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Effects of topical vitamin A on conjunctival goblet cell density after small incision cataract surgery

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ABSTRACT

Background: Conjunctival goblet cells could be impaired and resulted in unstable tear film after small incision cataract surgery (SICS). The purpose of this study was to describe the effects of topical vitamin A on the conjunctival goblet cell density after SICS.

Methods: This is a randomized clinical trial with pre-and post-control group design conducted in Bali Mandara Hospital on September 2015 until January 2016. A total of 38 patients were divided into two groups; 19 patients were given topical vitamin A and 19 patients were given placebo. Impression cytology specimen was obtained before SICS, after SICS and 4 weeks after intervention. Impression cytology was examined in Pathological Anatomy Laboratory of Sanglah General Hospital. Mean density of goblet cells before and after SICS were calculated using paired t-test. The difference of before and after intervention in each group were calculated using independent t-test.

Results: Mean density of conjunctival goblet cells before SICS was 15.5 ± 13.4 cells/10 HPF and after SICS was 15.7 ± 12.7 cells/10 HPF ($p = 0.938$). Mean difference in conjunctival goblet cells density before and after SICS in the intervention group was 0.2 ± 11.7 cells/10 HPF and in the control group was -10.2 ± 8.1 cells/10 HPF ($p = 0.003$). The mean difference between the two groups was 10.4 ± 3.2 cells/10 HPF.

Conclusions: The mean difference of conjunctival goblet cells density between two groups after intervention was found to be statistically significant.

Keywords: SICS, impression cytology, topical vitamin A.

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INTRODUCTION

Small incision cataract surgery (SICS) is a cataract extraction technique which most efficient and economical for developing countries such as Indonesia. During surgery, incision on conjunctiva, sclera and cornea may damage the tissue. As a result of the damage, limbal stem cells and conjunctival goblet cells reduce the secretion of mucin and disrupt the tear film stability.[1,2] Post surgery inflammation, tissue edema, wound healing, topical anesthesia with preservative benzalkonium chloride (BAK), and other toxic substances because mucin becomes less hydrophilic so that tear film stability decreases and dry eye condition appears.[3]

National Health Survey in 2001 showed that the prevalence of cataract in Indonesia was approximately 4.99%. The prevalence of cataract in Java-Bali was 5.48% higher compared to other provinces in Indonesia. The prevalence of cataract in rural area was 6.29%, while in urban area was 4.5%.[4]

Decrease of conjunctival goblet cells density and alteration in morphology of epithelial cells often found in dry eye condition.[5] Ocular surface disorders cause both normal epithelial secretion and non-secretion modified so it turns to non-secretion keratinization called squamous metaplasia. A decrease in density of goblet cells also reduce mucin secretion, consequently lead to dry eyes.[6,7]

Li et al[8] reported dry eye symptoms in patients after phacoemulsification with lower tear meniscus, decreased tear break up time (TBUT), decrease schirmer test 1, and squamous metaplasia detected by impression cytology. Impression cytology is a simple, noninvasive technique that allows the collection of the superficial layers of the conjunctival epithelium for the morphological examination of the ocular surface. It has high specificity to detect early changes in conjunctiva and cornea which not detected by tears routine tests. Many researchers said that impression cytology may become the first line examination for diagnosis of dry eye.[5]

Artificial tears containing vitamin A is known to have an important role in regulating proliferation and differentiation of corneal epithelial cells, helping normal cell growth, and maintaining conjunctival goblet cells.[8-10]

This study was conducted to investigate the effect of topical vitamin A to conjunctival goblet cells density with cytology impression technique in patients post SICS.

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METHODS

This is a clinical trial study with pre-and post design, extension randomized, double blind, pre-and post test control group design aimed to determine changes in the density of goblet cells in the treatment group compared to the control group. This study was approved by Research Ethical Committee Faculty of Medicine Udayana University/Sanglah General Hospital Denpasar. The study was conducted at eye outpatient clinic of Bali Mandara Hospital Province Bali from September 2015 until January 2016.

