IJPRP

Indian Journal of Pathology: Research and Practice

Volume 7 Number 4, April 2018
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.7418.13

Original Research Article

Conjunctival Impression Cytology in Evaluating Tear Film Status in Small Incision Cataract Surgery

Serah Joseph[1], C.M. Kiran[2], Bhagwati Wadwekar[3], Surendra Nirmale[4]

[1]Senior Resident [3]Assistant Professor [4]Professor, Dept. of Ophthalmology, [2]Associate Professor, Dept. of Pathology, Pondicherry Institute of Medical Sciences, Puducherry – 605014, India.

Corresponding Author:

C.M. Kiran,
Associate Professor,
Dept. of Pathology,
Pondicherry Institute of Medical Sciences, Puducherry – 605014, India.
E-mail:
kirencm@yahoo.com

(Received on 26.02.2018,
Accepted on 24.03.2018)

Abstract

Background: Dry eye is a clinical condition resulting from decreased tear production or increased loss of tears due to evaporation. Conjunctival impression cytology (CIC) helps in evaluating the abnormal tear film status apart from the routine tear function tests.

Aim: We aimed to know the influence of small incision cataract surgery on tear film and assess the changes in tear film by CIC.

Materials and Methods: 101 patients diagnosed with cataract were studied over a period of 2 years. Schirmer's test, tear film break up time, fluorescein staining and lissamine green staining were done pre and post operatively. Conjunctival impression cytology smears stained with periodic acid Schiff (PAS) stain were analyzed pre and post operatively.

Results: Pre-operatively CIC showed 15 patients (14.8 %) with no cells, 72 patients (71.2 %) with squamous cells and 14 patients (13.8 %) with squamous and goblet cells. Post-operatively 36 patients (35.6 %) showed no cells, 52 patients (51.4 %) showed squamous cells and 13 patients (12.8 %) showed squamous and goblet cells. Pre-operatively Schirmer's test showed 90 patients (89.1 %) to be normal and 11 patients (10.9 %) abnormal. Post-operatively 85 patients (84.1 %) were normal and 16 patients (15.9 %) were abnormal. Tear film break up time, flourescein staining and lissamine green staining showed no changes pre and post operatively. CIC and Schirmer's test showed a significant positive correlation with p values of 0.001 and 0.034 respectively. Tear film break up time, fluorescein staining and lissamine green staining showed a negative correlation.

Conclusions: Manual small incision cataract surgery is associated with a significant worsening of tear film stability and CIC is a vital tool with diagnostic accuracy in evaluating tear film status.

Keywords: Cataract; CIC; Dry Eye; Goblet; Squamous; Tear Film.

Introduction

Dry eye per se is not a disease entity, but a symptom complex occurring as a sequelae to deficiency of tears or any abnormalities involving the tear film. The international dry eye workshop 2007 defines dry eye as a multifactorial disease of tears and ocular surface that result in symptoms of discomfort, visual disturbances and tear film instability. The tear film is important in protecting and maintaining a healthy ocular surface. Its stability is attributed to the

harmonious interaction of lipids, mucin and aqueous layer. Interventions such as cataract surgery and corneal refractive surgeries cause significant change in tear film status and result in dry eye. Moreover, the usage of antibiotic drops containing preservatives and the surgical incision site serving as an irregular surface contribute to tear film instability and aggravate dry eye [1,2,3].

Cataract is the most common age related disorder and most of the patients undergoing cataract surgery are old. Secretion of tears decreases with age and cataract surgery further adds up to the toll [1]. The surgical incision site causes tear film instability further aggravating the dry eye. Phacoemulsification is most popular and surgery of choice in the western world and few studies show association of dry eye with phacoemulsification [1,3,5]. In developing countries like India, manual small incision cataract surgery (SICS) is still the choice especially in camp settings done on a large scale [5]. Considering the number of patients undergoing cataract surgery, it becomes important to identify dry eye and initiate appropriate treatment at an early stage.

Routine tear function tests used in assessing dry eye include Schirmer's test, tear film break up time (TBUT), fluorescein staining and lissamine green staining. Conjunctival impression cytology (CIC) is a minimally invasive technique enabling conjunctival changes to be seen at cellular level. Conjunctival goblet cell density and extent of squamous metaplasia can be assessed accurately. To the best of knowledge only one study related to dry eye after small incision cataract surgery has been identified [6]. Hence, we aimed to evaluate the tear film changes in patients before and after small incision cataract surgery and determine whether patients with no apparent dry eye pre-operatively developed altered conjunctival goblet cell density post-operatively.

