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THE EFFECTS OF SMOKING ON CORNEAL ENDOTHELIAL CELLS

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ABSTRACT

Background: smoking is a known contributor to various ocular conditions, such as cataract, glaucoma, macular degeneration, retinal vascular occlusion, and anterior ischaemic optic neuropathy. Chronic smoking affects every tissue in the eye and has been shown to decrease antioxidants and increase free radicals in the blood, ocular tissues, and aqueous humour. **Aim:** the present study aimed to evaluate the effects of chronic smoking on corneal endothelial cells and central corneal thickness**. Methods:** this observational cross-sectional study was conducted on 160 patients attending the out-patients department at Benghazi Ophthalmology Teaching Hospital, from December 2023 to April 2024. 80 patients were smokers and 80 were non-smokers and were aged between 20 and 70 years and they underwent a complete ophthalmological examination and visual acuity was determined. **Results:** there was no statistically significant difference between smokers and non-smokers group regarding age. The mean duration of smoking was .The mean cell density was 2311(311) cell/mm^2 for the smoker and 2697 (33) cell/mm^2 for the non-smoker and the difference was statistically significant ($p < 0.0001$). The difference in the average cell size was also statistically significant ($p< 0.0001$) between the smoker and non-smoker [419 (88) um^2 and 359 (33) um^2 respectively]. Among the smoker, there was non-significant association between the type of smoking (cigarette, shisha or combined) and corneal measures. **Conclusion:** chronic smoker have a significantly lower the cell density and cell hexagonality values but a larger average cell size than non-smokers. Preoperative corneal examination should be performed more carefully among smokers while planning intraocular surgeries such as cataract surgery, refractive surgery and keratoplasty.

KEYWORDS: Cigarette smoking, ocular surface, central corneal thickness, endothelial cells.

INTRODUCTION

Chronic smoking habit leads to numerous preventable serious health problems. Despite all struggles on this issue, it is still a current problem with high mortality and morbidity rates. According to the WHO report, smoking causes 5.4 million premature deaths each year. Many toxic substances are found in cigarette smoke and particles. On the one hand, these substances cause vasospasm, platelet aggregation in tissues leading to hypoxia, on the other hand, it causes oxidative damage on protein, lipid and DNA by producing reactive oxygen metabolites. It has also been reported that these toxic substances reduce the level of antioxidants in blood, aqueous humor and ocular tissues (**Karakurt et al., 2019).**

Various studies have shown that chronic smoking habit causes cardiovascular, respiratory and neoplastic diseases, as well as cataract, open-angle glaucoma, age-related macular degeneration, retinal vein occlusion, optic neuritis, dry eye, graves ophthalmopathy, and ocular inflammation **(Velilla et al., 2013)**. In addition to these eye diseases, long-term exposure to cigarette smoke has been reported to cause some ocular surface disorders **(Özcura et al., 2009)**.

Corneal endothelial cells have important functions in providing visual acuity, regulating intraocular pressure, and providing corneal integrity. In order to perform these tasks, they must be both adequate in number and regular in structure. The studies have shown that corneal endothelial cells are highly susceptible to hypoxia and oxidative stress **(Joyce, 2012).**

The density of corneal endothelial cell exhibits a physiological reduction between 0.5% and 0.8% annually due to aging. Endothelial cells, which are highly susceptible to negative conditions such as trauma, ocular surgery, hypoxia and oxidative stress, have important functions in providing corneal integrity and optical transparency. The place of damaged endothelial cells in the endothelial cell layer, which has no regeneration ability, is compensated by the enlargement of the remaining cells. The typical hexagonal structure of

enlarged endothelial cells is thereby impaired. Nowadays, non-invasive specular microscopes are used to evaluate the morphology and number of the endothelial layer. Corneal thickness can also be measured with these instruments **(Karakurt et al., 2019).**

