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Long-Term Effects of Topical Anti-Glaucoma Medications on Conjunctival Cell Morphology by Impression Cytology

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ABSTRACT

Background: Glaucoma is a major cause of irreversible blindness worldwide. Reduction of intraocular-pressure (IOP) using topical anti-glaucoma medications remains the mainstay of treatment. However, prolonged use of these medications may induce adverse effects on the ocular surface.

Methodology: This cross-sectional observational study, conducted at the B.P. Koirala Lions Centre for Ophthalmic Studies, Maharjgunj Medical Campus from February 2023 to August 2024. Patients of primary open angle glaucoma receiving topical anti-glaucoma medications for at least three months were enrolled. Conjunctival- Impression Cytology samples were obtained and graded according to Nelson's grading system. Data analysis was done using SPSS version 16. A p-value <0.05 was considered statistically significant.

Results: 128 eyes from 128 glaucoma patients were analyzed, including 54 males (42.2%) and 74 females (57.8%), with mean age of 58.16 ± 14.44 years. Patients using prostaglandin-analogues demonstrated 39.3% Grade-2 and 27.3% Grade-3 changes. Duration of medication use exceeding three years was significantly associated with higher Nelson grades ($p < 0.05$). Older patients, particularly those aged 61–70 years, exhibited greater cytological changes ($p = 0.005$). A significant positive correlation was observed between duration of medication use and severity of conjunctival changes ($r = 0.597$, $p < 0.001$). Males exhibited more severe conjunctival changes than females ($p = 0.009$).

Conclusions: Prostaglandin-analogues showed most severe conjunctival cytological alterations. Longer duration of medication use, advancing age, and male gender were significantly associated with conjunctival changes.

Keywords: Anti glaucoma medications; conjunctival morphology; impression cytology, ocular surface disorder.

INTRODUCTION

Glaucoma is characterized by progressive damage to the optic nerve head, leading to irreversible blindness.[1] The number of individuals aged 40-80 years affected by glaucoma is expected to reach 111.8 million by 2040.[2,3] Risk factors for glaucoma are family history, age, race and ethnicity, cardiovascular diseases and diabetes mellitus.[4-6]

Use of topical anti glaucoma medications delays visual field loss by lowering intraocular pressure and reduces the risk of progression by 17% in patients.[7]The prolonged application of these medicine can lead to various ocular surface disorder, manifesting as changes in conjunctival cell morphology.[8] Ocular surface abnormalities will lead to dry eye related problems. That in turn will cause less compliance to anti-glaucoma drugs causing progression of glaucomatous optic neuropathy and ultimately results in glaucoma blindness.

Impression cytology is a non-invasive technique utilized to assess the morphology of conjunctival cells, goblet cell density and epithelial cell characteristics, which are indicatives of ocular surface integrity.[9] Nelson's grading system is a standardized method for categorizing these cytological changes.[10]

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This study aimed to investigate the long-term effects of various topical anti-glaucoma medications on conjunctival cell morphology using impression cytology.

METHODS

This was a hospital-based cross sectional observational study conducted at the B.P. Koirala Lions Centre for Ophthalmic Studies, Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal. Ethical approval to conduct the research was taken from Institutional Review committee of Institution of Medicine, Tribhuvan University (Ref No: 426(6-11) E2 on 1/3/2023). Informed consent were taken from patients before enrolling in the study. All patients diagnosed with primary open-angle glaucoma (POAG) those receiving topical anti-glaucoma medications for a minimum duration of three months and who presented during from February 2023 to August 2024 were included in the study. A total of 128 eyes from 128 patients were enrolled. In cases where both eyes were treated, eyes which has been treated longer was included. Patients with obvious ocular surface disorders, history of ocular surface surgeries and cyclodestructive procedures in the past, long term use of any medications apart from anti glaucoma medications were excluded. More than 3 months uninterrupted use of antiglaucoma drugs is considered as long term use.

Patients using single drops were categorized according to the types of medications they are using. When multiple anti glaucoma medications are used in a single patient, it is considered as a combination therapy.

Conjunctival impression cytology was performed on the inferonasal bulbar conjunctiva of each enrolled eye, which was randomly selected. Cellulose acetate filter paper was used to collect epithelial cells from the ocular surface. The collected samples were then sent to pathology department Maharajgunj Medical Campus for cytological examination.

