Original Research Article

The Effect of Tobacco Smoking on Corneal Endothelium and Central Corneal Thickness

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Abstract

We aimed to study the impact of tobacco smoking towards central corneal thickness, morphology, and corneal endothelial cell density (ECD) in healthy subjects. This cross-sectional study recruited consecutive patients who visited the Ophthalmology Clinic, Universiti Kebangsaan Malaysia Medical Centre, from April 2017 to August 2018 who were active smokers and non-smokers. Active smokers were further divided into heavy and light smokers according to the number of pack-years smoked. The nicotine dependency level was measured using the Fagerstrom test and divided into low, moderate, and severe dependency. Non-contact specular microscopy was performed. Parameters studied included the ECD, percentage of hexagonality (HEX), coefficient of variation of cell size (COV), and central corneal thickness (CCT). A total of 84 eyes from 84 subjects were included, with 42 in the smoker and 42 in the non-smoker groups. The mean age was 38.3 ± 6.7 and 39.2 ± 10.7 years in the smoker and non-smoker groups, respectively. The mean ECD, HEX, and COV were 2783 ± 316 vs. 2877 ± 333 cells/mm^2, 55.0 ± 6.7% vs. 55.0 ± 7.7% and 32.7 ± 4.4% vs. 32.2 ± 4.1% in the smoker and non-smoker group, respectively. However, none were statistically significant ($p>0.05$). The mean CCT was also not statistically different, with 532.6 ± 30.8 µm in the smoker group and 530.5 ± 30.8 µm in the non-smoker group. No statistically significant difference was found between light and heavy smokers and addiction levels. There was a statistically significant but weak negative correlation between ECD and duration of smoking ($r = -.370$, $p = 0.016$) and a weak positive correlation between COV and number of pack-years smoked ($r = .344$, $p = 0.025$). Smoking did not significantly affect corneal ECD, cellular morphology, or CCT.

Keywords: Cellular morphology; central corneal thickness; corneal endothelial density; hexagonality; tobacco smoking

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Introduction

Cigarette smoking is a well-known risk factor for a wide range of serious illnesses, including certain types of malignancies and cardiovascular diseases, (1) and its health effects are widely known and well documented. Since 1964, every report by the U.S. Surgeon General has identified smoking as the leading cause of preventable death and disease (2). There are substantial benefits associated with the cessation of smoking, and numerous reports have shown these noteworthy benefits, which include a reduction in the number of deaths annually from cardiovascular diseases, cerebrovascular accidents, cancers, and respiratory illnesses like chronic obstructive pulmonary diseases (3).

Tobacco contains more than 4000 hazardous toxic substances that can cause harmful side effects. It is important to note that over 40 of these chemicals have been identified as carcinogenic, while many others can pose a serious risk to our pulmonary and cardiovascular systems. It is crucial that we take steps to minimise our exposure to such substances to safeguard our health and well-being. They include nicotine, tars, formaldehyde, carbon monoxide,

nitrosamines, polycyclic aromatic hydrocarbon, and hydrogen cyanide (4). These toxic compounds have various detrimental effects on different organs, including the eyes.

Tobacco exposure has led to yearly mortality of around 6 million worldwide (5). By 2025, the global estimated number of smokers is expected to increase to 1.6 billion, which will then increase the number of mortality related to cigarette smoking to nearly 8.3 million by 2030 (6). Developing countries are vulnerable to the harmful effects of smoking-related diseases (5), experiencing significant negative impacts on their health, economy, and society. For the past three decades, smoking-related diseases have become the leading cause of Malaysian mortality. We have spent almost 2.92 billion Malaysian Ringgit treating chronic obstructive pulmonary disease, ischaemic heart disease, and lung cancer (7). It is concerning that the prevalence of smoking among adult males in Malaysia was as high as 46.4%. However, it is positive to note that there was a gradual decrease of 2.8% from 1996 to 2006. This highlights the need for continued efforts to reduce smoking rates and promote healthier lifestyles (7). This ongoing high prevalence of current male smokers in Malaysia should raise awareness not only among the public health authorities but also among every healthcare worker, including ophthalmologists.

