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Effects of Tobacco on Corneal Endothelial Cells – a Hospital-based Cross-sectional Study from Western Gujarat, India

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Abstract:

Background: The aim was to evaluate various detrimental effects of tobacco chewing and smoking on corneal endothelial cells and morphology, as tobacco causes various ocular conditions, including cataract, glaucoma, dry eye, optic neuropathy, age-related macular degeneration and many more.

Materials and methods: The cross-sectional study was conducted in the ophthalmology department, focusing on individuals who consumed tobacco, through either smoking or chewing. Each group included 100 patients. Specular microscopy was performed to measure the endothelial cell density, coefficient of variation coefficient of variation, and percentage of hexagonal cells.

Results: Endothelial cell density and hexagonal cells were significantly higher in non-tobacco users than tobacco users, with p-values < 0.001. Corneal density, left eye coefficient of variation and hexagonal cells comparisons between smokers and tobacco chewers revealed no significant differences, with p-values > 0.05. Only right eye coefficient of variation showed a significant difference, with a p-value < 0.001.

Conclusion: This study revealed poor corneal endothelia cell health in smokers and tobacco users. Strict law should be enforced to halt tobacco consumption.

Key words: hexagonal cells (HEX), coefficient of variation (CV), corneal endothelial cell, tobacco.

Introduction

The use of tobacco products is one of the most significant risks to public health on a global scale. Using tobacco products is a substantial contributor to the development of several chronic illnesses, including cancer, lung disease, cardiovascular disease, and stroke. In terms of both consumption and production, India is the world's second-biggest tobacco market [1].

Cigarette smoke and the particles it produces include many hazardous chemicals. On one hand, these compounds promote vasospasm and platelet aggregation in tissues, ultimately leading to hypoxia. On the other hand, they cause oxidative damage to protein, lipid, and DNA by creating reactive oxygen metabolites [2, 3].

The effects of smoking on ocular illnesses are strongly dose-dependent; higher levels of smoking not only increase the likelihood of developing cataracts, age-related macular degeneration and dry eye but also exacerbate the severity of these conditions [4]. Research has shown that the levels of antioxidants found in blood, aqueous humour, and ocular tissues are decreased in the presence of these harmful chemicals. Smoking has significant negative effects on the eyes because it raises the concentration of oxygen radicals in the aqueous humour while lowering the quantity of ascorbic acid, an essential antioxidant [5, 6].

Numerous studies have shown that smoking increases the risk of developing age-related macular degeneration, cataracts, open-angle glaucoma, retinal vein occlusion, optic neuritis, dry eye, Graves' ophthalmopathy, and ocular inflammation because of all these toxic effects. Long-term exposure to cigarette smoke has been linked to a variety of problems that affect the ocular surface [7–10].

The first layer of the eye that experiences the effects of environmental stress is the ocular surface mucosa, which consists mostly of the cornea. The clear front section of the eye is believed to be responsible for two-thirds of the eye's total optical power. The corneal endothelium is a monolayer that lines the posterior aspect of the cornea and is important for maintaining the cornea in a dehydrated condition [11–13]. Because it is an avascular tissue, the cornea is more vulnerable to ischemia and oxidative stress (e.g. due to gases, vapours, or cigarette smoke) than other tissues in the eye [13–15]. Smoking may induce instability in the tear film, leading to dry eye symptoms such as foreign body sensation, stinging, burning, grittiness, and lacrimation [16].

Gujarat is one of the states where tobacco is used highly in both smoked and smokeless forms. According to GATS 2, 38.7% of men, 10.4% of women and 25.1% of all adults either smoke tobacco or use smokeless tobacco [17]. Studies on the effects of tobacco consumption (either by smoking or in smokeless form) on corneal endothelium in western India in Gujarat are scarce. Therefore, the present study was conducted to compare the effects of tobacco on corneal endothelial cell number and morphology.

Materials and methods

This was a cross-sectional study with a comparison group conducted for two years in the Ophthalmology Department of the GMERS Medical College, Dharpur, Patan, Gujarat, India. Persons who consumed tobacco (a person smoking at least one cigarette per day and a person chewing at least one packet of tobacco per day) for at least the last six months were eligible for the study group. Individuals who were neither active nor former smokers were also enrolled in the comparison group. Individuals who did not provide consent, who were on any ocular medication and had any ocular conditions were excluded from the study. Sample size was calculated as 100 based on the GATS-2 [17] study. Finally, 200 individuals (100 tobacco consumers and 100 non-tobacco consumers) were included in the analysis.