Oxidative stress has very harmful effects on tissues. Smoking causes a release of a large number of free radicals, leading to peripheral vasoconstriction and consequently a reduction in tissue oxygenation. Moreover, a low concentration of nicotine induces both sympathetic and parasympathetic stimulation, especially more in the sympathetic ganglion. This sympathetic stimulation causes an increase in heart rate and peripheral resistance. In addition, nicotine may also cause these effects by leading to a stimulation in the adrenal gland. Carbon monoxide (CO) has been found to be 100 times higher in smokers than in non-smokers. Several studies have shown that CO contributes to increase in carboxyhaemoglobin and development of hypoxia in tissues **(Kara et al., 2017)**. Hypoxia and oxidative stress resulting from these mechanisms might be expected to have negative effects on highly sensitive corneal endothelial cells. Furthermore, other studies have shown that toxic substances in cigarette induce apoptosis in endothelial cell cultures **(Ilhan et al., 2016).**

AIM OF THE STUDY

The present study aimed to evaluate the effects of chronic smoking on corneal endothelial cells, central corneal thickness.

Patients and Methods

This observational cross-sectional study was conducting in Benghazi Opthalmology Teaching Hospital, Faculty of medicine, Benghazi University, from December 2023 to April 2024 on 160 participants (80 smokers and 80 non-smokers) aged 20–70 years, who underwent routine eye examination during this study period.

Written informed consent was obtained from all participtants and the study was approved by the Research Ethical Committee of Faculty of Medicine, Benghazi University (Institutional review board).

Inclusion criteria

One hundred and sixty male patients aged between 20 and 70 years were included in the study; 80 were smokers (study group) and 80 were non-smoker (control group). The smoker participants were healthy without any ophthalmic disorders and with a history of at least 5 years continuous smoking.

Exclusion criteria

Patients who had ocular trauma or underwent any other ocular surgery, used contact lenses, had additional ocular pathology and those with a systemic disease such as hypertension or diabetes mellitus, any drug or substance addiction were non eligible to be included in the study. Moreover, healthy individuals who were a former smoker were excluded from this study.

A full history was taken; any chronic illness, duration and type of smoking (cigarette, shisha, or both, taking in consideration that 1 shisha stone equals 10 cigarettes). A complete ophthalmological examination was performed for the patients and visual acuity was determined. Their intraocular pressures were measured with the Goldmann applanation tonometer. Their anterior segment and fundus examinations were performed using a biomicroscope. Central corneal thickness (CCT), cell density (cells/mm^2) (CD), coefficient of variation (CV) of endothelial cells, percentage of hexagonal cells (HEX), and average cell size variability (AVG), were evaluated by non-contact specular microscopy (Topcon SP-3000P, Topcon Corporation, Tokyo, Japan). If both eyes were qualified to and eligible to be included in the study, only one eye was randomly selected to perform the specular microscopy.

Moreover, smoking history was taken from all smoking participants; pack/year (calculated by multiplying the number of smoking years by the average number of cigarette packs used per day) **(Bernaards et al., 2001).**

The Fagerstrom questionnaire (Table 1) was used for the Nicotine Dependency of smoking patients. In this test, 6 questions were answered by the entire smoker population. Based on their responses, Nicotine addiction was graded in three groups as low (0–3 points), moderate (4–6 points), and severe (7–10 points), according to the total test scores **(Meneses-Gaya et al., 2009).**

Table 1: Fagerstrom Nicotine Dependence Scale (Meneses-Gaya et al., 2009).