The severity of conjunctival cytological changes were assessed using Nelson's grading system.[10] This system classifies conjunctival morphology based on characteristics such as goblet cell density, epithelial cell size and shape, and the presence of inflammatory cells. Grades typically range from zero (normal) to 3 described as below.

Grade 0: The epithelial cells are small and round with eosinophilic-staining cytoplasm. The nuclei are large, basophilic, with N/C ratio of 1/2. The goblet cells are abundant, plump, and oval and have an intensely PAS-positive cytoplasm.

Grade 1: The epithelial cells are slightly larger and more polygonal and have eosinophilic-staining cytoplasm. The nuclei are smaller, with N/C ratio of 1/3. The goblet cells are decreased in number, however, they still maintain their plump oval shape, with an intensely PAS-positive cytoplasm.

Grade 2: The epithelial cells are larger and polygonal, occasionally multinucleated with variably staining cytoplasm. The N/C ratio is between 1/4 -1/5. The goblet cells are markedly decreased in number and are smaller, less intensely PAS-positive with poorly defined cellular borders.

Grade 3: The epithelial cells are large and polygonal with basophilic-staining cytoplasm. The nuclei are small, pyknotic, and completely absent from many cells. The N/C ratio is larger than 1/6. Goblet cells are completely absent.

Data were entered and analyzed in SPSS (version 16). Descriptive statistics were utilized to summarize the demographic characteristics of the study population, including age, gender, and laterality of the affected eyes. To investigate the association between different types of anti-glaucoma medications and the severity of conjunctival cytological changes, the Chi-square test was employed. Furthermore, Spearman's correlation coefficient was calculated to determine the relationship between the duration of medication use and the severity of conjunctival alterations, while the Mann-Whitney U test compared cytological changes between males and females. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant for all analyses.

RESULTS

This study enrolled 128 eyes of 128 glaucoma patients. The study population included 74 (57.8%) female patients and 54 (42.2%) male patients. the mean age of the participants was 58.16 ± 14.44 years (ranging from 21 to 90 yrs).

Prostaglandin analogues were the most frequently prescribed anti glaucoma medication in this study, followed by combination therapy of various groups of drugs. Other medications included Beta-blockers, fixed formulation of alpha agonist and beta blocker, Alpha-agonists and Carbonic anhydrase inhibitors (CAI) as shown in Figure 1.

