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Original Article

Evaluation of the effect of chronic smoking habit on corneal endothelial cells, central corneal thickness and dry eye tests

Yucel Karakurt[1], Mukadder Sunar[2], Nurdan Gamze Tasli[1], Turgay Ucak[1], Sumeyye Burcu Agcayazi[3], Adem Ugurlu[3], Erel Icel[1]

[1]Erzincan Binali Yıldırım University, Faculty of Medicine, Department of Ophthalmology, Erzincan, Turkey
[2]Erzincan Binali Yıldırım University Faculty of Medicine, Department of Anatomy, Erzincan, Turkey
[3]Erzincan Mengücek Gazi Hospıtal Department of Ophthalmology, Erzincan, Turkey

Abstract

Aim: The aim of our study was to evaluate the effects of chronic smoking habit on corneal endothelial cells, central corneal thickness, and dry eye tests.

Material and Methods: A total of 160 eyes of 160 chronic smokers and 160 eyes of 160 control cases were included in the study. The smokers were grouped into 4 groups according to pack-year smoking as follows: 0-10, 10-20, 20-30, >30. Endothelial cell density (CD), average cell size (AVG), percentage of hexagonal cell (HEX), and coefficient of variation (CV), central corneal thickness (CCT) were measured using a specular microscope. The Tear Break-Up Time (TUBT) and Schirmer tests were performed.

Results: In Group 1 and Group 2, there was no significant difference between the smoker and the control groups in terms of CD, AVG, HEX and CV. In Group 3 and Group 4, there was a significant decrease in CD and HEX values, while there was a significant increase in AVG and CV values. CCT values were not significantly different in the Groups 1,2 and 3, whereas there was a significant decrease in the Group 4. TBUT and Schirmer tests were significantly decreased in all groups.

Conclusion: It was found that as the number of pack-year increased, CD and HEX values decreased and AVG and CV values increased. Dry eye tests were affected in a shorter period of time, while CCT was affected in a longer period of time than the time required for CD, HEX, AVG and CV parameters to be affected.

Keywords: Corneal Endothelium; Smoking; Specular Microscopy.

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 (1). Many toxic substances are found in cigarette smoke and particles (2,3). 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 (4,5). It has also been reported that these toxic substances reduce the level of antioxidants in blood, aqueous humor and ocular tissues (6,7). Various studies have shown that chronic smoking habit causes cardiovascular, respiratory and neoplastic diseases (8-10), as well as cataract, open-angle glaucoma, age-related macular degeneration, retinal vein occlusion, optic neuritis, dry eye, graves ophthalmopathy, and ocular inflammation as a result of all these toxic effects (11-17). In addition to these eye diseases, long-term exposure to cigarette smoke has been reported to cause some ocular surface disorders (18-20).

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 (21,22). The studies have shown that corneal endothelial cells are highly susceptible to hypoxia and oxidative stress (23). Despite numerous studies showing the negative effects of chronic smoking habit on ocular surface, there are a very few studies evaluating corneal

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Corresponding Author: Yucel Karakurt, Erzincan Binali Yıldırım University, Faculty of Medicine, Department of Ophthalmology, Erzincan, Turkey **E-mail:** dryucelkarakurt@gmail.com

endothelial cells. To the best of our knowledge, patients were not compared with control groups by grouping according to pack-year calculation. In this study, we aimed to investigate the effects of toxic substances in cigarette smoke on corneal endothelium, central corneal thickness, and dry eye parameters by dividing the participants with chronic smoking habit into groups based on the pack-year calculation.

MATERIAL and METHODS

The study was conducted in Erzincan Mengucek Gazi Training and Research Hospital between May 2016 and December 2017. The study was approved by the local ethics committee (Date: 22/04/2016, Number: 33216249-604.01.02-17066) and informed consent was obtained from all participants. This study was supported by the Scientific Research Project Coordination Unit of Erzincan Binali Yildirim University.

This prospective, cross-sectional study included a total of 320 participants, of whom, 160 were volunteer participants between 20-70 years of age, who had been smoking for at least 5 years, had no ocular trauma or underwent any ocular surgery, had no additional ocular pathology, had no systemic disease that could impair the ocular health, and 160 were non-smokers who also did not expose to cigarette smoke. One eye of each participant was included in the study. Patients who had ocular trauma or underwent any other ocular surgery, used contact lenses, had additional ocular pathology and a systemic disease that could impair the ocular health were not included in the study.