The Topcon SP-1P Specular Microscope was used for specular microscopy. Endothelial cell morphology analyses were performed using automated measurements with the retracing method built-in image analysis software. Three images from the cornea were taken, and at least 100 continuous cells were generated. Endothelial cell density was calculated by the formula.

$$\text{CD (cell/ } mm^2) = \frac{10^6}{\text{average cell area}}$$

Patients with a cell density of more than 1,000 cells/mm^2 were considered normal. Coefficient of variation (CV) in cell size (standard deviation divided by the mean cell area) was used as an index of the extent of variation in the cell area. A CV less than 33 was considered normal. The percentage of hexagonal cells (HEX or 6A) was counted. A HEX or 6A of more than 50% was considered normal. Centre corneal thickness (CCT) more than 0.49 mm was considered normal.

All participants were given a participant information sheet (PIS) in their native language and informed consent was taken. Ethical permission was taken from Institutional Ethics Committee (IEC). Confidentiality and privacy of the participants were maintained at every level. Epi info CDC 7 version was used to enter and analyse data. Mean and standard deviation (SD) were used to represent continuous variables. Proportions were used for categorical variables. The t-test was applied to evaluate the relationship between continuous variables. The chi-square test was used to assess the relationship between category variables. A *p*-value less than 0.05 was considered statistically significant.

Results

Table I shows that there was no significant difference for mean age or gender between study groups. The *p*-values for both RE and LE cell counts are less than 0.001, indicating that the cell counts were significantly higher in non-tobacco users than tobacco users. Similar results were obtained for CV in cell size and HEX.

Table II compares different variables between smokers and non-tobacco users. Non-tobacco users have significantly higher RE and LE cell counts than smokers. Smokers have significantly higher RE and LE coefficients of variation in cell size than non-tobacco users. Non-tobacco users exhibit significantly higher HEX values in both RE and LE compared to smokers.

	Tobacco users (n = 100)	Non-tobacco users (n = 100)	Total (n = 200)	*p*-value
Age				
	53.71 ± 11.78	55.72 ± 11.62	54.72 ± 11.72	0.23*
Gender				
Male	86 (86.0%)	88 (88.0%)	174 (87.0%)	0.67**
Female	14 (14.0%)	12 (12.0%)	26 (13.0%)	
Cell count				
RE cell count	2560.73 ± 336.06	2786.97 ± 306.90	2673.85 ± 340.44	<0.001*
LE cell count	2551.36 ± 381.94	2779.53 ± 270.81	2665.45 ± 349.49	<0.001*
CV in cell size				
RE-CV	34.51 ± 6.16	31.19 ± 2.71	32.85 ± 5.03	<0.001*
LE-CV	34.42 ± 3.65	31.12 ± 2.30	32.77 ± 3.46	<0.001*
HEX				
RE-HEX	44.36 ± 13.02	55.22 ± 8.11	49.79 ± 12.11	<0.001*
LE-HEX	44.99 ± 13.09	54.76 ± 7.14	49.88 ± 11.60	<0.001*

*p-value is calculated by independent sample t-test; **p-value is calculated by chi square (χ2) test.

Tab. I. Comparison of different variables between tobacco and non-tobacco users.

	Smokers (n = 62)	Non-tobacco users (n = 100)	*p*-value*
Corneal density			
RE cell count	2481.87 ± 308.55	2786.97 ± 306.90	<0.001
LE cell count	2493.39 ± 382.30	2779.53 ± 270.81	<0.001
CV in cell size			
RE-CV	34.13 ± 4.00	31.19 ± 2.71	<0.001
LE-CV	34.68 ± 3.92	31.12 ± 2.30	<0.001
HEX			
RE-HEX	42.90 ± 13.16	55.22 ± 8.11	<0.001
LE-HEX	44.76 ± 11.92	54.76 ± 7.14	<0.001

*p-value is calculated by independent sample t-test

Tab. II. Comparison of different variables between smokers and non-tobacco users.

