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Conjunctival Impression Cytology after Antiglaucoma Treatment with Travoprost with Benzalkonium Chloride Versus Travoprost with Stabilized Oxy Chloride Complex in Newly Diagnosed Primary Open Angle Glaucoma Patients Over 6 Months

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Abstract: ***Aim: Microscopic evaluation of conjunctival morphological changes with conjunctival impression cytology in case of Primary Open Angle Glaucoma (POAG) treated with travoprost with Benzalkonium chloride (BAC) versus travoprost with Stabilised Oxy Chloride (SOC). Method: Our study involved 56 patients (112 eyes) who received antiglaucoma treatment by instillation of one drop of travoprost (0.004%) with SOC (0.005%) in group 1 (56 eyes of 28 patients) and travoprost with BAC (0.015%) in group 2 (56 eyes of 28 patients) every 24 hours. Conjunctival impression cytology was carried outat baseline and after 6 months to analyze cellular density and morphologic parameters. Result: Conjunctival impression smears at the beginning of the study were normal in 100% of eyes in both the groups. At the end of 6 months, 48.3% and 82.1% of eyes in group 1 and 2 respectively showed abnormal change in morphology and decrease in number of goblet cells on conjunctival impression study. Conclusion: Stabilised OxychloroCompound preservative in Travoprost preserves the ocular surface integrity better compared to Benzalkonium chloride in the Travoprost though the adverse effects of original drug molecule could not be negated in both groups.***

Keywords: Conjunctival impression cytology, Primary open angle Glaucoma, Prostaglandin analogue, Preservatives in ocular drugs

1. Introduction

Glaucoma is the second leading cause of blindness in the world and is predicted to account for over 11 million patientsby 2020. [1]Medical treatment is considered an effective way of controlling glaucoma in its initial stage. [2]Topical medical treatment is mainly used as first-choice therapy to avoid the onset of further irreversible optic nerve damage and visual field defects. Most of the patients are on long term treatment medically. Surgery is reserved in case of intolerance, inadequate response to topical therapy,contra indications or progression of disease. The benefits of reducing microbial contamination through use of preservatives are offset by the known ocular side effects of preservatives.[3] The toxic action of preservatives on the ocular surface has been widely demonstrated in vitro as well as in vivo, in both humans and animals. [4-6]

Benzalkonium chloride (BAC) is among the most common preservatives used in ophthalmic preparations.It kills bacteria; the same mechanism that eradicates microbes is also toxic to many cell types of the eye like conjunctival epithelium and corneal epithelium.The ocular effects are dose-dependent and can range from apoptosis to necrosis. The local inflammation causes changes that can mimic the appearance of dry eye signs and symptoms. The discomfort associated with dry eye decreases the patient's quality of life, and it also reduces their desire to comply with treatment.[7]

Oxidants, such as stabilized oxychloro complex (SOC) and sodium perborate, are usually small molecules that penetrate cell membranes and disrupt cellular function by modifying lipids, proteins, and DNA. Their membrane destabilizing activity is less potent than that of detergent preservatives. At low levels, oxidative preservatives have an advantage over the detergent preservatives by providing enough activity against microorganisms while exerting only negligible toxic effects on eukaryotic cells.[8]

Conjunctiva is a semipermeable natural barrier to topical medication. Conjunctiva responds to stress by becoming inflamed or by loss of vascularization or by exhibiting a spectrum of metaplasia like loss of goblet cells, stratification and keratinization,[9]subtle signs of ocular toxicity such as superficial punctate keratitis indicate chronic cell injury. In the cornea, application of preservatives induces reduction in cell proliferation and viability, hence corneal healing is impaired and the epithelial barrier is compromised.

In order to determine the effect of topical treatment on the conjunctiva, we carried out impression cytology on the conjunctiva. It is a valuable diagnostic tool for the early stages of the ocular surface disorderbecause it is non-invasive and can be used over longer periods[10, 11]

2. Methods

Our study involved 56 patients (112 eyes) between ages 40 to 65 years who received antiglaucoma treatment for

primary open angle glaucoma in form of instillation of one drop of travoprost (0.004%) with SOC (0.005%) ortravoprost with BAC (0.015%) every 24 hours at night around 9pm. These patients were not receiving any other topical ocular treatment.

Patients with history of ocular surgery, previous topical drug administration within last 3 months, ocular surface disease, collagen vascular disease, known hypersensitivity to therapy, contact lens use and allergic conjunctivitis were excluded.

General ophthalmic examination was carried out in each patient at every visit for Best corrected visual activity (BCVA), meibomitis, limbal marginal keratinisation, tear meniscus height, presesnce of conjunctivalhyperemia, superficial punctate keratitis (SPK's), conjunctivalisation, Schirmer-1 test, fluorescein staining, Tear film breakup time (TBUT), intraocular pressure (IOP), gonioscopy.