Pack-year was calculated by multiplying the number of pack per day smoked by the individuals in the smoker group. Based on pack-year calculation, the patients were divided into four groups as follows: Group 1: 0-10 packs-years, Group 2: 10-20 packs-year, Group 3: 20-30 packs-year, Group 4: 30 packs-year and more. Since endothelial cell number decreases as age increases as the natural result of aging, similar control groups were formed for each group in terms of age and gender.

A complete ophthalmological examination was performed on 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), endothelial cell density (cells/mm2) (ECD), percentage of hexagonal cells (HEX), and average cell size variability % (AVG), coefficient of variation (CV) of endothelial cells were measured using a noncontact specular microscope (CEM-530 Specular Mıcroscope, NIDEK, Japan). Eye dryness of the patients was evaluated with the Tear Break-Up Time (TUBT) test and Schirmer test. While administering the TUBT test, the patient was asked to blink 3 times after the placement of fluorescein strip in the lower eyelid fornix. After blinking for 3 times, the patient was asked to stare straight ahead without blinking. Under cobalt blue light, the layer of the tear film was viewed and the time between the last blinking and the first dry spot formation in the film layer was recorded. While administering the Schirmer test, a drop of proparacaine 0.5% was dropped on the eye of the patient. The Schirmer test strip was placed on the 1/3 outer part of the lower eyelid. The strip is removed after 5 minutes and the wet portion recorded in mm.

Statistical Analysis

The statistical analysis of the data was performed using the SPSS 22.0 (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 patient groups, while the Chi-square test was used to evaluate the categorical data. A P value of < 0.05 was considered statistically significant.

RESULTS

There was no statistically significant difference between the groups in terms of age,gender, vision acuity and intraocular pressure measurements when the four groups were compared with the control groups. The mean age of the smokers in Group 1 was 28.21 years; of these, 47.5% were male and 52.5% were female. The mean age of the participants in the control group that was formed for Group 1 was 27.85 years; of these 50% were male and 50% were female. The mean age of the smokers in Group 2 was 48.83 years; of these, 50% were male and 50% were female. The mean age of the participants in the control group that was formed for Group 2 was 46.05; of these, 47.5% were male and 52.5% were female. The mean age of the smokers in Group 3 was 51.15 years; of these, 45% were male and 65% were female. The mean age of the participants in the control group that was formed for Group 3 was 50.90 years; of these, 45% were male and 65% were female. The mean age of the smokers in Group 4 was 59.1 years; of these, 47.5% were male and 52.5% were female. The mean age of the participants in the control group that was formed for the Group 4 was 58.9 years; of these, 47.5% were male and 52.5% were female (Table 1).

Table 1. The demographic findings of all participants

AGE	Smoking	Control	P
Group 1	28.21±7.18	27.85±6.8	0.924
Group 2	45.83±7.18	46.05±5.8	0.896
Group 3	51.15±9.74	50.9±9.97	0.910
Group 4	59.1±8.2	58.9±9.9	0.942
GENDER			
Group 1	19/21	20/20	0.823
Group 2	20/20	19/21	0.823
Group 3	18/22	18/22	> 0.05
Group 4	19/21	19/21	> 0.05

In the Group 1, CD was 2886.4±112.4 in the smoker group, while it was 2934.75±271,3 in the control group. The number of endothelial cells in the smoker group was decreased numerically, but there was no significant

difference between the groups when compared statistically (p=0.451). AVG was 357.45±20.82 in the smoker group, while it was 349.13±20.5 in the control group. AVG in the smoker group was increased numerically, but there was no significant difference between the groups when compared statistically (p=0.381). HEX was 67.25±3.83 in the smoker group, while it was 70.45±4.43 in the control group. HEX in the smoker group was decreased numerically, but there was no significant difference between the groups when compared statistically (p=0.294). CV was 30.72±3.88 in the smoker group, while it was29.11±2.98 in the control group. CV in the smoker group was increased numerically, but there was no significant difference between the groups when compared statistically (p=0.318). CCT was 556.05±28.6 in the smoker group, while it was 559.88±38.9 in the control group. CCT in the smoker group was decreased numerically, but there was no significant difference between the groups when compared statistically (p=0.608). The TBUT was 11.47±2.7 in the smoker group, while it was 13.85±2.9 in the control group. There was a statistically significant difference between the groups (p=0.035). The Schirmer test was 9.2±1.88 in the smoker group, while it was 12.7±2.2 in the control group. There was a statistically significant difference between the groups (p=<0.001) (Table 2).