The samples of this study were all patients who planned for cataract surgery with SICS techniques that came to eye outpatient clinic of Bali Mandara Hospital and met the inclusion and exclusion criteria. The inclusion criteria were male and female above 50 years old, patients who were planned for cataract extraction with SICS techniques, patients with topical antibiotics and steroid after surgery. Patients with history of glaucoma and refractive surgery, history of blunt and sharp trauma, chemical injury, radiation, history of systemic diseases such as allergy, diabetes mellitus, hypertension and sjogren syndrome, abnormality of conjunctiva, cornea and sclera such as pterygium, blepharitis, uveitis, contact lens used > 1 year, history of glaucoma eye drop used such as timolol and betaxolol and patients who did not complete the research protocols were excluded from this study. Total samples were 19 samples for each group, counted with pocock formula.

Samples which already diagnosed as cataract, scheduled for SICS, and met the inclusion and exclusion criteria need to sign the informed consent. Impression cytology was performed before SICS, 1 week after SICS (before intervention), and 4 weeks after interventions.

Patients were given pantocain 2% eye drop before the blepharostat was put to the eyelids. Nitrocellulose filter was applied in the area of the superior conjunctiva and gently press the filter paper for 3-5 seconds. Subsequently the filter paper was placed on the glass object and fixated with alcohol 95%. The impression cytology then taken to the Pathologic Anatomy Laboratory in Sanglah General Hospital to be analyzed with papanicolau staining and examination under the microscope.

All subjects were checked for the first impression cytology before surgery. One week after the surgery, all subjects were again undergone procedures of impression cytology examination then started the interventions by given packages of eyedrops with code A or B according to sequence permutation block. We could not find out the content of each eye drop that given to the patients until the study was completed. All subjects were followed up on the second week and fourth week after intervention, then the final impression cytology examination was performed. Interpretation of conjunctival impression cytology was made by one Anatomy Pathologist in Sanglah General Hospital (EK). Once the study completed, the composition of both code A and B eyedrop were informed to us by the factory.

All data obtained were analyzed with SPSS 17.0. Mean density of goblet cells before and after SICS were calculated using paired t-test. The difference before and after intervention in each group were calculated using independent t-test.

Table 1 Characteristics of subjects

Characteristics	Treatment group (post op + topical Vit.A) n = 19	Control group (post op + placebo) n = 19
Age, mean ± SD (years old)	68.68 ± 6.447	69.74 ± 7.687
Sex, n		
Male	8	12
Female	11	7

Table 2 Impression cytology before and after SICS

	Before SICS n = 19	After SICS n = 19	p
Conjunctival goblet cell density, mean ± SD (cells/10 HPF)	15.5 ± 13.4	15.7 ± 12.7	0.938*

*paired t-test

RESULTS

Characteristics of subjects

The eligible samples for this study were 38 subjects. The characteristics of subjects presented in Table 1. The mean age in the treatment group was 68.68 ± 6.447 years old, while the control group was 69.74 ± 7.687 years old. Majority of female found in the treatment group (11 subjects), while most of male subjects were in control group (12 subjects).

Conjunctival goblet cell density before and after small incision cataract surgery (SICS)

The differences of conjunctival goblet cell density before and after SICS was analyzed with paired t-test since normal data distribution was obtained after normality test with Shapiro-Wilk ($p > 0.05$). The result was presented in Table 2.

Mean conjunctival goblet cell density before and after SICS was 15.5 ± 13.4 cells/10 HPF and 15.7 ± 12.7 cells/10 HPF, respectively. There were no statistically significant differences between two groups ($p > 0.05$).