Certain ocular diseases have been attributed to cigarette smoking. There is an increasing consideration that smoking is a significant risk factor for many ischemic disorders of the eye, affecting ocular vasculature on several levels, including non-arteritic anterior ischemic optic neuropathy (8) and idiopathic ophthalmoplegia, which decreases blood supply to the extraocular muscles and functional paresis (9). For those concerned about these conditions, it may be helpful to consider reducing or quitting smoking and adopting a healthier lifestyle. Tobacco smoking increases the risk of cataract development (10), probably through its effect on the oxidant-antioxidant interaction and oxidative damage in the lens. Smoking further impairs the lens function by causing additional oxidative challenges and depletion of endogenous antioxidant pools. Heavy metals like lead, cadmium, and copper may accumulate in the lens and cause further toxicity (11).

Smoking has been implicated in the development of ophthalmologic manifestation of Graves's disease in genetically predisposed individuals. There were significantly more smokers in patients with ophthalmopathy than in patients with Graves thyrotoxicosis or the healthy control group (12).

A strong correlation was found between advanced stages of age-related macular degeneration (ARMD) and tobacco exposure, related to its effect on increasing oxidative stress and lipid peroxidation and reducing plasma concentrations of antioxidants (11). Active smokers were at increased risk for late-stage exudative ARMD (13). The consumption of tobacco has been found to have adverse effects on the choroidal blood flow in the eye, which in turn increases the susceptibility of the macula to degenerative changes. This occurs due to the generation of free radicals and subsequent oxidation of the outer retina, which is known to be rich in polyunsaturated fatty acids.

We aimed to compare the corneal endothelial density and morphological changes between chronic smokers. We grouped them according to their level of addiction level and number of pack-years, and the control group of non-smokers, in a Malaysian population. The result of this study might shed some light on the health of corneal endothelial cells in smokers to ensure post-operative success in ocular surgeries and to predict the prognosis in various cases, especially in refractive surgery. It is also important to note when performing pre-operative evaluation for cataract or refractive surgeries in chronic smokers and informing them about additional risks related to post-operative recovery.

Materials and Methods

- Subject selection

This observational cross-sectional study was done in the Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) from April 2017 to August 2018. Patients or relatives who visited UKMMC and UKMMC staff from all departments were invited to participate in this study. Ethical approval was obtained from the Universiti Kebangsaan Malaysia Research and Ethics Committee (Ethical approval code: FF-2017-455). This study adhered to the tenets of the Declaration of Helsinki and Malaysian Guidelines for Good Clinical Practice (GCP). A signed written informed consent was obtained before enrolment. Sample size calculation was based on comparing two means. Based on Ilhan et al. (2016), the estimated total sample size would be 82 patients for this study, with 41 subjects in each group of smokers and non-smokers (14).

The inclusion criteria were age between 18 to 60 years old, current smokers for at least five years without any eye disease apart from mild refractive error (refractive

error between -3.00 to +3.00D), healthy non-smokers without any eye disease apart from mild refractive error, and ability to provide written informed consent. A history of contact lens use, history of intraocular surgery, ocular trauma, and diabetes mellitus as it is well established that diabetes can affect endothelial cell density (ECD) (15), patients with pre-existing ocular disease such as glaucoma, uveitis, corneal opacity, keratoconus, Fuchs endothelial dystrophy, presence of on-going ocular infection or inflammation and current use of topical ophthalmic drug were excluded.

The data collected include age, ethnicity, occupation, smoking status and chronicity, nicotine dependence score, visual acuity, intraocular pressure, ECD, percentage of hexagonal cells, coefficient of cell size variation, and central corneal thickness (CCT).