Table 2. The corneal variables of the smokers and controls in Group 1

Variables	Smokers	Control	P value
Number (eyes)	40	40	-
Packs-year	6.07±1.9	0	-
CD	2886.4±112.4	2934.75±271.3	0.451
AVG	357.45±20.82	349.13±20.5	0.381
HEX	67.25±3.83	70.45±4.43	0.294
CV	30.72±3.88	29.11±2.98	0.318
CCT	556.05±28.6	559.88±38.9	0.608
TBUT	11.47±2.7	13.85±2.9	0.035
SCHIRMER	9.2±1.88	12.7±2.2	< 0.001

In Group 2, CD was 2486.1±139.1 in the smoker group, while it was 2534.75±278.9 in the control group. The number of endothelial cells in the smoker group was decreased numerically, but there was no significant difference between the groups when compared statistically (p=0.341). AVG was 371.45±26.86 in the smoker group, while it was 359.13±39.54 in the control group. AVG in the smoker group was increased numerically, but there was no significant difference between the groups when compared statistically (p=0.261). HEX was 65.15 ± 4.33 in the smoker group, while it was 69.45±5.46 in the control group. HEX in the smoker group was decreased numerically, but there was no significant difference between the groups when compared statistically (p=0.382). CV was 31.4±4.88 in the smoker group, while it was 30.18±3.99 in the control group. CVin the smoker group was increased numerically, but there was no significant difference between the groups when compared statistically (p=0.522). CCT was 546.05±28.6 in the smoker group, while it was 550.88±38.9 in the control group. CCT in the smoker group was decreased numerically, while there was no significant difference between the groups when compared statistically (p=0.412). The TBUT was 8.27±1.7 in the smoker group, while it was 9.85±2.6 in the control group. There was a statistically significant difference between the groups (p=0.029). The Schirmer test was 8.2±1.08 in the smoker group, while it was 10.7±2.68 in the control group. There was a statistically significant difference between the groups (p=<0.001) (Table 3).

Table 3. The corneal variables of the smokers and controls in Group 2

Variables	Smokers	Control	P value
Number (eyes)	40	40	-
Packs-year	14.7±3.32	0	-
CD	2486.1±139.1	2534.75±278.9	0.341
AVG	371.45±26.86	359.13±39.54	0.261
HEX	65.15±4.33	69.45±5.46	0.382
CV	31.4±4.88	30.18±3.99	0.522
CCT	546.05±28.6	550.88±38.9	0.412
TBUT	8.27±1.7	9.85±2.6	0.029
SCHIRMER	8.2±1.08	10.7±2.68	< 0.001

In Group 3, CD was 2350.6±349.9 in the smoker group, while it was 2713.6±178.7 in the control group. There was a statistically significant difference between the groups (p=<0.001). AVG was 438.5±95.5 in the smoker group, while it was 370.18±26.4 in the control group. There was a statistically significant difference between the groups (p=<0.001). HEX was 59.7±4.7 in the smoker group, while it was 66.48±4.34 in the control group. There was a statistically significant difference between the groups (p=<0.001). CV was 35.98±2.29 in the smoker group, while it was 31.05±3.89 in the control group (p= <0.001). CCT was 538.3±28.6 in the smoker group, while it was 551.2±38.9 in the control group. CCT in the smoker group was decreased numerically, but there was no significant difference between the groups when compared statistically (p=0.122). TBUT was 7.2±1.1 in the smoker group, while it was 9.55±2.3 in the control group. There was a statistically significant difference between the groups (p=<0.001). The Schirmer test was 7.3±1.48 in the smoker group, while it was 9.67±1.61 in the control group. There was a statistically significant difference between the groups (p=<0.001) (Table 4).