Table 3 Impression cytology before and after interventions

	Treatment group n = 19	Control group n = 19	Mean difference	95% CI	p
Impression cytology before SICS, mean ± SD (cells/10 HPF)	13.1 ± 14.4	17.9± 12.2	-4.8 ± 4.3	-13.7 to 3.9	0.267*
Impression cytology before intervention (1 week after SICS), mean ± SD (cells/10 HPF)	13.8 ± 12.6	17.6± 12.8	-3.8 ± 4.1	-12.2 to 4.5	0.358*
Impression cytology after intervention (4 weeks after SICS), mean ± SD (cells/10 HPF)	13.9 ± 8.1	7.5± 6.3	6.4 ± 2.3	1.6 to 11.2	0.010*
Difference impression cytology (before and after intervention), mean ± SD (cells/10 HPF)	0.2 ± 11.7	-10.2 ± 8.1	10.4 ± 3.2	3.7 to 16.9	0.003*

*Independent t-test

Conjunctival goblet cell density before and after interventions

The differences of conjunctival goblet cell density before and after interventions was analyzed with independent t-test since normal data distribution was obtained after normality test with Shapiro-Wilk ($p > 0.05$). The results presented in table 3.

There was no significant difference in conjunctival goblet cell density before SICS and 1 week after surgery between two groups, but a significant difference of conjunctival goblet cells density was found in treatment group than in the control groups after intervention ($p = 0.010$). There was also a significant difference in mean of conjunctival goblet cells between two groups after intervention. ($p = 0.003$)

DISCUSSION

Senile cataract defined as cataract that appeared in individual with age above 50 years old.[11] Mean of age according to study by Zaman et al (2009)[12] on sutureless manual extra capsular extraction was 57.7 ± 12.2 years old. Study by Retnaniadi et al in Malang about the effect of incision of cataract surgery to dry eye found that mean of age was 52-84 years old, with the most was 60-69 years old.[13] The result of this study was in accordance with the studies above that the mean age in the treatment group was 68.68 ± 6.447 years old and in control group was 69.74 ± 7.687 years old.

Study by Khan et al. (2014)[14] found that the decrease of conjunctiva goblet cell density was associated with age and the presence of symptoms of dry eye and squamous metaplasia. Goblet cells density was found to decrease significantly in the case of dry eye.[15] The mean age of patients in this study were between 65 and 75 years old which had higher risk of dry eye.

Study by Sunariasih (2014)[16] in Bali revealed that cataract was found more in men than women. Tsai et al (2003)[17] described that cataract in females was significantly higher than in males. Athanasiov et al (2010)[18] reported no statistically significant differences between the incidence of cataracts and sex. In this study, males were found more compared to females overall, with majority of gender in the treatment group was female whereas in the control group was male. This proves the absence of relations between sex with the incidence of cataracts.

Study by Sinha et al (2014)[19] in India regarding dry eye after cataract surgery by comparing impression cytology parameters in patients underwent MSICS and phacoemulsification, obtained decreased of mean goblet cells density in both group 3 months after surgery. According to Zu-Gou et al (2002)[20], there was improvement of tear secretion 7 days after surgery and returned to the level before surgery, then no significant difference of tear secretion was found between before and 30 days after surgery.

In this study, there was no significant difference between the mean of conjunctival goblet cells density before SICS and after SICS, but there was significant difference of goblet cells density after the interventions. The results of this study were different with previous study because the impression cytology was examined after 1 week postoperatively and interventions were already given after 30 days. Antibiotics and anti-inflammation also had been given for 1 week so the inflammatory process that occurred had already decreased and made the results between two groups after SICS before intervention were not statistically different.

Study by Kim et al (2009)[21] about the effectiveness of vitamin A and cyclosporine A 0.05% eye drops for dry eye syndrome showed both were effective for the improvement of the symptoms, TBUT, corneal staining scores and impression cytology. Study by Kobayashi et al (1997)[22] revealed increase of goblet cells, decrease of keratin cells and increased of non-keratine cells in the cytology analysis after treatment with retinol palmitate to dry eye patients. Study by Kim et al (2012)[9] on the effect of vitamin A to the wound healing after alkali trauma

in mice revealed an increased degree of impression cytology and the density of goblet cells in the vitamin A group than in control group.