The standard of National Health Interview Survey (NHIS) on current smoking definition, which screens for lifetime smoking of ≥100 cigarettes, was used. The definition comprised two questions with the present wording in use since 1992. The first question is, "Have you smoked at least 100 cigarettes in your entire life?" Respondents answering "yes" were classified as ever-smokers, and those who answered "no" were classified as non-smokers. Ever smokers were then asked a second question: "Do you now smoke cigarettes every day, some days, or not at all?" Respondents who answered "every day" or "some days" were classified as current smokers (16). Smokers were divided into light smokers who have smoked less than 15 pack-years and heavy smokers who have smoked more than 15 pack-years. Subjects, who were on electronic cigarettes, nicotine replacement therapy, and other non-cigarette smoking products, for example, cigars and water pipe, were excluded from this study.

The Fagerstrom Test for Nicotine Dependence (FTND) is an instrument commonly used to assess the intensity of physical dependence. Patients with more intense physical dependency on nicotine will have a higher Fagerstrom score. It is a valuable tool for identifying individuals who have the greatest concentration of tobacco products and who, therefore, might be at greater risk of disease. It is also closely related to biochemical indices of the heaviness of smoking (17). Nicotine dependence is divided into low, moderate, and severe.

All participants underwent slit lamp examination and applanation tonometry. Corneal ECD, endothelial cell morphology, and CCT were measured using non-contact specular microscopy (Topcon SP-1P, Japan). Only measurement from the right eye of each subject was taken.

- Statistical analysis

Statistical analysis was performed using SPSS for Mac, Version 25 (SPSS Inc, Chicago, IL, USA). The normality of the distribution for continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous data was presented as mean ± standard deviation, while categorical variables were shown as frequency and percentages. The independent t-test was used to compare the parametric variables between the two groups. Non-parametric tests like the Whitney U test were used for non-normally distributed data. Subgroup analysis was calculated using one -way ANOVA test to compare means in more than two groups. Pearson or Spearman correlation was used to determine the strength of the relationship between the variables. P values <0.05 were regarded as statistically significant.

Result

A total of 84 subjects were recruited in the study. Forty-two subjects were in the smoker group, and the other 42 subjects were in the non-smoker group. The mean age was 38.3 ± 6.7 years and 39.2 ± 10.7 years in the smoker and non-smoker group. There was no significant difference in age and ethnicity among the study participants (P>0.05). Table 1 showed the summary of the 84 eyes studied in both groups.

Table 2 compared the mean values of corneal endothelial parameters between smoker and non-smoker groups. The mean corneal ECD was lower in the smoker group compared to non-smokers, but it was not statistically significant. There was no difference between smokers and non-smokers regarding the percentage of hexagonality and coefficient of variation. Smokers were found to have slightly thicker CCT, but it was not statistically significant.

We compared the mean values of corneal endothelial cell parameters between light smokers who smoked less than 15 pack-years and heavy smokers who smoked more than 15 pack-years. The result showed that heavy smokers had a lower mean value for ECD and percentage of hexagonality and a higher value of the coefficient of variation. However, it was not statistically significant. CCT was thicker in heavy smokers but not statistically significant (Table 3). No statistically significant difference was found in the corneal endothelial parameters when compared across addiction levels (Table 4).

On average, the smoker group consumed 14.3 ± 8.8 cigarettes per day and had been smoking for 21.1 ± 6.2 years. The average number of pack years was 15.7 ±