Table 4. The corneal variables of the smokers and controls in Group 3

Variables	Smokers	Control	P value
Number (eyes)	40	40	-
Packs-year	26.2±7.07	0	-
CD	2350.6±349.9	2713.6±178.7	< 0.001
AVG	438.5±95.5	370.18±26.4	< 0.001
HEX	59.7±4.7	66.48±4.34	< 0.001
CV	35.98±2.29	31.05±3.89	< 0.001
CCT	538.3±28.6	551.2±38.9	0.122
TBUT	7.2±1.1	9.55±2.3	< 0.001
SCHIRMER	7.3±1.48	9.67±1.61	< 0.001

In Group 4, CD was 2225.6±319.2 in the smoker group, while it was 2683.6±278.7 in the control group. There was a statistically significant difference between the groups (p=<0.001). AVG was 468.5±95.5 in the smoker group, while it was 383.24±21.2 in the control group. There was a statistically significant difference between the groups (p=<0.001). HEX was 59.7±4.7 in the smoker group, while it was 66.48±4.34 in the control group. There was a statistically significant difference between the groups (p=<0.001). CV was 38.99±2.14 in the smoker group, while it was 32.25±3.29 in the control group (p=<0.001). CCT was 530.3±22.3 in the smoker group, while it was 550.1±32.9 in the control group. There was a statistically significant difference between the groups (p=<0.001). The TBUT was 6.82±1.3 in the smoker group, while it was 9.25±2.41 in the control group. There was a statistically significant difference between the groups (p=<0.001). The Schirmer test was 7.1±1.4 in the smoker group, while it was 9.41±1.84 in the control group. There was a statistically significant difference between the groups (p=<0.001) (Table 5).

Table 5 The corneal variables of the smokers and controls in Group 4

Variables	Smokers	Control	P value
Number (eyes)	40	40	-
Packs-year	39.8±7.38	0	-
CD	2225.6±319.2	2683.6±278.7	< 0.001
AVG	468.5±85.1	383.24±21.2	< 0.001
HEX	59.7±4.7	66.48±4.34	< 0.001
CV	38.99±2.14	32.25±3.29	< 0.001
CCT	530.3±22.3	550.1±32.9	0.040
TBUT	6.82±1.3	9.25±2.41	< 0.001
SCHIRMER	7.1±1.4	9.41±1.84	< 0.001

When all groups were evaluated together, there was no statistically significant difference between the smoker groups and the control groups in Group 1 and Group 2 in terms of CD, AVG, HEX and CV, despite the numerical difference. In Group 3 and Group 4, there was a statistically significant decrease in CD and HEX values, while there was a statistically significant increase in AVG and CV values. When the CCT values were compared with that of the control group, there was no statistically significant difference in the Group 1,2 and 3, whereas there was a statistically significant decrease in Group 4. It was found that there was a statistically significant decrease in the TBUT and Schirmer tests in all groups.

DISCUSSION

The density of corneal endothelial cell exhibits a physiological reduction between 0.5% and 0.8% annually due to aging (24). 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 (25). 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.

Oxidative stress has very harmful effects on tissues. Smoking causes a large number of free radical release, leading to peripheral vasoconstriction and consequently a reduction in tissue oxygenation (26). 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 (27,28). 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 (29,30).

The effect of chronic smoking habit on corneal endothelium has been demonstrated in some studies. The study by Kara et al. found no significant difference between smokers and non-smokers when CCT, CD, HEX, AVG values were compared. Whereas, a significant decrease was found in TBUT values (29). When the cigarette exposures were calculated by packs-year, the mean packs-year was calculated as 13.92 packs-year (14.5 (duration of smoking) X (19.2 (number of cigarettes per day)/20)). These results seem to be similar with the results of our patients in the Group 2 (14.7±3.32 pack-year). The study by Sayin et al. found no significant difference between smokers and non-smokers when CCT, CD and AVG values were compared. Whereas, a significant decrease was found in TBUT and Schirmer test values (31). When the cigarette exposures were calculated by packs-year, the mean packs-year was calculated as 10.5 packs-year (12.3 (duration of smoking) X (17.5 (number of cigarettes per day) / 20)). These results seem to be similar with the results of our patients in the Group 2 (14.7±3.32 packs-year). The study by Golabchi et al. found a significant decrease in CD and a significant increase in AVG in smokers; however, no statistically significant difference was found in HEX and CV despite a numerical decrease (32). In this study, the pack-year calculation was 17.36 and seems to be similar with the results of our patients in the Group 2 (14.7±3.32 packs-year). Unlike our study, no statistically significant result was found for CCT despite a numerical increase. The study by Sopapornamorn et al. found that endothelial cells were not affected by smoking (33). When the cigarette exposures were calculated by packs-year, the mean packs-year appears