Questions	Answers	Points
How soon after you wake up do smoke your first cigarette?	Within 5 min	3
	6 - 30 min	2
	31 – 60 min	1
	After 60 min	0
Do you find it difficult to refrain from smoking in places where it is forbidden, e.g. in church, at the library, in a cinema, etc.?	Yes	1
	No	0
Which cigarette would you hate to give up most?	The first one in the morning	1

	All others	0
How many cigarettes do you smoke per day?	10 or less	0
	11-20	1
	21–30	2
	31 or more	3
Do you smoke more frequently during the first hours after awakening than during the rest of the day?	Yes	1
	No	0
Do you smoke if you are so ill that you are in bed	Yes	1
most of the day?	No	0

Statistical Analysis: the statistical analysis of the data was performed using the SPSS version-22 (Chicago, IL, USA) software. The normality of the variables was analysed using the Kolmogorov-Smirnov test. The Independent-t test was used to compare the continuous data, while the Chi-square test was used to compare the categorical data. The association between smoking type and corneal measures and also between nicotine dependency with addiction levels were assessed. P value of < 0.05 was considered statistically significant.

RESULTS

Table 2: Demographic data of participants.

		Smokers (n = 80)	Non-smokers (n = 80)	p
Age (years), mean ± SD (min–max)		48.6 ± 9.1 (25–70)	46.9 ± 8.5 (20–65)	> 0.05
Type of smoke	Cigarette	42 (52.5%)	-	-
	Shisha	30 (37.5%)	-	-
	Combined	8 (10%)	-	-
Smoking (packs/years), median (min–max)		30 (3–160)	-	-

SD: standard deviation

The mean age for the smokers was 48.6 ± 9.1 years and for the non-smokers was 46.9 ± 8.5 (20–65) and the differences between the studied groups was not statistically significant Table (2). The median of smoking (calculated by multiplying the number of smoking years by the average number of cigarette packs used per day) was 30 pack/year.

Table 3: The Corneal Variables of The Smokers And Controls.

Variables	Smokers (n = 80)	Non-smokers (n = 80)	P value
Packs-year	26.2±7.07	0	-
CD	2311.2±310.8	2697.9±163.4	< 0.001
AVG	419.5±88.6	359.18±33.2	< 0.001
HEX	58.9±3.7	64.9±5.7	< 0.001
CV	36.4±3.6	31.8±4.6	< 0.001
CCT	517.3±31.2	554.2±39.9	0.122

CD= cell density (cells/mm^2), CCT= central corneal thickness, AVG= average cell size variability
CV=coefficient of variation of endothelial cells, HEX= percentage of hexagonal cells

Table (3) showed that CD was 2311.2±310.8 cell/mm^2 in the smoker group, while it was 2697.9±163.4 cell/mm^2 in the control group and it was significantly higher in the smokers (p=<0.001). AVG was significantly larger among the smoker participants (419.5±88.6) than the non-smoker group (359.18±33.2) with a p value < 0.0001. HEX was 58.9±3.7 in the smoker group, while it was 64.9±5.7 in the control group with a statistically significant difference between the groups (p=<0.001). CV was 36.4±3.6 in the smoker group, while it was 31.8±4.6 in the control group (p= <0.001). CCT was 517.3±31.2 in the smoker group, while it was 554.2±39.9 in the control group. CCT in the smoker group was lower, but difference was non-significant between the groups (p=0.122).

Table 4: The Effect of The Smoking Type And Corneal Measures.

Variables	Cigarette s smokers (n = 42)	Shisha smokers (n = 30)	Combined smokers (n = 30)	P value
Age	31.9±8.4	33.4±9.2	34.1±7.9	0.617
CD	2814.2±270.5	2790±9.1	2785±6.8	0.587
AVG	419.5±88.6	418.5±75.3	419.8±92.1	0.115
HEX	42.6±5.2	45.6±1.9	43.1±4.3	0.945
CV	46.1±3.6	45.4±6.1	44.9±5.2	0.684
CCT	517.3±31.2	514.2±39.9	519.1±29.4	0.122

Table (4) demonstrated that there was non-significant differences between the different types of smoking (cigarette, shisha or combined) on corneal measures.

Table 5: Nicotine dependency among smokers.

