of 1:4 to 1:5. The goblet cells are markedly decreased in number (PAS, 400X).

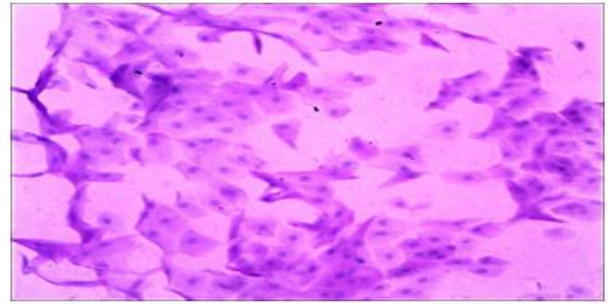


Figure 4: Microscopic picture showing Grade 3 (CIC positive for dry eye): The epithelial cells are larger and polygonal with abundant cytoplasm. The goblet cells are completely absent (PAS, 100X).

Out of the 45 females of clinically diagnosed dry eyes, CIC was positive in 23 cases (51.11%) of which 20 cases (86.96%) were above the age of 40 years. In the present study the proportionate positivity for conjunctival impression cytology in female patients above the age of 50 years was 56.53% as compared to female patients below the age of 50 years which was 43.47%, indicating decreased goblet cell density in postmenopausal women due to hormone depletion. History of estrogen therapy will also play as a risk factor, but in our study there was no history of hormonal usage.¹³

Out of the 35 males of clinically diagnosed as dry eye, CIC showed decreased goblet cell density in 20 cases, of which 19 cases (95.0%) were above the age of 40 years. A reduction in androgens that occur in increasing age may be a factor for this increase after 40 years.¹² In the present study the total numbers of clinically diagnosed dry eye patients, females were 45 (56.25%) and males were 35 (43.75%) and female male ratio was 1.28: 1 with a slight female preponderance, whereas study of Shrestha E et al showed female preponderance with 65.8% positive CIC.⁷

In the present study in clinically diagnosed dry eyes, CIC showed positivity in 43 cases (53.75%) and in Shrestha E et al study, P Varma et al study, and M Reddy et al study the positivity for CIC was 49.2%, 20.0%, and 76.6% respectively.^{6,7,14} Results of our study of dry eye in relation with CIC positivity is comparable to the study of Shrestha E et al.⁷

In our study normal eye subjects showed no positivity on CIC which correlated with the studies of P Varma et al, M Reddy et al and S. Gadkari et al.^{6,14,15}

CONCLUSION

Conjunctival imprint cytology is a simple and reliable procedure which helps in assessing the severity of dry eye by estimating the goblet cell density in clinically diagnosed cases. A total of 100 cases comprising 80 cases of dry eyes and 20 normal eye controls were subjected to conjunctival impression cytology during the study period

of 2 years from August 2011 to July 2013. Out of the 80 clinically diagnosed dry eyes CIC showed positivity (combined grade 2 and grade 3) in 43 cases (53.75%) and negative (combined grade 0 and grade 1) in 37 cases (46.25%). 39 cases (90.70%) of positive CIC were above the age of 40 years which showed that the goblet cell density decreases with increasing age. The proportionate positivity for conjunctival impression cytology in female patients above the age of 50 years was 56.53% as compared to female patients below the age of 50 years which was 43.47%, indicating decreased goblet cell density in postmenopausal women's suggesting the role of hormonal depletion in dry eyes. Results of this study were comparable with other studies.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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