TABLE 1: Demographic data of participant

	Smoker	Non-smoker	P value
	n = 42	n = 42	
Age (years ± SD)	38.3 ± 6.7	39.2 ± 10.7	0.875[c]
Ethnicity, n (%)			
Malay	38 (90.5)	32 (76.2)	0.091[b]
Chinese	4 (9.5)	6 (14.3)	
Indian	0 (0)	2 (4.8)	
Others		2 (4.8)	
Visual acuity, Logmar	0.08	0.06	0.411[c]
IOP, mmHG	13.4	13.6	0.696[a]
Smoking duration, years	21.1 ± 6.2		
Mean number of pack year	15.7 ± 6.2		
Mean number of cigarette/day	14.3 ± 8.8		
Mean addiction score	2.9 ± 2.6		
Smoking severity, n (%)			
Light smoker	27 (64.3)		
Heavy smoker	15 (35.7)		
Fagerstrom score, n (%)			
Low	27 (64.3)		
Moderate	10 (23.8)		
High	5 (11.9)		

[a]Student t test, [b]Mann Whitney U test, [c]Fisher's exact test

TABLE 2: Characteristic of endothelial cell parameters between smoker and non-smoker

	Smoker n = 42 Mean ± SD	Non-smoker n = 42 Mean ± SD	P value
Endothelial cell density, cell/mm^2	2783.88 ± 316.37	2877.07 ± 333.27	0.192[a]
Percentage of hexagonality, %	55.57 ± 6.74	55.33 ± 7.77	0.881[b]
Coefficient of variation, %	32.71 ± 4.41	32.29 ±4.12	0.647[a]
Central corneal thickness, µm	532.60 ± 30.86	530.55 ± 30.84	0.770[a]

[a]Independent t test, [b]Mann Whitney U test

TABLE 3: Comparison of mean values of corneal endothelial cell parameters between light smoker and heavy smoker

	Heavy smoker n = 15 Mean ± SD	Light smoker n = 27 Mean ± SD	P value[a]
Endothelial cell density, cell/mm^2	2732.73 ± 283.39	2812.30 ± 335.02	0.442
Percentage of hexagonality, %	54.53 ± 6.41	56.15 ± 6.97	0.464
Coefficient of variation, %	33.67 ± 3.13	32.19 ±4.96	0.303
Central corneal thickness, µm	534.53 ± 31.08	531.52 ± 34.72	0.781

[a]Independent t test

TABLE 4: Comparison of mean values of corneal endothelial cell parameters between level of addictions

Nicotine dependency	Low n = 27 Mean ± SD	Moderate n = 10 Mean ± SD	High n = 5 Mean ± SD	P value[a]
Endothelial cell density, cell/mm^2	2827.89 ± 321.28	2724.90 ± 337.82	2664.20 ± 241.97	0.463
Percentage of hexagonality, %	57.37 ± 7.06	51.80 ± 4.54	53.40 ± 5.68	0.058
Coefficient of variation, %	32.37 ± 5.00	34.10 ± 2.69	31.80 ± 3.77	0.517
Central corneal thickness, µm	536 ± 35.31	521.90 ± 21.16	353.60 ± 41.86	0.515

[a]One-way ANOVA

6.2. According to the Fagerstrom nicotine addiction test results, the addiction level was low in 27 subjects (64.3%), moderate in 10 subjects (23.8%), and high in 5 subjects (11.9%). The mean addiction score was 2.9 ± 2.6.

ECD had a weak negative correlation with age in the smokers group (r = -.173, p = 0.27) and the non-smokers group (r = -.275, p = 0.079). However, these correlations were not statistically significant. There was a statistically significant but weak negative correlation between ECD and age when both groups were combined (r = -.247, p = 0.024) (Figure 1A). This result showed that ECD reduced with ageing.

There was a statistically significant, weak, negative correlation between ECD and duration of smoking (r = -.370, p = 0.016, Figure 1B). A weak negative correlation was also seen between ECD and the number of pack years, but it was not statistically significant (r = -.248, p = 0.113).

A weak, negative correlation was found between the percentage of hexagonality and age (r = -.273, p = 0.08), duration of smoking (r = -.163, p = 0.3), and number of packyears (r = -.224, p = 0.153) in the smokers group but these were not statistically significant. There was a statistically significant, weak, negative correlation between the percentage of hexagonality and age when both groups were combined (r = -.388, p = 0.000, Figure 1C).

