Assessment of Corneal Endothelial Cell Density in Patients with Dry Eye Disease

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Abstract

Aim: **We aimed to improve the corneal function in patients with dry eye disease by studying the effect of dry eye disease and its severity on the cornea.** ***Materials and Methods:*** **This study was a case-control, cross-sectional study, that included 200 eyes of 100 patients they were divided into two groups:** ***Group 1:*** **50 patients with dry eye disease which then divided into (mild, moderate, and severe) and** ***Group 2:*** **50 patients as a control group.** ***Results*****: The endothelial cell count (cell density) showed a statistically significant difference between the two groups. It was lower in the dry eye group than in the normal group (p=0.005). The central corneal thickness (CCT), co-efficient variant (CV), and hexagonality (HEX) showed no significant difference between the two groups.** ***Conclusion*** **The Dry eye disease group showed a decrease in the CD. There was no effect on CCT, CV, or HEX. Severe dry eyes showed less CCT and CD than eyes with mild and moderate degrees of dryness. They showed higher HEX than that of mid and moderate dryness. However, there was no difference in CV between the three degrees of dry eye disease.**

Keywords**:** Central corneal thickness (CCT), endothelial cell density (CD), co-efficient variant (CV), hexagonality of cells (HEX).

Introduction

The cornea is the transparent anterior part of the eye. It consists of 6 layers. The corneal endothelium is the lining layer of the cornea. It is around 5μm thick. It is composed of a monolayer of hexagonal cells, and it plays a major role in maintaining corneal transparency, thickness, and hydration. The model of the arrangement of this cell layer makes the endothelium easily captured with a specular microscope[1]. The endothelial cell density normally ranges from 3000 to 4000 cells/mm^2 in children to 1000 to 2000 cells/mm^2 at the age of 80 years. The density of the endothelium reduces normally with age because of cell disintegration. The cornea endothelial cell density must be in the range of 400 to 700 cells/mm^2 for proper function. Disruptions to the endothelial mosaic can include an increase in the variation of cell shape (pleomorphism) or size (polymegathism) and endothelial cell loss[2-4]. Dry eye disease or dry eye syndromes can be classified into two major

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classes; Aqueous deficient dry eye (ADDE) and Evaporative dry eye (EDE). ADDE is divided into Sjögren Syndrome Dry Eye (SSDE) and non-Sjögren Syndrome Dry Eye (NSSDE)[4]. Aqueous deficient dry eye is due to decreased lacrimal tear production or volume. Aqueous deficient dry eye is caused by disease in the lacrimal gland (e.g., Sjögren syndrome), obstruction to lacrimal gland outflow (e.g., cicatricial pemphigoid), homeostatic disturbance induced by blockage of the afferent pathway (e.g., topical anesthesia or trigeminal nerve section), and by blockage of the efferent pathway (e.g., damage to the Pterygo-palatine ganglia on and third-order neurons). Also, systemic drug uptake may cause it[5]. Evaporative dry eye EDE is due to increased tear evaporation rate with normal function of the lacrimal gland. It is divided into intrinsic and extrinsic EDE. It can be caused by lid-related or ocular surface-related diseases[6-8]. Specular microscopy is used for analysis and imaging of corneal endothelium. It has qualitative and quantitative analysis of the endothelium. Qualitative analysis is about cell size, cell perimeter, average cell length, cell shape, and cell density[9]. Quantitative analysis of specular microscopy is about cell confirmation, cell boundaries and their intersection, and figuration of dark boundaries. It is done before intraocular surgery to evaluate the endothelium and detect any presence of abnormal endothelial structures. A number of inter and intra-endothelial cell structures can be seen like cornea guttae which are easily made out[10].