Nicotine dependency	No	%
Low (0-3 scores)	39	48.75
Moderate (4-6 scores)	19	23.75
Severe (7-10 scores)	22	27.5
mean of smoking time in the smoker group	14.3± 7.4 years	

Nicotine dependency was graded in three levels. The addiction level was low in 39 subjects, moderate in 19 subjects, and severe in 22 subjects. The mean of smoking time in the smoker group was 14.3±7.4 years (Table 5).

Table 6: The effect of nicotine dependence levels on corneal measures.

Variables	Nicotine dependency	Mean	P value
CD	Low	2582.45±319.70	0.918
	Moderate	2508.42±276.67	0.710
	Severe	2428.41±317.91	0.372
AVG	Low	393.94±56.27	0.975
	Moderate	403.08±43.47	0.643
	Severe	418.71±56.74	0.408
HEX	Low	49.00±9.85	0.978
	Moderate	47.96±6.94	0.974
	Severe	47.41±8.11	0.883
CV	Low	38.85±7.98	0.843
	Moderate	38.42±4.30	0.733
	Severe	39.47±4.72	0.955
CCT	Low	506.00±42.01	0.017
	Moderate	526.23±33.48	0.063
	Severe	504.82±35.37	0.949

Table (6) showed that, the different levels of nicotine dependence (low, moderate or severe) did not seem to significantly affect the corneal measures (p> 0.05).

DISCUSSION

Oxidative stresses have harmful effects on tissue organs. Smoking introduces a large number of free radicals into the body which causes peripheral vasoconstriction and, consequently, decreases tissue oxygenation **(Yanbaeva et al., 2007)**. In low concentrations, nicotine stimulates both sympathetic and parasympathetic ganglia; however sympathetic effects are much higher. This sympatric stimulation leads to increasing heart rate and peripheral resistance. In addition, nicotine stimulates adrenal gland that leads to such effects. Carbone monoxide (CO) is as high as 100 folds higher in smokers comparing with healthy individuals; leading to increment of carboxyhemoglobin and finally hypoxia in tissues. Interestingly, nicotine but not CO impairs vascular regulations especially vasoconstriction induced by oxygen. Free radicals in cigarette are more than 7000 substances which are responsible for oxidative damage to molecules; resulting in diseases including; cancer, pulmonary diseases, neurological disorders and cardiovascular diseases. It has been shown that smokers are at higher risk of ischemic optic neuropathy, glaucoma, and retinal vein occlusion. Furthermore, smoking accelerates development of cataract and age-related macular degeneration **(Golabchi et al. 2018).**

Corneal endothelial stratum has a pivotal role in the transparency of the cornea. Endothelial cell parameters including CCT, CV, AVG, CD, and hexagonality could be evaluated by specular microscopy. Specular microscopy was performed based on two methods; contact and non-contact. In contact methods, after corneal anesthesia, the magnified corneal endothelial picture is taken. Although in a non-contact form the magnification is lower, it is easier to perform **(Rio-Cristobal and Martin, 2014)**. In children, cell density is near to 4000 cell/mm^2 and it starts to decline with aging. Thus, it is normal to find CD about 2400-2600 cell/mm2 in the population older than 75 years. The normal endothelial cells have hexagonal and regular shapes **(McCarey et al., 2008)**.

This observational cross-sectional study was conducting in Benghazi Opthalmology Teaching Hospital, Faculty of Medicine, Benghazi University, from December 2023 to April 2024 on 160 participants (80 smokers and 80 non-smokers) aged 20–70 years. All the participants underwent routine eye examination during this study period to evaluate the effects of chronic smoking on corneal endothelial cells, central corneal thickness.

The current study, regarding the age, there was no statistically significant difference between the two studied groups. There was a statistically significant difference between the smoker group and control group regarding CD, AVG (p=<0.001). The type of smoking (cigarette, shisha or combined) did not significantly affect the corneal measures, and also the level of smoking severity (low, moderate and sever) showed no effect on the corneal measures. Similarly, (**Alajeeli and**

Al-Ahmad, 2022; Ilhan et al., 2016) reported that the difference in age between both groups (smokers and non-smokers) was statistically non-significant (p value > 0.05).