A weak, positive correlation was observed between coefficient of variation and age (r = 0.290, p = 0.062), duration of smoking (r = 0.283, p = 0.069), which was not statistically significant, and number of pack years (r = 0.344, p = 0.025, Figure 1D) which was statistically significant. There was a statistically significant, weak, positive correlation between the coefficient of variation and age when both groups were combined (r = 0.287, p = 0.008) (Figure 2). No significant correlation was seen between CCT and age, duration of smoking and chronicity of tobacco.

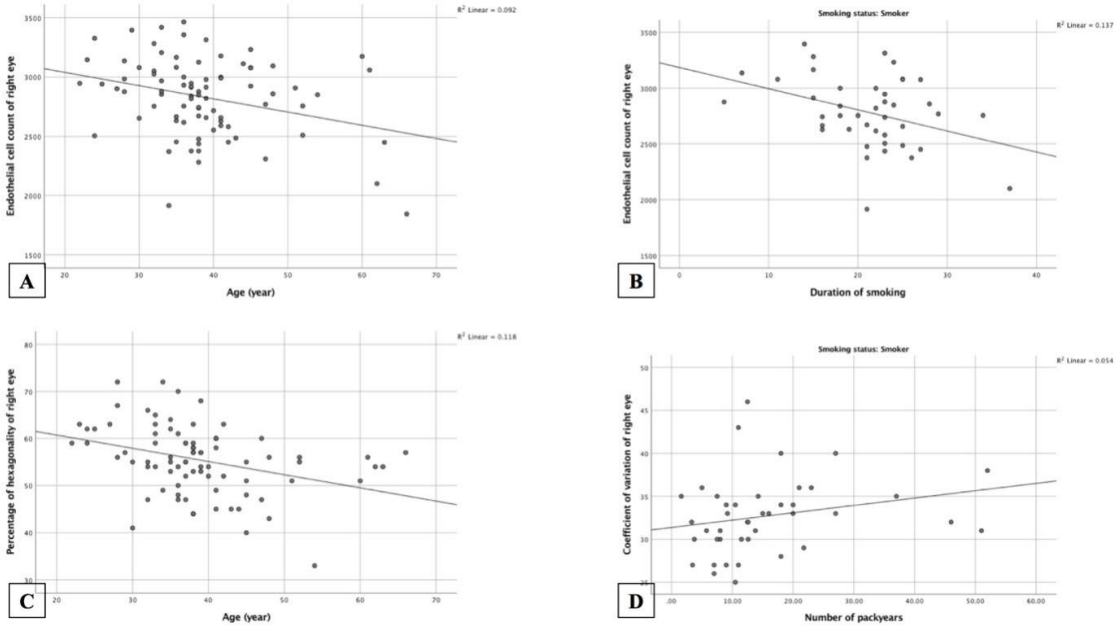

FIGURE 1: Scatterplot showing corneal endothelial parameters and various factors. (A) Scatterplot showed the relationship endothelial cell density and age (r = -0.247, p = 0.024); (B) Scatterplot showed the relationship between endothelial cell density and duration of smoking (r = -.370, p = 0.016); (C) Scatterplot showed the relationship between percentage of hexagonality and age when both groups was combined (r = -.388, p = 0.000); (D) Scatterplot showed the relationship between coefficient of variation and number of pack years (r = 0.344, p = 0.025).