Materials and Methods

This is a case-control, cross-sectional study that included 200 eyes of 100 patients from the Ophthalmology Department, Suez Canal University Hospitals, Ismailia, Egypt from September 2019 to January 2021. *Inclusion Criteria:* Age between (40-60), both sexes, Patients with dry eye syndrome "keratoconjunctivitis sicca". *Exclusion Criteria:* Pre-existing corneal abnormalities, previous ocular inflammations or trauma, Glaucoma, patients who performed any previous anterior segment surgeries, contact lens wearers, and smokers. The patients who fulfilled the criteria were divided into two groups: *Group 1:* 50 patients with dry eye disease which was then divided into (mild, moderate, and severe) and *Group 2:* 50 patients as a control group. All Patients were gone under full ophthalmic examination. Dryness tests and Schirmer test. Non-contact specular examination by (NIDEK CEM-530) was done on all the patients which showed the central corneal thickness (CCT), endothelial cell density (CD), co-efficient variant (CV), and hexagonality of cells (HEX). The *Schirmer test* was used to detect normal and dry eye; normal: more than 10 mm, mild to moderate: equal or more than 10 mm, severe: equal or less than 5 mm, very severe: equal or less than 2 mm[11]. And other dry eye tests; *Tear meniscus height*: The tear meniscus height was measured. The height of less than 0.25 mm was suggestive of dry eye[12], *Fluorescein clearance test*: It included the use of Schirmer paper and the application of 5µL of Fluorescein. The wetting of the strip and the disappearance of dye were both measured in 10 minutes. A value of 3 mm or greater at the first 10-minute interval was the standard for normal. At the 20-minute interval, when the dye could not be detected, clearance was normal[13,14], *The tear break-up time test*: Fluorescein was instilled, and the patient was asked not to blink. TBUT was measured as the first dark spot after blinking. Any dark spot before 10 seconds was considered a dry eye[15,16].

Ethical considerations

The study was conducted after approval by the Local Research Committee, the Studies Committee as well and the Research Ethics Committee. Informed written consent has been obtained from all patients and contained the following: The aim, procedures, and duration of the study explained in a simple way. The patients had the right to refuse participation without affecting the medical care expected to be offered to him/her. The patients could leave the study at any time and for no reason. Confidentiality of data and results of all study populations was preserved by ensuring anonymity of data and minimal access to data by the research team only. The mobile number of the principal researcher as well as the phone number of the Research Ethics Committee was provided for any future inquiries and complaints by the study participants. Any potential risk attributed to the local medications used and any side effects were managed promptly, in addition, the procedure was performed by a qualified experienced ophthalmologist.

Results

The study included 16 males and 36 females in the study group and 20 males and 30 females in the control group. The study group was divided into 3 groups regarding the degree of dryness. It included mild (18%), moderate (62%), and severe dryness (20%). Regarding BCVA either in the right eye or in the left eye, there were statistically significant differences between the two studied groups (p<0.05) as the mean right BCVA was (0.58±0.36) in the control group compared with (0.37±0.33) in the study group also the left BCVA was (0.57±0.4) in the control group compared with (0.37±0.33) in the study group.

Table 1: Age and gender distributions between the two groups (n=100)

Variables	Normal eyes (n=50)		Dry eyes (n=50)		Test	p-value
Age						
Mean ±SD	52.5±5.38		54.2±6.9		1.4^	0.2
Min-Max	43-59		40-60			
	N	**%**	**N**	**%**	**Test**	**p-value**
Gender						
Male	20	40.0	16	32.0	0.6^^	0.4
Female	30	60.0	34	68.0		

^independent student t-test ^^X^{2}: Chi square test

On doing the Schirmer test on the studied eyes of the two groups, it was found that the means±SD of the Schirmer test of the study group was highly significant (p<0.001) lower than that of the control group (6.5±1.62 & 14.58±2.9 respectively). On examining the central corneal thickness, coefficient variant (CV), and hexagonality of cells (HEX) of the studied eyes in the two groups there were no statistically significant differences between them (p=0.3, p=0.1 and p=0.4 respectively). However, the corneal endothelial cell count (CD) of the study group (2610.12±342.4) was significantly lower than (P=0.005) that of the control group (2731.53±255.36). The CCT in the severe dry eye was significantly lower than mild dry eye and significantly lower than moderate dry eye (505.9±34.3, 557.06±36.1 and 536.9±33.8 respectively, on the other hand, the HEX was significantly higher in severe dry eye than mild dry eye (66.7±8.2 vs. 57.9±13.03). Also, CD in the severe dry eye was significantly lower than mild and moderate dry eye (2428.9±282.5, 2625.8±325.1 and 2664.0±348.7 respectively), while no significant difference in CV with respect to the difference in degree of dryness. The most frequent degree of dryness among patients with dry eye disease was moderate (62%) followed by severe one (20%) while mild dryness was least frequent (18%) (Figure 1).