Recently, limited studies have been conducted on the effects of smoking on corneal epithelial cell parameters. The current study found a non-significant difference between smokers and non-smokers in term of CCT (P = 0.122). These results are in agreement with previous studies (**Ilhan et al., 2016; Kara et al., 2017; Sayin et al., 2014).**

Moreover, the results of the present study showed a significant difference in the mean values of AVG and CD between smokers and non-smokers. In smokers, AVG was more than that of non-smokers but CD was significantly less than that for non-smokers (p < 0.001). These findings are in agreement with (**Ilhan et al. 2016).**

In contrast, (**Kara et al., 2017; Sayin et al., 2014)** did not found a significant difference between smokers and non-smokers in mean values of AVG and CD (P=0.441 and P=0.156 respectively). These controversies might be due to applying different methods and relatively small sample size of that study and inclusion criteria of the subjects in relation to smoking history individuals in the study.

In the current study, the severity of nicotine dependence was found to have no effect on corneal measures, this was in controversy with (**Golabchi et al., 2018)** who reported that strong nicotine dependency had more association with the changes in endothelium parameters including CD and AVG.

Furthermore, another study (**Sopapornamorn et al. 2008)** proposed that smoking had no effect on corneal endothelial cells. However, their study included only 38 patients with a history of smoking and 23 of them were former smoker, this in contrast to our inclusion criteria; chronic smoker or non-smoker.

Therefore, we consider that tobacco smoking and nicotine derivatives may play a role in the cell death and apoptosis of the corneal endothelium supporting (**Csordas et al., 2011)** who reported that cigarette smoke extract induced cell death in human umbilical vein endothelial cells.

The current study showed that the type of smoking (cigarette, shisha or combined) had no significant different the effect on corneal measures, which in agreement with (**Alajeeli and Al-Ahmad., 2022)** who reported that there no significant relation between the type of smoking and corneal measurement.

In the current study, there was no statistically significant difference among the smoker group in the terms of severity addiction levels on the corneal measures where p value was > 0.05. In the same context (**Alajeeli and Al-Ahmad, 2022)** found that neither central corneal thickness nor endothelial cell density where found to be significantly different statistically in the smoker group in the term of addiction levels where p value was > 0.05. **Also (El Saman et al., 2019)** reported the difference between addiction levels and central corneal thickness or endothelial cell density was not statistically significant.

(Ilhan et al., 2016) showed that there was no significant difference in terms of the mean values of ECD and CV between light and heavy smokers. It is possible that exclusion of individuals with glaucoma, contact lens wear, and systemic medication may cause an unnaturally low rate of problems in the smoking group. In addition, the smoking criteria, that is, light or heavy, were determined by pack-years, but other factors such as smoking commencement age, the number of smoking years, and inhalation patterns may change the effects on the corneal endothelial cell layer. Former smokers were not included, which limits the study. The other limitation is related to measurement variability.

In addition to the systemic effects of smoking, preoperative chronic smoking could alter endothelium healing process due to its adverse effects on matrix metalloproteinase and consequently collagen synthesis. Thus, it is important to counsel the smoker patients before ophthalmologic surgeries such as cataract or corneal transplantation procedures **(Golabchi et al., 2018).**

CONCLUSION

In conclusion, the study showed that cell density and cell hexagonality values were decreased, whereas average cell size values were increased significantly in chronic smokers compared to ono-smokers. Preoperative corneal examination should be performed more carefully while planning intraocular surgeries such as cataract surgery, refractive surgery and keratoplasty.

Recommendations

Further studies are needed to determine the effects of acute and chronic smoking and passive smoking on the ocular surface and studying the effect of quitting smoking on endothelial cell parameters. Larger studies are needed to reveal the effects of chronic smoking on corneal health more explicitly.

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