to be 10.61±7.87.These results seem to be similar with the results of our patients in the Group 2 (14.7±8.32 packs-year). The study by Zoega et al. showed that the risk of developing corneal guttata increased by more than 2 times in patients using 20 packs of cigarette per year and more (34).Considering the pack-year calculation in this study, it seems to be similar with our study. The study by Ilhan et al. found a significant decrease only in CD in smoker group, and found no statistically significant difference in HEX and CCT values despite a numerical decrease. No statistically significant difference was found in CV values despite a numerical increase. The pack-year calculation in their study appears to be 12.0±10.3 (28). It seems to be consistent with the Group 2 in our study. Considering the above-mentioned studies and our study in general, we can speculate that the toxic effects of smoking on corneal endothelial cells show a cumulative effect and exhibit statistically significant results only with more than 20 packs of cigarettes per year.

The studies have shown that chronic smoking habit reduces corneal and conjunctival sensitivity, increases conjunctival squamous metaplasia, and impair the structure of tear proteins (35,36). Moreover, other studies have shown that chronic smoking habit causes ocular surface diseases and dry eye by disrupting the tear layer (37,38). In our study, it was seen that the results of TBUT and Schirmer tests were similar with the literature in all groups.

The study by Wang et al. found a reduction in corneal thickness due to smoking. They said that it was due to hypoxia and impairment of collagen biyosynthesis (39). The study by Knuutinen et al. showed impaired collagen biosynthesis and turnover of extracellular matrix (40). Whereas, the studies by Sayın et al. and Kara et al. found no significant difference in CCT. In our study, there was no significant difference in CCT in the Groups 1,2 and 3; however, there was a significant decrease in the Group 4. Given these results, we can speculate that the time required for corneal thickness to be affected is longer than the time required for other parameters to be affected.

This study also demonstrates that the pack-year calculation is one of the parameters that should be paid attention while evaluating the toxic effects of smoking. In order to avoid unexpected corneal decompensations after a successful ocular surgery, corneal endothelial cells should be carefully evaluated in patients with a history of long-term smoking.

It can be said that not administering the Fagerström Test for Nicotine Dependence is one of the weaknesses of our study.

CONCLUSION

In conclusion, our study showed that CD and HEX values were decreased, whereas AVG and CV values were increased as the number of packs-year increased in chronic smokers. These changes are statistically significant, especially with more than 20 packs of cigarettes per year. A statistically significant effect on corneal thickness is observed in smokers who smokes higher number of pack of cigarettes per year. A statistically significant effect on TBUT and Schirmer tests from dry eye parameters is observed in the earlier period. Especially in patients with long-term smoking habit, preoperative corneal examination should be performed more carefully while planning intraocular surgeries such as cataract surgery, refractive surgeryand keratoplasty. Prospective studies with larger samples are needed to reveal the effects of chronic smoking on corneal health more explicitly.

A part of this study has been accepted as a poster in Turkish Ophthalmology Society 16. March Symposium

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Yucel Karakurt ORCID: 0000-0001-8104-3819
Mukadder Sunar ORCID: 0000-0002-6744-3848
Nurdan Gamze Tasli ORCID:000-0001-8587-3430
Turgay Ucak ORCID: 0000-0002-4977-4942
Sumeyye Burcu Agcayazi ORCID: 0000-0003-4673-9233
Adem Ugurlu ORCID:0000-0002-8900-7043
Erel Icel ORCID: 0000-0001-7150-5581

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