Table 2: Best corrected visual acuity between the two groups (n=100)

BCVA	Normal eyes (n=50)	Dry eyes (n=50)	Test ^	p-value
Right eyes BCVA				
Mean ±SD	0.58±0.36	0.37±0.33	842	**0.004***
Min-Max	0.02-1.0	0.02-1.0		
Left eyes BCVA				
Mean ±SD	0.57±0.4	0.37±0.3	905	**0.02***
Min-Max	0.05-1.0	0.02-1.0		

*^ MW: Mann-Whitney test, *statistically significant difference (p<0.05)*

Table 3: Schirmer test in both groups (n=200):

Schirmer test	Normal eyes (n=100)	Dry eyes (n=100)	Test^	p-value
Mean ±SD	14.58±2.9	6.5±1.62	16.9	**<0.001****
Min-Max	10-20	3-9		

*^ independent student t-test **highly statistically significant (P<0.001)*

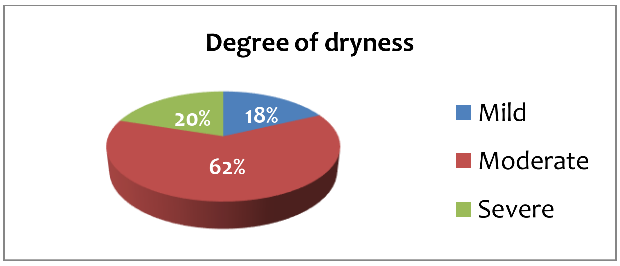

Figure1: Degree of dryness in dry eyes group (n=50)

Discussion

Corneal endothelium is very important for normal corneal function. Its number decreases normally with age. It doesn't have the ability to regenerate. Dry eye syndrome is a very common disease that affects a large number of the population. There are plenty of dryness causes like blepharitis, long contact lens wearing, LASIK surgeries, and smoking. Non-contact specular microscopy plays an important role in the assessment of the corneal endothelium[2,6]. This study was conducted at the Outpatient Ophthalmology **Department**, Faculty of Medicine, Suez Canal University from September 2019 to January 2021. It included (200) eyes of (100) patients. They were divided into two groups; group (1) (study) included 100 eyes with dry eye disease with a mean age of (54.2±6.9), and group (2) (control) included 100 eyes of control group with mean age (52.5±5.38). The study included 16 males and 36 females in the study group and 20 males and 30 females in the control group. The dry eye group was divided into 3 groups regarding the degree of dryness. It included mild (18%), moderate (62%), and severe dryness (20%).

Table 4: CCT, CV, HEX and CD parameters in both groups (n=200)				
Corneal thickness, CV, HEX	Normal eyes (n=100)	Dry eyes (n=100)	Test^	p-value
Central corneal thickness				
Mean ±SD	529.58±35.67	534.3±37.64	0.9	0.3
Min-Max	298-566	460-616		
CV				
Mean ±SD	31.5±5.2	32.8±6.4	1.5	0.1
Min-Max	14-41	25-56		
HEX				
Mean ±SD	63.48±7.2	62.4±10.6	0.8	0.4
Min-Max	47-73	33-75		
CD				
Mean ±SD	2731.53±255.36	2610.12±342.4	2.8	**0.005***
Min-Max	2080-3502	1951-3275		

*^independent student t-test *statistically significant difference (p<0.05)*

Table 5: CTT, CV, HEX and CD parameters in dry eye group (n=100)				
CCT, CV, HEX, CD	Mild (n=18)	Moderate (n=62)	Severe (n=20)	^p-value
CCT(μm)				
Mean ±SD	557.06±36.1	536.9±33.8	505.9±34.3	<0.001**
Min-Max	519-616	473-593	460-553	
	P1=0.03 P2=<0.001** P3=0.001*			
CV				
Mean ±SD	35.1±11.04	32.3±5.0	32.2±4.1	0.2
Min-Max	25-56	25-43	27-42	
HEX				
Mean ±SD	57.9±13.03	62.4±10.2	66.7±8.2	**0.04***
Min-Max	33-75	38-75	45-75	
	P1=0.1 P2=0.01* P3=0.1			
CD (cell/mm²)				
Mean ±SD	2625.8±325.1	2664.0±348.7	2428.9±282.5	**0.03***
Min-Max	1951-2946	1951-3275	2050-2795	
	P1=0.7 P2=0.07 P3=0.0.007*			

P1= Mild/Moderate P2= Mild/Sever P3=Moderate/Sever ^One way ANOVA with post HOC
**Statistical significant difference (p<0.05) **highly significant (P<0.001)*

This study used non-contact specular microscopy to evaluate the central corneal thickness (CCT), endothelial cell density (CD), co-efficient variant (CV), and hexagonality of cells (HEX). We started by comparing the data between the control group; group (2) and the whole group of the patients with study disease; group (1). In our study, the corneal endothelial cell count (CD) of the study group was (2610.12±342.4) and of control eye group was (2731.53±255.36). The endothelial cell count showed a statistically significant difference between the two groups. It was lower in the dry eye group than in the normal group (p=0.005). Kheirkhah et al.[17] performed a study on endothelial cells using confocal microscopy. They agreed with us as they found that the corneal endothelium in the study is significantly lower than in the normal eye. Corneal ECD in the dry-eye disease group was (2,595.8 ±356.1 cells /mm²) and in the control group was (2,812.7 ±395.2 cells/mm²) (*P*=0.046). In our study, the central corneal thickness (CCT) of the study group was (534.3±37.64) and of the control group was (529.58±35.67).