	Tobacco chewers (n = 51)	Non-tobacco users (n = 100)	*p*-value*
Corneal density			
RE cell count	2589.00 ± 367.91	2786.97 ± 306.90	<0.001
LE cell count	2550.27 ± 447.92	2779.53 ± 270.81	<0.001
CV in cell size			
RE-CV	34.84 ± 7.80	31.19 ± 2.71	<0.001
LE-CV	34.55 ± 3.58	31.12 ± 2.30	<0.001
HEX			
RE-HEX	43.22 ± 15.79	55.22 ± 8.11	<0.001
LE-HEX	43.39 ± 15.36	54.76 ± 7.14	<0.001

*p-value is calculated by independent sample t-test.

Tab. III. Comparison of different variables between tobacco chewers and non-tobacco users.

	Smokers (n = 62)	Tobacco chewers (n = 51)	*p*-value*
Corneal density			
RE cell count	2481.87 ± 308.55	2589.00 ± 367.91	0.19
LE cell count	2493.39 ± 382.30	2550.27 ± 447.92	0.23
CV in cell size			
RE-CV	34.13 ± 4.00	34.84 ± 7.80	<0.001
LE-CV	34.68 ± 3.92	34.55 ± 3.58	0.51
HEX			
RE-HEX	42.90 ± 13.16	43.22 ± 15.79	0.25
LE-HEX	44.76 ± 11.92	43.39 ± 15.36	0.06

*p-value is calculated by independent sample t-test.

Tab. IV. Comparison of different variables between smokers and tobacco chewers.

Table III compares different variables between tobacco chewers and non-tobacco users. The *p*-value of <0.001 indicates a significant difference. Thus, non-tobacco users have significantly higher RE and LE cell counts than tobacco chewers. Tobacco chewers exhibit significantly higher RE and LE coefficients of variation in cell size compared to non-tobacco users. Non-tobacco users have significantly higher HEX values for both eyes compared to tobacco chewers.

The comparison of different variables between smokers and tobacco chewers is depicted in Table IV.

The *p*-value for RE and LE cell count is less than 0.05, indicating that the difference is not significant. The *p*-value for RE-CV is less than 0.001, indicating a significant difference, with tobacco chewers having a significantly higher mean CV than smokers, but the same is not true for LE-CV. The *p*-value for RE and LE-HEX is less than 0.05, suggesting that the difference is also significant.

Discussion

Tobacco consumption is an established risk factor for numerous ocular and systemic diseases. Tobacco (whether smokeless or in smoking form) contributes to various pathological conditions that lead to dysfunctional tear film development and corneal decompensation. According to the findings of the Beaver Dam Study, those who were either active smokers at the time of the study or had a smoking history were more likely to have ocular surface abnormalities and accompanying symptoms than non-smokers, [18] which is true for our study also.

Sayin N et al. [19] observed that there was no significant difference between groups for mean age and gender. The same distribution was found in our study also. In our study, both eye cell counts were significantly higher in non-tobacco users than tobacco users, with *p*-values less than 0.001. Similar observations were reported by Jha A. et al. [20] and Ilhan N. et al. [21].

In this study, CV for both eyes was significantly higher in tobacco users compared to non- tobacco users, with *p*-values less than 0.001. In a study by Jha A. et al. [19], these differences were statistically significant, but there was no statistically significant difference in studies by Ilhan N. et al. [21], Cankurtaran V. et al. [22] and Kara S. et al. [23].

In our study, both eye HEX values were significantly higher in non-tobacco users compared to both smokers and tobacco chewers, with *p*-values less than 0.001. We obtained similar findings as in the Jha A. et al. [20] study but different from the findings of Ilhan N. et al. [21] and Cankurtaran V. et al. [22].

Conclusion

We observed significant differences in cell counts, coefficient of variation, and hexagonal cells values between tobacco and non-tobacco users, the latter consistently showing better outcomes.

Specifically, non-tobacco users exhibit significantly higher cell counts in both eyes, lower coefficient of variation, and higher hexagonal cells values, indicating better endothelial cell health. Smokers and tobacco chewers both have lower cell counts, higher coefficient of variation, and lower hexagonal cells values compared to their non-using counterparts, with all differences being statistically significant. These findings highlight the detrimental impact of tobacco use on ocular cell health, reinforcing the need for public health interventions to reduce tobacco consumption.

Disclosure
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