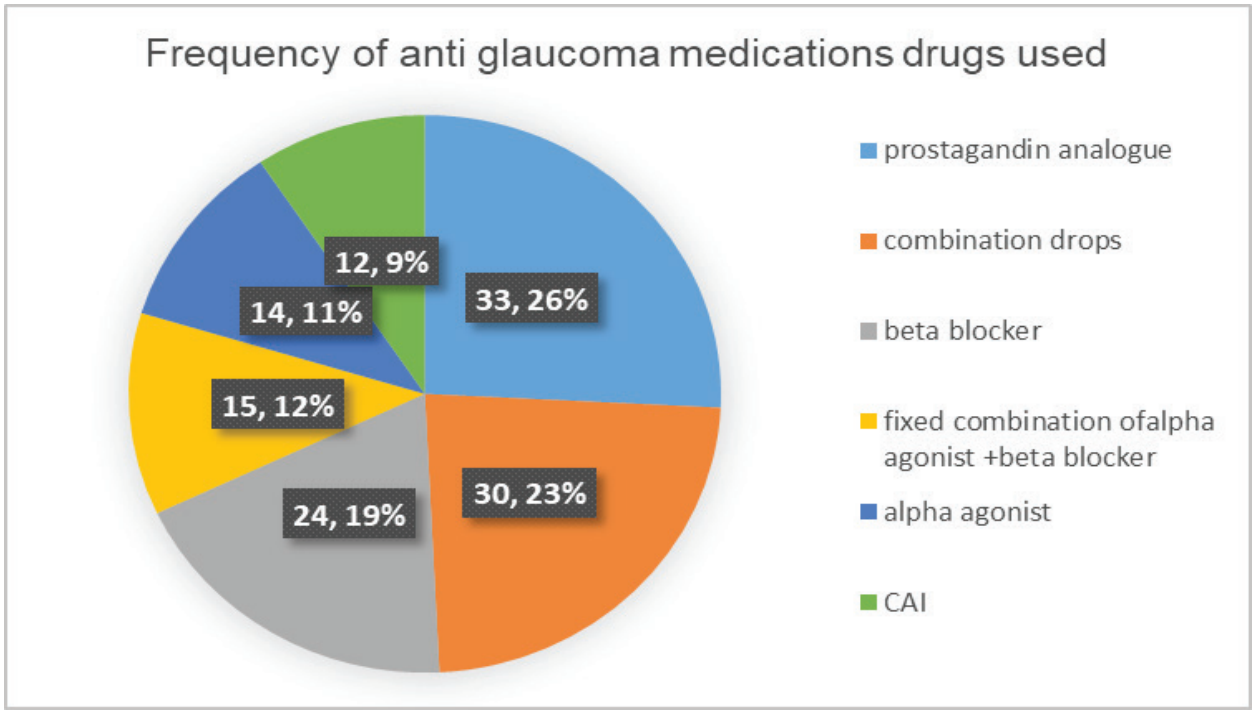

Figure 1.The Various Groups of Anti Glaucoma Medications Used.

Conjunctival cytological changes were assessed using Nelson's grading system. The severity of these changes varied significantly across different types of medication. Patients using Prostaglandin analogues exhibited the highest severity of cytological changes, with 39.3% classified as Grade 2 and 27.3% as Grade 3. In contrast, Carbonic anhydrase inhibitors showed a lower incidence of severe changes, with 50% in Grade 0 and 41% in Grade 1 as shown in Table 1.

Table 1.Conjunctival Cytology Changes According to Nelson's Grading Among Various Anti-glaucoma Drugs.

Medication Type	Grade 0 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Total (n)	p-value
Alpha agonist	7.1	35.0	28.5	28.5	14	
Alpha agonist + beta blocker fixed combination	20.0	40.0	40.0	0.0	15	
Prostaglandin analogue	12.0	21.0	39.3	27.3	33	
Carbonic anhydrase inhibitors	50.0	41.0	9.0	0.0	12	
Combination therapy	36.0	36.0	10.0	16.0	30	0.004
Total	**25.8**	**35.9**	**22.7**	**15.6**	**128**	

(Chi Square test)

A statistically significant association was found between the duration of drug use and the severity of Nelson grades ($p < 0.05$). Specifically, patients who had been using anti-glaucoma medications for more than three years demonstrated higher Nelson's grades, indicating more pronounced conjunctival alterations.

Table 2.The Cytological Grading in Relation to Duration of Use of Medications.

Duration of treatment (yrs)	Conjunctival cytological changes according to Nelson's criteria				p-value
	Grade 1	Grade 2	Grade 3	Grade 4	
<1	2	1	0	0	0.9
1-3	13	9	0	1	0.1
>3	18	36	29	19	<0.05
Total	33	46	29	20	

(Chi-square test)

Spearman's correlation analysis revealed a significant positive correlation between the duration of medication use and the severity of conjunctival changes ($r = 0.597$, $p < 0.001$). This moderate to strong correlation suggests the cumulative impact of long-term anti-glaucoma therapy on ocular surface health.

Furthermore, older age groups, particularly those aged 61–70 years, (Chi- Square test, p value 0.005) were associated with greater cytological changes, suggesting that age is a contributing factor to ocular surface damage in glaucoma patients.