Materials and Methods

This was a hospital based prospective study in our institute in the Departments of Ophthalmology and Pathology during the period August 2014 and July 2016. The Institutional Ethics Committee of our institute approved the research study and informed consent was obtained from the patients in accordance with the Declaration of Helsinki. 101 patients diagnosed with significant cataract were taken up and included in the study. Patients with pre-existing dry eye, glaucoma, ocular allergy, pterygium, blepharitis and those with complicated cataract (trauma, uveitis, drug induced, chemical burns, radiation, ocular surface diseases) were excluded from the study. Patients using contact lenses and those who had complications during surgery were also excluded from the study. The study was done pre-operatively (one day prior to surgery) and post-operatively (five weeks after surgery).

All the 101 patients were subjected to routine tear function tests like Schirmer's test-I, tear film break up time (TBUT), fluorescein staining and lissamine green staining pre and post-operatively. Conjunctival impression cytology (CIC) was also done pre and post-operatively.

Schirmer's test-I was done using a Whatman filter paper. The filter paper strip was inserted over the lateral 1/3rd of lower conjunctival fornix. The wetness on the strip was measured 5 minutes after insertion. A wetness of size $\geq$ 15 mm was considered normal. A wetness ranging from 6 - 14 mm was considered as mild to moderate dry eye and wetness $\leq$ 5 mm was considered as severe dry eye.

Tear film break up time was measured by noting the time interval between the last complete blink and the first appearance of a random dry spot using a stop watch. A break up time more than 10 seconds was considered normal and noted as 'good'. A break up time less than 10 seconds was considered as dry eye and noted as 'bad'.

Fluorescein staining was done by touching the inferior conjunctival fornix with a wet fluorescein strip. Any break in the corneal epithelial barrier permits rapid penetration of fluorescein and stains those denuded areas when viewed through cobalt blue filter of a slit lamp biomicroscope. Corneal staining with fluorescein was noted as 'yes' and absence of fluorescein staining was noted as 'no'.

Lissamine green staining was done by touching the inferior conjunctival fornix with a wet lissamine green strip. Scoring was given depending on the staining pattern in accordance with Van Bijisterveld grading system. A score < 3 was considered 'normal' and a score $\geq$ 3 being 'abnormal'.

Conjunctival impression cytology was done after instilling local anaesthetic eye drops (4 % xylocaine). A wire speculum was placed in the eye and the patient was asked to look upward and outward. An ultipore nylon filter paper was placed on the nasal and inferior part of bulbar conjunctiva for about two seconds and then peeled off the conjunctiva. The filter paper was then placed over a glass slide and gentle pressure was applied. The imprint cytology slides were immediately placed in a fixative containing 95 % ethyl alcohol for about 2 hours. The alcohol fixed slides were then stained with periodic acid Schiff (PAS) stain and the slides were assessed thoroughly for the presence of goblet cells and squamous epithelial cells under x 400 magnification in Olympus CX 41 microscope. A magenta colored cytoplasm was considered as positive staining for goblet cells.

All the routine tear function tests were repeated post-operatively (after five weeks) and the results were analyzed.

Results

A total of 101 patients were analyzed. The mean age of presentation was 64 years with a M:F ratio of 1.2:1.

Pre-operatively conjunctival impression cytology showed 15 patients (14.8%) in 'no cells' category, 72 patients (71.2 %) in 'only squamous cells' category and 14 patients (13.8 %) in 'squamous plus goblet cells' category. Post-operatively 36 patients (35.6 %) showed 'no cells', 52 patients (51.4 %) showed 'only squamous cells' and the remaining 13 patients (12.8 %) showed both 'squamous and goblet cells'. The cross tabulation of pre-operative and post-operative CIC patients is shown in table 1. Goblet cells are large round cells with eccentrically placed nuclei and abundant cytoplasm (magenta color) indicating mucin (Figure 1).

Pre-operatively Schirmer's test showed 90 patients (89.1 %) with normal eye, 9 patients (8.9%) with mild to moderate dry eye and 2 patients (1.9 %) with severe dry eye. Post-operatively, of the 90 patients with normal eye, 85 patients remained in the same group with 4 patients shifting to moderate dry eye and 1 patient shifting to severe dry eye. Thus totally 85 patients (84.1 %) had normal eye, 13 patients (12.8 %) had mild to moderate dry eye and the remaining 3 patients (2.9 %) had severe dry eye. The cross tabulation of pre-operative and post-operative Schirmer's test is shown in table 2.

Pre-operatively tear film break up time was good in 90 patients (89.1%) indicating normal eye and bad in the remaining 11 patients (10.8 %) indicating dry eye. Post-operatively, no significant change was noted.