A study of vitamin A suggests that vitamin A plays a role in the synthesis of glycosaminoglycans and glucoprotein on the cornea and also stimulate cDNA synthesis to increase the fibroblast on substantia propria layer. Vitamin A also important for energy metabolism that affects the cornea and conjunctiva tran differentiation, causing increased expression of epidermal growth factor receptor (EGFR) on corneal endothelium, which has the additional effect on wound healing of the cornea caused by EGF. Vitamin A is essential in maintaining normal function of vision and immune integrity. Vitamin A deficiencies will result in a loss of conjunctival goblet cells and conjunctiva epithelial thickening, squamous metaplasia, and cornification. The protective function of topical vitamin A to the proliferation of goblet cells is to inhibit the conjunctival epithelial cornification.[23,24] Vitamin A in this study help to maintain the density of goblet cells post SICS.

Studies on the effect of artificial tears after cataract surgery for symptoms and signs of dry eye were still limited. Study in cyclosporine A 0.05% 2 times a day for 1 month before surgery and 1 month after surgery resulted in improvement in the complaints but not TBUT.[25] There is still no study about conjunctival goblet cells density analysis on the use of artificial tears after cataract surgery, so this is the first study to analyze the conjunctival goblet cells density with additional topical vitamin A therapy after SICS.

This study was conducted in a period of approximately 5 weeks postoperatively where cytology impression measurements performed at 1 week after SICS and resumed 4 weeks after administration of eye drops. In this study, the mean density of conjunctival goblet cells density after intervention showed an improvement in the treatment group but there was reduction in the control group. Dry eye can deteriorate after cataract surgery if not treated on time, and this could happen as early as possible within one week postoperatively with a peak of about one month.[8] It can be concluded from this study that in the treatment group, vitamin A can help the process of goblet cells proliferation.

The normal mean number of goblet cells in the bulbar conjunctiva in adults were reported between 1-10/mm. Several studies have reported the density of goblet cells in different units. Normal goblet cells density in units of cells/mm^2 was reported as > 100/mm^2. It has been reported that normal conjunctival goblet cells density was 1.24 ± 1.62 and in the inferior conjunctival fornix was 30.21±14.32.[6] In this study, although both groups were still in normal density of conjunctival goblet cells, the number of conjunctival goblet cells in the treatment group were increased.

The use of artificial tears containing vitamin A in this study proved to maintain conjunctival goblet cells density and improve dry eye complaints such as watery and foreign body sensation. The limitations of this study were the difficulty to control the compliance of the patient and the study was performed in the short period of time. There are also variations in number of conjunctival goblet cells on impression cytology. Further study is needed with long term follow up of the intervention of artificial tears and its composition that can prevent or reduce postoperative dry eye state of other eye surgery.

CONCLUSIONS

Mean conjunctival goblet cells density difference before and after SICS was found to be not statistically significant. The mean difference of conjunctival goblet cells density between two groups after intervention was found to be statistically significant.