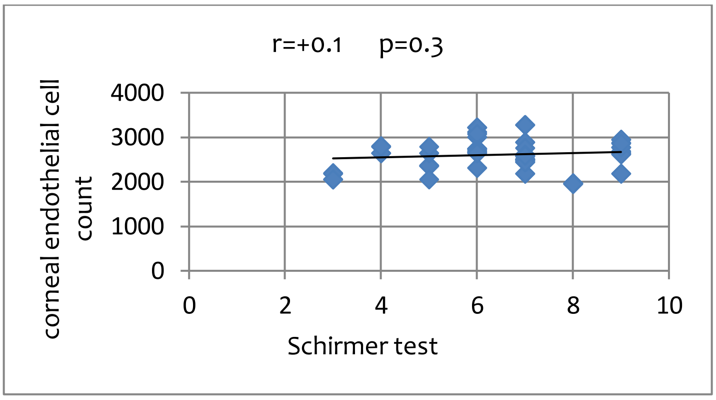

Figure 2: Correlation between Schirmer test results (severity of dryness) and cell density (CD) among the dry eye group (n=100).

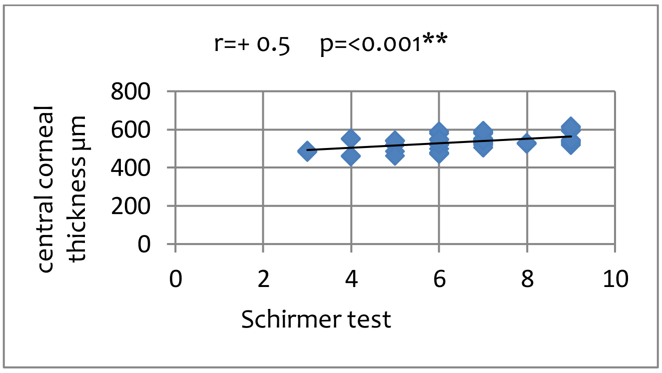

Figure 3: Correlation between Schirmer test results (severity of dryness) and CCT among dry eye group (n=100). ****highly significant (P<0.001)***

There was no statistically significant difference between them (P=0.3). Koray Karaday, et al[18] performed a study to find the effect of artificial tear application on central corneal thickness in dry eye patients. They found that CCT was lower in dry eyes. It increased after artificial tears application. The mean CCT in the dry eye group was (531.2 ±16.5) and (559.6 ±37.8) after 1 week of therapy. The CCT in the control group was (549.2 ±37.8) and (552.3 ±39.8) after 1 week of therapy. So, it increased after therapy (P=0.0001). In our opinion, they disagreed with us as the study was not directly related to dry eye disease but to artificial tears application. In our study, the coefficient variant (CV) of the study group was (32.8±6.4) and of the control group was (31.5±5.2). There was no statistically significant difference between them. (P=0.1). The hexagonality (HEX) of the study group was (62.4±10.6) and of the control group was (63.48±7.2) where there was no statistically significant difference between them. (P=0.4). As far as we know there were no studies about (CV) or (HEX) in dry eye disease patients directly or indirectly. Despite excluding smoking, contact lens wear, LASIK surgery to minimize the study bias, there were some studies that showed its role in dry eye disease and on corneal endothelial cells. Nihat Sayin, et al[19] performed a study about the relationship between smoking and corneal endothelium using non-contact specular microscopy.