The diagnosis of Primary open angle glaucoma was confirmed by Applanation tonometry, gonioscopy, optic nerve head evaluation, visual fields defect [Glaucoma hemifield test (GHT) outside normal limits and/or pattern standard deviation (PSD) with p<5%], Ocular coherence tomography (OCT) and fundus examination for glaucomatous optic neuropathy (rim thinning, excavation and/or retinal nerve fibre layer defects). Patients with Normal tesion glaucoma (NTG) were included but those with only Ocular hypertension (OHT) were not included in the study. Informed and written consent was obtained from all patients with consent form approved by the Institutional ethical committee.

Patients were divided into two groups: Group 1(56 eyes of 28 patients): Patients on Travo-Z with stabilised Oxychloro complex (0.005%) and Group 2 (56 eyes of 28 patients): Patients on Travatan (0.004%) with Benzalkonium chloride (0.015%).

3. Procedure

Impression cytology was carried out in each patient in both eyes before starting the treatment (untreated patients). In the same patients, another sample was taken after 6 months of treatment. Patients with abnormal impression cytology at baseline were excluded. Compliance and adherence of treatment were confirmed with every patient. The samples were collected on Millipore which was cut into small rectangles (5 x 3 mm) and marked in a corner in order to guarantee correct orientation. One drop of 0.5% proparacaine was instilled in inferior fornix of the patient's eye as topical anaesthetic. Next, the filter paper was applied on infero temporal part of bulbar conjunctiva exerting light pressure for at least 3 seconds. The Millipore paper was then pressed onto a microscope slide for the sample to adhere to its surface, and subsequently the paper was removed. One drop of 96% ethanol was placed on the sample for 10 minutes to act as fixative and then the sample was stained using periodic acid–Schiff–haematoxylin. The samples were first hydrated in decreasing concentrations of ethanol, then oxidized in 0.5% periodic acid (10 min.), rinsed in distilled water, stained with Schiff reagent (20 min.), soaked in 0.5% sodium bisulfite (two or three rinses), and then counterstained with Carazzihematoxylin (5 min.). The specimens were then dehydrated (96º to 100º ethanol) and immersed in xylene. Finally, they were mounted with Entellan and cover slipped. Further analysis for morphology of conjuncival epithelial cells, nucleocytoplasmic ratio, mean individual epithelial cell area (MIECA) and number of goblet cells/mm^2 was done. Grading of impression cytology was done using Nelson's Grading system.

4. Statistical Analysis

A total of 56 patients (112 eyes) were included in the study. The data was entered in MS EXCEL spread sheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. A paired test was used to assess the changes at baseline and at 6 months. A Chi square test was applied to find out difference of results between two groups at the end of the 6 months. P-value has been calculated using two tailed test. A p-value of less than 0.05 was considered significant.

5. Results

All results are provided as means and standard deviations for continuous variables and frequencies and percentages (%) for ordinal variables, unless otherwise indicated. The overall mean age of the patients was 57.14 years in group 1 and 54.75 years in group 2. Approximately two-thirds of the patients were females (62.5%). There were 60.71% females in group 1 and 64.28% females in group 2.

Conjunctival impression cytology in both groups is illustrated in table 1. The conjuctival impression cytology using Nelson's grading was graded as: Normal = grade 0 and grade 1 while Abnormal = grade 2 and grade 3 (Table 2). Percentage of eyes with normal and abnormal conjunctival impression cytology at baseline and at 6 months in both groups is shown in (figure 1 and 2). A Conjunctival impression cytology picture of both groups at baseline is shown in (figure 3).

In group 1, 100% of eyes at baseline had normal score while at 6 months,51.7% had normal score.(p-value < 0.000001) In group 2, 100% of eyes at baseline had normal score compared to only 17.9% at 6 months. (p-value < 0.000001)

6. Discussion

We investigated the degree of squamous metaplasia occurring in patients treated with travoprost for glaucoma at the end of 6 months. The degree of squamous metaplasia provides us with information about the state of the ocular surface, as this is directly related to the severity of the metaplasia.[12, 13] In more specific terms, the study of goblet epithelial cells is highly relevant because a loss or a decrease in their density is an early sign of squamous metaplasia.[14, 15]

Conjunctival impression smears at the beginning of the study were normal in 100% of eyes in both the groups. At the end of 6 months, 48.3% and 82.1% of patients in group 1

and 2 respectively showed abnormal conjunctival impression study.

In our study, we demonstrated that the cytology grading at 6 months. This supports the significance of long duration of topical therapy in causing conjunctival alterations.