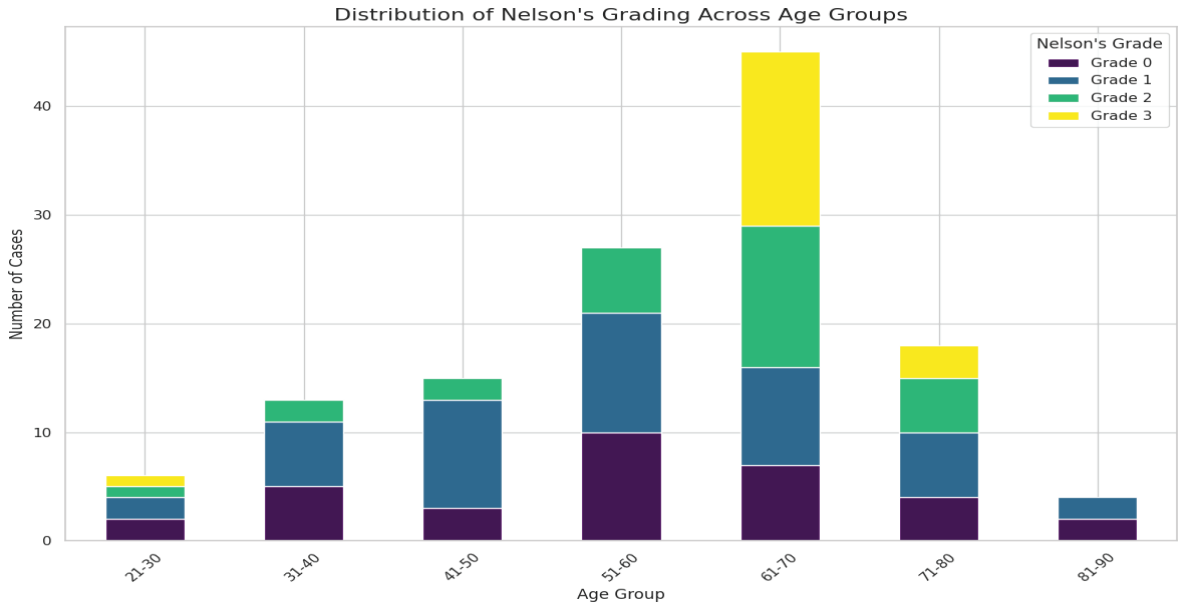

Figure 2.Bar Diagram Showing Conjunctival Morphological Changes Across Age Groups.

Males showed higher grades of conjunctival changes than females. The difference in Nelson's grading among males and females is statistically significant (Mann Whiteney U test, p=0.009)

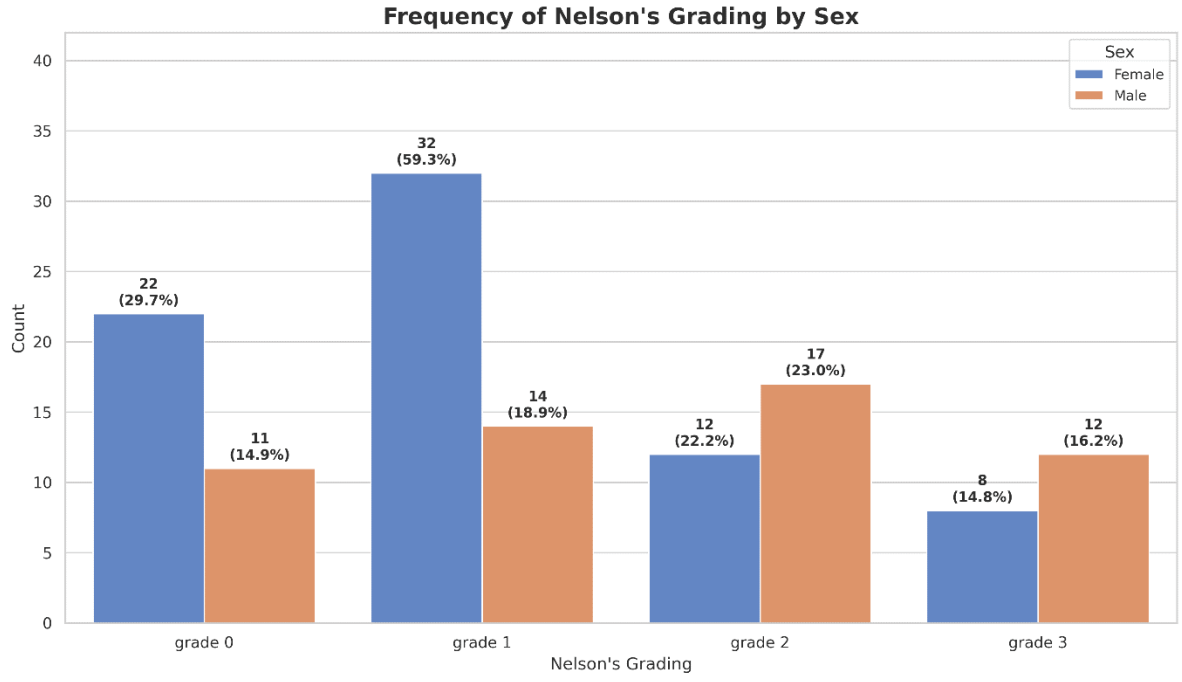

Figure 3. The Distribution of Conjunctival Cytological Changes (Nelsons grading) Among Males and Females.

DISCUSSION

Primary treatment of glaucoma is using anti glaucoma medicine to control the IOP. This study provides valuable insights into the long-term effects of topical anti-glaucoma medications on conjunctival cell morphology in patients with primary open-angle glaucoma. Our findings highlights the significant impact of medication type, duration of treatment, patient age and gender on ocular surface health, as assessed by impression cytology using Nelson's grading system[10].