Fluorescein staining pre-operatively showed 99 patients (98 %) failing to take up the stain (normal eye) 2 patients (1.9 %) taking up the stain (dry eye). Post-operatively, 95 patients (94 %) showed no uptake of the stain (normal eye) and 6 patients (5.9 %) showed stain uptake (dry eye).

Lissamine green staining pre-operatively showed 99 patients (98 %) with a score < 3 (normal eye) and 2 patients (1.9 %) with a score > 3 (dry eye). Post-operatively, no significant change was observed.

Marginal homogeneity test showed conjunctival impression cytology more statistically significant with a p value of 0.001 followed by Schirmer's test with a p value of 0.034 (Table 3). Tear film break up time, fluorescein staining and lissamine green staining showed a negative correlation with p values of 1.000, 0.687 and 1.000 respectively.

Table 1: Cross tabulation of conjunctival impression cytology : pre-operative versus post-operative

Parameter		Post-operative CIC			Total
		NC	SC	S+G	
Pre-operative CIC	NC	15	0	0	15
	SC	19	51	2	72
	S+G	2	1	11	14
Total		36	52	13	101

NC - No cells, SC - Squamous cells, S+G - Squamous+Goblet cells

Table 2: Cross tabulation of Schirmer's test : pre-operative versus post-operative

Parameter		Post-operative Schirmer's test			Total
		Normal	Mild to moderate dry eye	Severe dry eye	
Pre-operative Schirmer's test	Normal	85	4	1	90
	Mild to moderate dry eye	0	9	0	9
	Severe dry eye	0	0	2	2
	Total	85	13	3	101

Table 3: Marginal homogeneity test of conjunctival impression cytology and Schirmer's test

Parameter	Conjunctival impression Cytology	Schirmer's test
Distinct values	3	3
Off-diagonal cases	24	5
Observed MH statistic	29.000	0.000
Mean MH statistic	40.000	3.000
SD of MH statistic	2.739	1.414
Standard MH statistic	- 4.017	- 2.121
Asymp. Sig. (2 tailed)	0.001	0.034

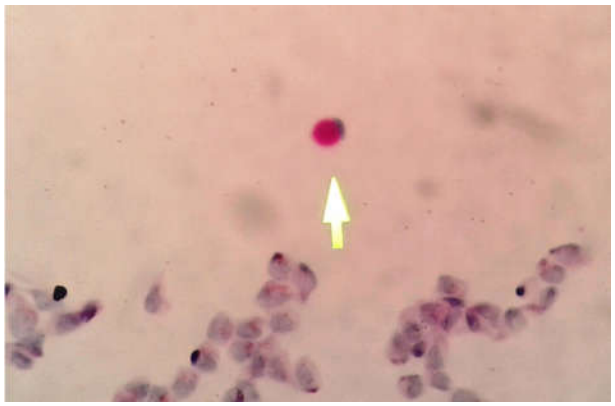

Fig. 1: Goblet cell denoted by an arrow showing abundant cytoplasmic mucin and sheets of squamous cells below (PAS x400).

Discussion

The overall impact of a moderate dry eye in patients has in fact been quantified as being similar to that of moderate angina [7]. Post-operative dry eye following cataract surgery has been implicated as a most important obstacle to patients satisfaction despite an excellent visual recovery. CIC is more objective showing early cytological changes in conjunctiva and cornea which fail to be picked up by routine tear function tests thus increasing its diagnostic accuracy. Schirmer's test and other routine tear function tests are less specific, less sensitive and have a less predictive value [5]. CIC is therefore considered a gold standard for diagnosing dry eye as established by Kumar et. al.

A comparative study of dry eye by Sinha M et. al. showed significant deterioration of CIC and Schirmer's test values which correlated with our study [1]. Other studies by Li et. al., Yu Yinhui et. al. and Ram et al also showed similar findings [3,8,9]. In our study, CIC and Schirmer's test values showed a significant positive correlation proving the fact that surgery resulted in marked deterioration of tear film stability. Other tear function tests showed a negative correlation as highlighted in studies by Doreen et al and Fuentes-Paez et. al. [10,11]. Li et. al. in his study showed a significant reduction in the goblet cell density three months after surgery along with demonstration of squamous metaplasia. Similar findings were reported by Maumenee et al and Oh Taehoon et. al. [12,13].

Analysis of our data using marginal homogeneity test showed Schirmer's test to be statistically significant (p value - 0.034). In contradiction to our study, Versura et . al. showed a poor diagnostic performance of Schirmer's test [14]. A retrospective study by Sullivan et al concluded that patients can have a gross symptomatic dry eye despite the Schirmer's values being greater than 15 mm [15]. Similar findings were observed by Fuentes-Paez et. al. Ram et al in his study of 23 patients demonstrated decreased Schirmer's scores favouring our study.