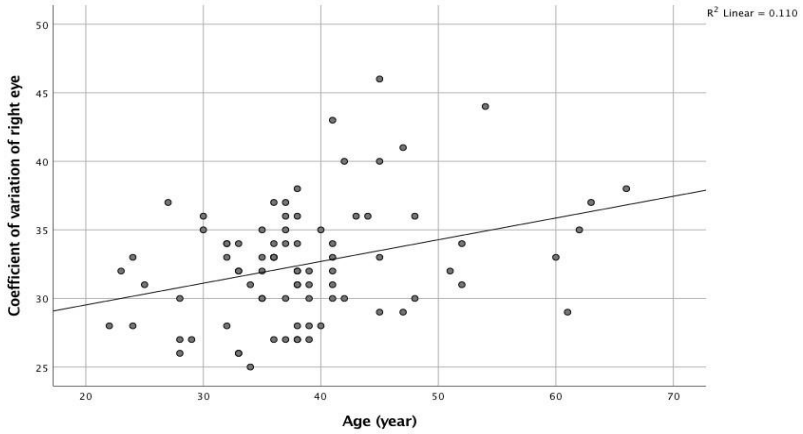

FIGURE 2: Scatterplot showed the relationship between coefficient of variation and age when both groups was combined. (r = 0.287, p = 0.008)

Discussion

Smoking has been established to be linked to many ocular diseases. Numerous studies in the scientific literature have provided extensive evidence of the various harmful physiological effects that cigarette smoking can have on the human body (18). However, the long-term impact and relationship between tobacco and corneal endothelium need to be better established. A study by Kwon et al. (2016) found that, in an eye bank corneal donor database, the ECD was not affected by smoking (19). Another survey by Sopapornamorn et al. (2008) found no relationship between corneal endothelial parameters and smoking (20). These two studies only specified whether there was a history of smoking. They did not address the length of exposure and severity of smoking. A population-based survey by Zoega et al. (2006) on the prevalence of primary central corneal guttata in a white population showed that the risk of developing corneal guttata, the abnormal excrescences of the basement membrane, and fibrillar collagens from distressed corneal endothelial cells, were found to be increased two-fold in smokers who smoked more than 20 pack-years (21).

The mean corneal ECD in normal adults was reported to be 2400 (range, 1500 – 3500) cells/mm^2 (22). We found higher values in our patients, with 2783 ± 316 cells/mm^2 in the smoker group and 2877 ± 333 cells/mm^2 in the non-smoker group. This finding is comparable to a study done in Malaysia (23), which showed a mean ECD of 2648 ± 310 cells/mm^2. The depletion of endothelial cells, which can be attributed to various factors such as ageing, trauma, intraocular surgery, and glaucoma, has resulted in alterations in the morphology of corneal endothelial cells.

Our results clearly did not show significant difference in ECD between smokers and non-smokers. This result is consistent with a few previous studies. A study by Sayin et al. and Kara et al. reported no significant difference in ECD and coefficient of cell size variation between smokers and normal subjects (24,25).

A study by Ilhan et al. (2016) found a significant reduction in ECD in smokers compared to non-smokers (14). However, no significant difference was reported in the percentage of hexagonality and coefficient of variation between smokers and non-smokers. This finding also agreed with a study by Golabchi et al. (2018), who found tobacco smoke and nicotine derivatives involved in corneal endothelial cell death and apoptosis (26). Similar findings were also found in experimental endothelial cell cultures (27). Smoking accentuates free radicals production and decreases antioxidant levels in the blood, aqueous humour, and ocular tissue. Increased oxidative stress was shown in the glaucoma patient's trabecular meshwork and posterior pole. Inflammatory and apoptotic marker levels were also increased in smokers' aqueous humour and plasma (28).

The percentage of hexagonality and coefficient of variation between the two groups in our study were not significantly different. This result showed that cigarette smoking did not increase the risk of endothelial cell damage. This is consistent with other studies, which found a lower percentage of hexagonal cells in smokers compared to normal healthy subjects, postulated to be caused by chronic hypoxia in smokers. Setala et al. (1998) and Lee et al. (2001) found chronic hypoxia may cause a reduction in the percentage of hexagonal endothelial cells (29,30). The

induced hypoxia disturbed cell stability, causing polymegathism, pleomorphism, and cell death. The tendency of corneal endothelial cells to form regular hexagons diminished. Hypoxia in chronic smokers, from the nicotine effect, is having an adrenergic impact, causing peripheral vasoconstriction and subsequent decrease in tissue-oxygen tension. This shifts the cellular metabolism towards an aerobic pathway as the tissue becomes poorer in oxygen and disrupts the corneal oxygenation regulation.