The predominant use of prostaglandin analogues (PGAs) as the most frequently used medication is seen in current clinical practices, where PGAs are often considered first-line therapy due to their potent IOP-lowering efficacy and once-daily dosing.[11] Long term studies with latanoprost have not revealed any cardiac or pulmonary effects unlike timolol which may cause systemic symptoms like bradycardia, arrhythmia, congestive heart failure and syncope. So Better formulation, easy dosage, better compliance and less systemic side effects may explain the more use of prostaglandin analogue in glaucoma patients. However, our study also revealed that PGAs were associated with the highest severity of conjunctival cytological changes, with a substantial proportion of patients exhibiting Grade 2 and Grade 3 alterations similar to some published reports.[12] This finding is consistent with previous researchs indicating that PGAs, particularly those containing preservatives like benzalkonium chloride (BAK), can induce significant ocular surface toxicity, leading to inflammation, goblet cell loss, and squamous metaplasia.[13,14]The chronic exposure to these agents can disrupt the delicate balance of the ocular surface, contributing to symptoms of ocular surface disease (OSD) and potentially affecting patient adherence to treatment.[15,16] A report form Dogan et al has found that there is increased apoptosis of conjunctival epithelial cell examined by conjunctival biopsy in patients of glaucoma treated with anti-glaucoma medications.[17] Similarly, Thakur et al have also observed the similar finding in eastern Nepal, where they had followed the newly diagnosed cases of glaucoma and ocular surface changes were seen within six months of treatment. Fixed combination therapy and prostaglandins drops showed a significant changes.[18] A report from south India has shown similar that ocular surface disorder was prominent in patient on topical anti glaucoma drops and it was pronounced as the number drops and duration of treatment increased.[19] Comparing the conjunctival morphology in people on anti glaucoma medications and those without these medicine significant changes were see as reported by Pai et al.[20]

The significant association between the duration of drug use and the severity of Nelson grades is a critical finding. Patients using anti-glaucoma medications for more than three years demonstrated more pronounced conjunctival alterations. Nelson' et al had also found the similar fact.[21] This suggests a cumulative toxic effect of these medications on the ocular surface over time. This long-term impact highlights the importance of regular ocular surface assessment in glaucoma patients, especially those on prolonged therapy, to mitigate potential complications and improve their quality of life.[22]

Furthermore, our study identified older age groups (61–70 years) as being more susceptible to greater cytological changes. This could be attributed to age-related physiological changes in the ocular surface, such as decreased tear production, reduced goblet cell density, and compromised epithelial barrier function, which may exacerbate the adverse effects of anti-glaucoma medications.[23] The combined effect of aging and medication induced toxicity contributes to the increased severity of OSD in elderly glaucoma patients.

When comparing the changes among males and females using anti glaucoma medication, the changes were more severe among males than females, which was unpredictable. Dry eye symptoms are reported more prevalent in females than males.[24] Studies in past have shown that females have more dry eye symptoms especially after the age of 40-50 years[25] due to hormonal changes, which is contradictory to our findings. This discrepancy in our study, might be explained by occupational or environmental exposures might be more in males.

Our results are largely in agreement with other studies that have utilized impression cytology to evaluate ocular surface changes in glaucoma patients. For instance, a study by Hong et al. similarly found a statistically significant difference in cytological changes among different anti-glaucoma drug groups.[26] While some studies, such as that by Turacli et al., reported no significant differences.[27] variations in study populations, methodologies, and duration of follow-up could account for these discrepancies. The reliability and non-invasiveness of impression cytology make it a valuable tool for monitoring ocular surface health in this patient population.

This study consisted of a small simple size and it is

a single center study so generalizability to wider population may not accurate. Secondly, age related ocular surface changes may be a confounding factor in older population of this study.We did not look into age changes in particular. Thirdly, preservative free anti-glaucoma medications are not available in Nepal. Otherwise, comparison with preservative free drops would have given a more conclusive result.

CONCLUSIONS

Long-term use of topical anti-glaucoma medications significantly affects conjunctival cell morphology, with the severity of changes influenced by type of medication, duration of treatment and patient age. Prostaglandin analogues were associated with the most severe ocular surface alterations, particularly in older patients and those on medication for more than three years. These findings emphasize the need for careful consideration of ocular surface health in the long-term management of glaucoma, advocating for regular monitoring and potentially the use of preservative-free formulations or alternative treatment strategies to minimize ocular surface toxicity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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