In our study, tear film break up time showed a negative correlation. Versura et al and Sullivan et al showed a poor performance of tear film break up time [15,16]. Li et. al. and Ram et al demonstrated decreased tear film break up time values compared to preoperative values [3,10]. Fluorescein staining and lissamine green staining were not statistically significant in our study. Bhargava R et al showed the sensitivity of lissamine green staining in diagnosing dry eye to be 34.6 % with a specificity of 86 % [7]. The sensitivity of Rosebengal staining was 63.7 % with a specificity of 64.1 % and a predictive value of 41.6 % in a study by Prachi et. al.

However our study had certain limitations like short follow up. Steroid antibiotic drops used after surgery could have been partially responsible for changes in the tear film status. We did not establish the association between duration of surgery and loss of goblet cell density.

Conclusion

Conjunctival impression cytology being a non invasive method has numerous implications other than evaluating a dry eye. It can be employed in the diagnosis of a wide range of ocular surface disorders, documenting sequential changes in the corneal and conjunctival surfaces over time, staging conjunctival squamous metaplasia and monitoring effects of treatment. Cytokeratin expression using immunocytochemical staining aids in the diagnosis of certain benign conditions simulating malignant melanoma. Despite certain minor limitations our study signifies the importane of conjunctival impression cytology in evaluating dry eye as literature pertaining to dry eye post cataract surgery is sparse.

References

1. Sinha M, Sinha A, Chowdhury B. Comparative evaluation of dry eye following cataract surgery : A study from North India. IOSR Journal of Dental and Medical Sciences 2014; 13(6):13-18.
2. Denise S, Ryan, Kraig S, Bower, Rose K, Sia. Goblet cell response after photorefractive keratectomy and laser in situ keratomileusis. J Cataract Refract Surg 2016;42: 1181–89.
3. Li XM, Hu L, Hu J, Wang W. Investigation of dry eye disease and analysis of the pathogenic factors in patients after cataract surgery. Cornea 2007;26(9):16-20.
4. Servet Cetinkaya, Emine Mestan, Nursen Oncel Acir. The course of dry eye after phacoemulsification surgery. BMC Ophthalmology 2015;68(15):58-63.
5. Sharma A, Hindman HB. Aging: A predisposition to dry eyes. Journal of ophthalmology 2014;37(2):46-50.
6. Gogate P, Deshpande M, Nirmalan PK. Why do phacoemulsification? Manual small-incision cataract

surgery is almost as effective, but less expensive. Ophthalmology 2007;114(5):965-8.

7. Kumar P, Bhargava R, Kumar M, Ranjan S, Kumar M. The correlation of routine tear function tests and conjunctival impression cytology in dry eye syndrome. Korean J Ophthalmol 2014;28:122-29.
8. Bhargava R, Kumar P. Can conjunctival impression cytology be the first line diagnostic test for evaluation of dry eye syndrome ? Clin Ophthalmol Res 2015;1(1):1-4.
9. Yinhui Yu, Huixia Hua, Menghan Wu. Evaluation of dry eye after femtosecond laser assisted cataract surgery. J Cataract Refract Surg 2015;41:2614–23.
10. Ram J, Gupta A, Brar G, Kaushik S, Gupta A. Outcomes of phacoemulsification in patients with dry eye. J. Cataract Refract. Surg 2002;28(8)1386-89.
11. Doreen, Schmidl, Katarzyna, Jadwiga Witkowska, Semira Kaya. The association between subjective and objective parameters for the assessment of dry eye syndrome. Investigative ophthalmology and visual science 2015; 6(4):63-67.
12. Fuentes-Paez G, Herreras JM, Cordero Y, Almaraz A, Gonzalez MJ. Lack of concordance between dry eye syndrome questionnaires and diagnostic tests. Arch Soc. Esp Oftalmol 2011;86:3-7.
13. Maumenee AE. Keratinization of the conjunctiva. Trans Am Ophthalmol Soc 1979;77(1):133-7.
14. Taehoon Oh, Younhea Jung, Dongjin Chang. Changes in the tear film and ocular surface after cataract surgery. Jpn J Ophthalmol 2012;56:113-18.
15. Versura P1, Campos EC. Osmolarity system for diagnosing dry eye. Expert Rev Mol Diagn. 2013;13(2):119-29.
16. Sullivan BD, Crews LA, Messmer EM, Foulks GN, Nichols KK. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. Acta Ophthalmol 2014;92:161-66.