Several factors could explain why our result showed no significant difference between smokers and non-smokers in ECD and corneal endothelial morphology. Our smaller sample size could explain this compared to other studies that showed significant differences in ECD. A further possible explanation would be that it is widely recognised that the cornea is an immune-privileged tissue, having an efficient physical barrier and lacking efferent lymphatics supply, and **(31)** is also devoid of blood vessels. Therefore, it is unknown how much of the toxic effect of nicotine from plasma blood can reach the cornea directly and cause significant damage to the corneal endothelium. A small amount of toxins from cigarette smoking might enter the aqueous humour. However, at the present moment, no study has been done to identify the level of the potential toxic agents from cigarette smoking in aqueous humour.

We also studied if there was any relationship between endothelial cell parameters and the chronicity of smoking by the number of pack years smoked. Our result showed a lower number of ECD, a lower percentage of hexagonality, and a higher coefficient of variation in the heavy smoker group, although it was not statistically significant. This finding could be due to the small sample size within the groups. Analysis by addiction level could not find any significant difference in ECD and cellular morphology. This is in agreement with studies by Kara et al. (2017)(24). However, Golabchi et al. (2018) found that smokers with severe nicotine dependency had significantly greater average cell size, which indicated polymegathism and lower ECD in comparison to non-smokers (26).

This study has several limitations, including its relatively small size. A multicentre study with different populations may be more valuable for future research strategies. A cross-sectional design may limit our results in the evaluation of causal relations. The other limitations were that the number of pack-years smoked and duration of smoking were determined from self-report.

Conclusion

In conclusion, this study showed no significant change in ECD, percentage of hexagonality, coefficient of variation, and CCT between smokers and non-smokers. Despite this result, smoking cessation is still an essential part of public health education as it is a modifiable behaviour. Eye care professionals should promote and integrate treatment for smoking cessation in patients' care to motivate and help them to quit smoking.

References

1. Bartecchi CE, MacKenzie TD, Schrier RW. The human costs of tobacco use. N Engl J Med 1994; 330(13): 907-12.

2. Fielding, J.E., Smoking: Health effects and control. N Engl J Med 1985; 313(8): 491-8.

3. Novello AC. Surgeon General's report on the health benefits of smoking cessation. Public Health Rep 1990; 105(6): 545-8.

4. Chiba M, Masironi R. Toxic and trace elements in tobacco and tobacco smoke. Bull World Health Organ 1992; 70(2): 269-75.

5. Wipfli H, Samet JM. Global economic and health benefits of tobacco control: Part 1. Clin Pharmacol Ther 2009; 86(3): 263-71.

6. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3(11): e442.

7. Lim HK, Ghazali SM, Kee CC, et al., Epidemiology of smoking among Malaysian adult males: Prevalence and associated factors. BMC Public Health 2013; 13: 8.

8. Chung SM, Gay CA, McCrary JA 3rd. Nonarteritic ischemic optic neuropathy. The impact of tobacco use. Ophthalmology 1994; 101(4): 779-82.

9. Hoffmann A, Barth A, Perthel U, Steffen HM, Brunner R, Allolio B. Vascular risk factors in patients with ophthalmoplegia. Med Klin 1990; 85(8): 459-62.

10. Christen WG, Manson JE, Seddon JM, et al., A prospective study of cigarette smoking and risk of cataract in men. JAMA 1992; 268(8): 989-93.

11. Solberg Y, Rosner M, Belkin M. The association between cigarette smoking and ocular diseases. Surv Ophthalmol 1998; 42(6): 535-47.