The time needed for metaplasia to start is speculated to be less than 3 months by a study done by Turacli et al [9] or even 1 month by a study done by Herreras JM et al.[16]In 2013 Leonardo M, Luca Agnifili et al[17] with in vivo confocal microscopy & impression cytology study showed that at 6 months goblet cell density remains stable in preservative free Tafluprost group while it significantly decreased in Latanoprost with BAC group. In 2008 study by Kahook MY et al[18] showed that more than 67% of conjunctivalgoblet cells were lost in rabbits receiving Latanoprost with BAC compared to only 14% loss in those receiving travoprost with SofZia. Brandt et al[19] showed significantly higher grades of conjunctival metaplasia in patients receiving anti-glaucoma drugs, even in those receiving a beta blocker alone.

HLA-DR-positive conjunctival epithelial cells and MUC5AC-expressing goblet cells were studied in impression cytology specimens as part of the study conducted in Finland, Sweden, and Germany. Significant changes toward normalization were seen during the treatment with preservative-free tafluprost in comparison with BAC-preserved latanoprost.[20] The results suggest that preservative-free tafluprost induces less harmful effects on the conjunctiva, which is the principal target of the toxic effects of topical ophthalmic preparations.

Prostaglandin analogs have progressively replaced beta-blockers as the first-line therapy of POAG, because they are the most effective IOP-lowering agents, lack relevant systemic side effects, and require only once-daily dosing.[21,22]Preservative-free prostaglandin analogs – such as travoprost – minimize the risk of ocular side effects and increase the likelihood of good treatment adherence. Hence, preservative-free solutions should be considered when available. They could be particularly beneficial to patients who 1) have pre-existing ocular surface disease, 2) are expected to develop ocular surface disease (dry eye) during long-term medication, 3) are using multiple concomitant topical ocular treatments, and/or 4) are about to undergo glaucoma surgery.[23,24]In general, the current glaucoma treatment guidelines call for therapies that can maintain visual function, minimize side effects, increase adherence, and improve quality of life of the patients. A correct choice of first-line therapy is fundamental for achieving these patient outcomes and reducing the economic costs in the long run. Preservative-free prostaglandin analogs currently provide the best monotherapy option for first-line treatment of POAG. [25] Limitation of our study was small sample size and short duration of follow-up period.

7. Conclusion

Conjunctival impression cytology showed worsening in both groups with higher incidence of worsening in Travoprost with benzalkonium chloride group as compared to Travoprost with stabilised oxychloro complex group. Thus, Stabilised Oxychloro Compound preservative in Travoprost preserves the ocular surface integrity better compared to Benzalkonium chloride in the Travoprost though the adverse effects of original drug molecule could not be negated in both groups.

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Table 1: Conjunctival impression cytology in both groups at baseline and 6 months

Treatment group	visit	CIC (no. of eyes)		'p' value (difference between baseline and 6 month)	'p' value (difference in two groups at 6 months)
		Normal (Grade 0-1)	Abnormal (Grade 2-3)		
1. Travoprost with SOC	Baseline	56 (100.00%)	0 (0.00%)	<0.000001	0.001
	6 months	29(51.7%)	27(48.3%)		
2. Travoprost with BAC	Baseline	56(100.00%)	0 (0.00%)	<0.000001	
	6 months	10(17.9%)	46(82.1%)		

SOC: stabilized Oxy chloride complex ;BAC : Benzalkonium chloride ; CIC :Conjunctival impression cytology

Table 2: Nelson's classification for squamous metaplasia

Grade	Features
0	>500 goblet cells/mm^2
	Small, round epithelial cells with large nuclei
1	350-500 goblet cells/mm^2
2	100-350 goblet cells/mm^2
3	<100 goblet cells/mm^2
	Large, polygonal epithelial cells with small nuclei

Grade 2 or more =abnormal.

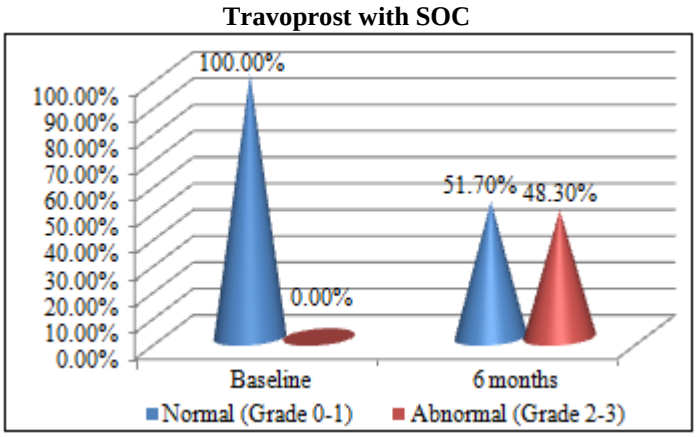


Figure 1: Percentage of eyes with normal and abnormal conjunctival impression cytology at baseline and at 6 months. (Group 1)