REFERENCES

1. Ganvit SS, Ahir HD, Sadhu J, Pandya NN. Study of dry eye changes after cataract surgery. *Int J Res Med*. 2014;3(2):142-5.
2. Kavitha C, Venugopal, Pavana AK, Chandan N. Evaluation of dryness of eyes after manual small incision cataract surgery with corneoscleral tunnel incision. *Journal of Clinical and Diagnostic Research*. 2012;6(6):1029-33.
3. Zhang S, Li YZ. Research of ocular surface changes after incisions of cataract surgery. *Int J Ophthalmol*. 2010;10(9):1719-21.
4. Tana L, Delima, Hastuti E, Gondhowiardjo T. Katarak pada petani dan keluarganya di teluk jambe barat. *Media Litbang Kesehatan*. 2006;16(4):43-4.
5. Bhargava R, Kumar P, Kaur A, et al. The diagnostic value and accuracy of conjunctival impression cytology, dry eye symptomatology, and routine tear function tests in computer users. *Journal of Laboratory Physicians*. 2014;6(2):102-8.
6. Gillan WDH. Conjunctival impression cytology: a review. *The South African Optometrist*. 2008;67(3):136-41.
7. Peters E, Colby C. The tear film. In: Tasman W, Jaeger EA, editors. Duane's Ophthalmology. Philadelphia: Lippincott Williams & Wilkins; 2009.
8. Li XM, Hu J, Wang W. Investigations of dry eye disease and analysis of the pathologic factors in patients after cataract surgery. *Cornea*. 2007;26(9):16-20.
9. Kim EC, Kim TK, Park SH, Kim MS. The wound healing effects of vitamin A eyedrops after a corneal alkali burn in rats. *Acta Ophthalmol*. 2012;90(7):e540-6.
10. Vibhute S, Kawtikwar P, Kshirsagar S, Sakarkar D. Formulation and evaluation of tear substitutes. *International Journal of Pharmaceutical Sciences Review and Research*. 2010;2(1):17-20.
11. Ilyas S. Ilmu Penyakit Mata. Jakarta: Balai penerbit FKUI; 2009. p. 212-4.
12. Zaman M, Shah AA, Hussain M, Babar TF, Tariq M, Marwat K, et al. Outcome of sutureless manual extra capsular cataract extraction. *J Ayub Med Coll Abbottabad*. 2009;21(1):39-42.

13. Retnaniadi S, Herwindo DP. Pengaruh jenis insisi pada operasi katarak terhadap terjadinya sindroma mata kering. *Jurnal Kedokteran Brawijya*. 2012;27(1):34-7.
14. Khan AA, Kesarwani D, Vasenwala SM, Amitava AK, Siddiqui Z, Akhtar K. Conjunctival surface changes in diabetics: an unsual cytological study. *Annals of Pathology and Laboratory Medicine*. 2014;1(2):A1-5.
15. Kumar P, Bhargava R, Kumar M, Ranjan S, Kumar M, Verma P. The Correlation of routine tear function tests and conjunctival impression cytology in dry eye syndrome. *Korean J Ophthalmol*. 2014;28(2):122-9.
16. Sunariasih NN. 2014. Kadar malondialdehyde serum pasien katarak senilis setelah pemberian astaxanthin lebih rendah daripada sebelum pemberian astaxanthin (thesis). Denpasar: Universitas Udayana.
17. Tsai SY, Hsu WM, Cheng CY, Liu JH, Chou P. Epidemiologic study of age-related cataracts among an elderly chinese populations shih-pai, Taiwan. *Ophthalmology*. 2003;110(6):1089-95.
18. Athanasiov PA, Edussuriya K, Senaratne T, Sennanayake S, Sullivan T, Selva D, et al. Cataract in central srilanka: prevalence and risk factors from the kandy eye study. *Ophthalmic Epidemiology*. 2010;17(1):34-40.
19. 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):13-8.
20. Zu-Guo L, Luo L, Yangling V. Phacoemulsification Addition to Changes in Tear Film After. *J Ophthalmo*. 2002;38(5):274-7.
21. Kim EC, Choi JS, Joo CK. A comparison of vitamin A and cyclosporine A 0,05% eye drops for treatment of dry eye syndrome. *American Journal of Ophthalmology*. 2009;147(2):206-13.
22. Kobayashi TK, Tsubota K, Takamura E, Sawa M, Ohashi Y, Usui M. Effect of retinol palmitate as a treatment for dry eye; a cytological evaluation. *Ophthalmologica*. 1997;211(6):358-61.
23. Zhang Y, Huang W, Zhang S, Liu Y. Effect of vitamin A on the conjunctival goblet cells of rat after corneal transplantation. *Int J Ophthalmol*. 2008;1(2):109-12.
24. Roberts CW, Elie ER. Dry eye symptoms following cataract surgery. *Insight*. 2007;32(1):14-21.
25. Fakhraie G, Lopes JF, Spaeth GL, Almodin J, Ichhpujani P, Moster MR. Effects of postoperative cyclosporine ophthalmic solution 0,05% (restasis) following glaucoma surgery.