12. Hegedius L, Brix TH, Vestergaard P. Relationship between cigarette smoking and Graves' ophthalmopathy. J Endocrinol Invest 2004; 27(3): 265-71.

13. Hyman LG, Lilienfeld AM, Ferris FL 3rd, Fine SL. Senile macular degeneration: A case-control study. Am J Epidemiol 1983; 118(2): 213-27.

14. Ilhan N, et al., Effects of smoking on central corneal thickness and the corneal endothelial cell layer in otherwise healthy subjects. Eye Contact Lens 2016; 42(5): 303-7.

15. Shenoy R, Khandekar R, Bialasiewicz A, Al Muniri A. Corneal endothelium in patients with diabetes mellitus: A historical cohort study. Eur J Ophthalmol 2009; 19(3): 369-75.

16. Ryan H, Trosclair A, Gfroerer J. Adult current smoking: Differences in definitions and prevalence estimates - NHIS and NSDUH, 2008. J Environ Public Health 2012; 2012: 918368.

17. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The fagerstrom test for nicotine dependence: A revision of the fagerstrom tolerance questionnaire. Br J Addict 1991; 86(9): 1119-27.

18. U.S. Department of Health and Human Services. The Health Consequences of Smoking: 50 years of Progress. A report of the Surgeon General. Atlanta, GA USA: Department of Health and Human Prevention and Health Promotion, Office on Smoking and Health. Printed with correction. 2014.

19. Kwon JW, et al.. Analyses of factors affecting endothelial cell density in an eye bank corneal donor database. Cornea 2016; 35(9): 1206-10.

20. Sopapornamorn N, Lekskul M, Panichkul S. Corneal endothelial cell density and morphology in Phramongkutklao Hospital. Clin Ophthalmol 2008; 2(1): 147-51.

21. Zoega GM, Fujisawa A, Sasaki H, et al., Prevalence and risk factors for cornea guttata in the Reykjavik Eye Study. Ophthalmology 2006; 113(4): 565-9.

22. American Academy of Ophthalmology. Examination techniques for the external eye and cornea. Basic and Clinical Science Course. 2004-2005: 33-34.

23. Mohammad-Salih PA. Corneal endothelial cell density and morphology in normal Malay eyes. Med J Malaysia 2011; 66(4): 300-3.

24. Kara S, Gencer B, Türkön H, et al. The effect of smoking on corneal endothelial cells. Semin Ophthalmol 2017; 32(2): 223-7.

25. Sayin N, Kara N, Pekel G, Altinkaynak H. Effects of chronic smoking on central corneal thickness, endothelial cell, and dry eye parameters. Cutan Ocul Toxicol 2014; 33(3): 201-5.

26. Golabchi K, et al., The effects of smoking on corneal endothelial cells: a cross-sectional study on a population from Isfahan, Iran. Cutan Ocul Toxicol 2018; 37(1): 9-14.

27. Tithof PK, Elgayyar M, Schuller HM, Barnhill M, Andrews R. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a nicotine derivative, induces apoptosis of endothelial cells. Am J Physiol Heart Circ Physiol 2001; 281(5): H1946-54.

28. Law SM, Lu X, Yu F, Tseng V, Law SK, Coleman AL. Cigarette smoking and glaucoma in the United States population. Eye 2018; 32(4): 716-25.

29. Setälä K, Vasara K, Vesti E, Ruusuvaara P. Effects of long-term contact lens wear on the corneal endothelium. Acta Ophthalmol Scand 1998; 76(3): 299-303.

30. Lee JS, Park WS, Lee SH, Oum BS, Cho BM. A comparative study of corneal endothelial changes induced by different durations of soft contact lens wear. Graefes Arch Clin Exp Ophthalmol 2001; 239(1): 1-4.

31. Zhou R, Caspi RR, Ocular immune privilege. F1000 Biol Rep 2010; 2: 3