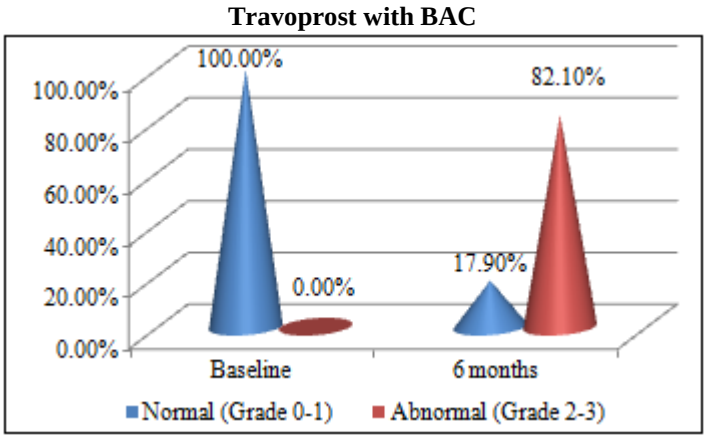
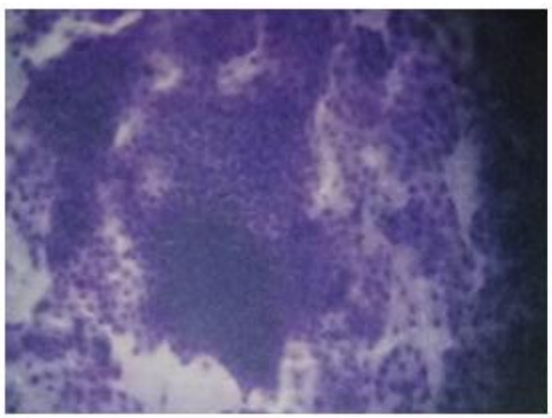
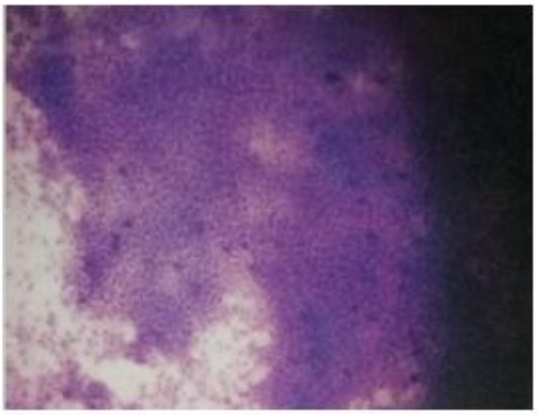
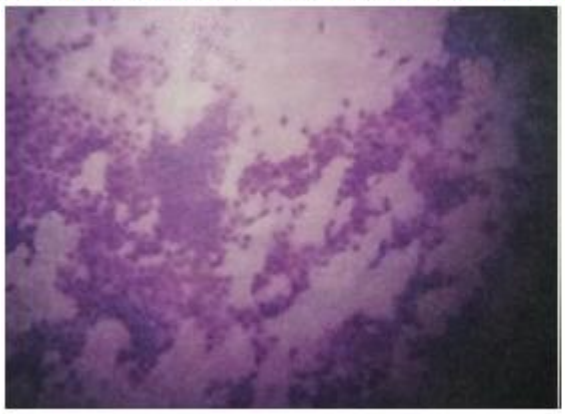


Figure 2: Percentage of eyes with normal and abnormal conjunctival impression cytology at baseline and at 6 months. (Group 2)


CIC of patient on travoprost with SOC at baseline grade 0


CIC of patient on travoprost with BAC at baseline grade 0


CIC of patient on travoprost with SOC at baseline grade 1

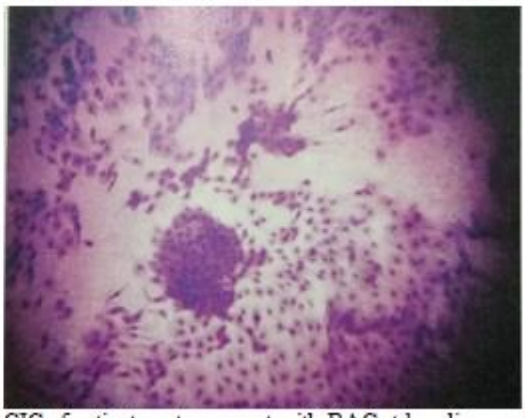

CIC of patient on travoprost with BAC at baseline grade 2

Figure 3: Conjunctival impression cytology (CIC) pictures