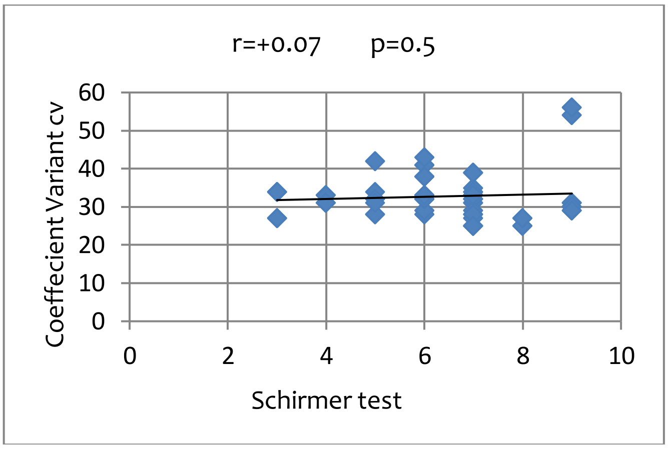

Figure 4: Correlation between Schirmer test results (severity of dryness) and Co-efficient variant (CV) among the dry eye group (n=100)

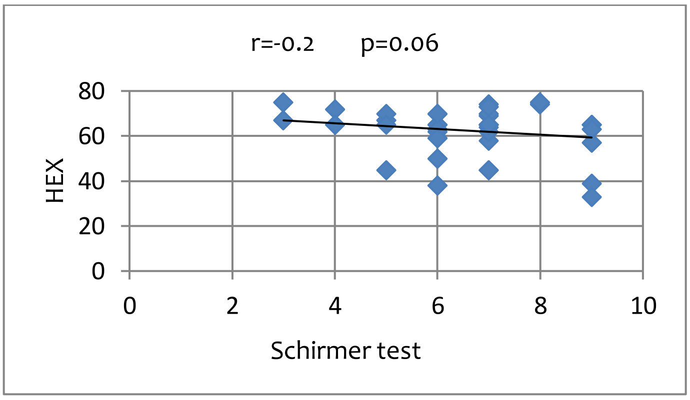

Figure 5: Correlation between Schirmer test results (severity of dryness) and Hexagonality (HEX) among the dry eye group (n=100).

They found that there was no statistically significant difference in corneal thickness, co-efficient variant, and cell density between the smoker group and the control group. They also found that there was a statistically significant difference in the hexagonality of cells in the smoker group than the normal one. The mean Schirmer score and TBUT value were significantly lower in the smoker group compared to the non-smoker group ($p = 0.015$) and ($p < 0.001$), respectively. Renu M. Magdum et al[20] performed a study about the effect of contact lenses on corneal endothelium. They found that there was a statistically significant difference in corneal cell density. It was (2570.91 ± 432.06) cells/mm^2 in lens users and (2723.17 ± 327.64 cells/mm^2 in the control group. They found that there was a statistically significant difference in the central corneal thickness and hexagonality. The CCT was (0.532 ± 0.0309 mm) in lens users and (0.514 ± 0.03 mm) in controls, while hexagonality was (54.81 ± 39.72%) in lens users and 67.65 ± 36.49% in controls. Sarah S. Jones et al [21] performed a study about the effect of LASIK on the corneal endothelium. They agreed and found that there was no statistically significant difference in morphology of the endothelium up to 12 weeks and the mean coefficient of variation was (0.35 ± 0.06) pre-operative and (0.35 ± 0.05) postoperative examinations. They found that there was no statistically significant difference in density. The mean ± SD preoperative endothelial cell density was (2,549 ± 365 cells/ mm^2). It was at the 2-week (2,561 ± 360 cells/mm^2) and at 12-week was (2,541 ± 364 cells/ mm^2). Then we divided the study group into mild, moderate, and severe groups and started to compare the data between them; new results appeared. In our study, the corneal endothelial cell count (CD) in severely dry eyes was significantly lower than in mild and moderate dry eyes (2428.9±282.5, 2625.8±325.1, and 2664.0±348.7) respectively. Also, corneal thickness in severely dry eyes was significantly lower than in mild dry eyes and significantly lower than in moderate dry eyes (505.9±34.3, 557.06±36.1, and 536.9±33.8) respectively. On the other hand, the hexagonality was significantly higher in severe dry eyes than in mild dry eyes (66.7±8.2 vs. 57.9±13.03). While no significant difference in coefficient variant (CV) in respect to the difference in degree of dryness. As far as we know there is no obvious reason for the correlation between dry eye disease and the decrease of the endothelial cell density. But Kheirkhah et al.[17] assumed that the reason may be due to a decrease in the sub-basal corneal nerve density in dry eye disease where the dry eye group showed significantly lower corneal sub-basal nerve density (17.1 ± 6.9 mm/mm^2) compared to the control group (24.7 ± 4.4 mm/mm^2), ($P < .001$). Lambiase and colleagues[22] have also reported significantly lower endothelial cell density in eyes with neurosurgical-induced neurotrophic keratitis than in the controls, with a significant correlation between the duration of the neurotrophic state and the endothelial loss. Being the first to do this study is the strength point of it. We hoped to use a larger number of eyes to study but it was not possible because of the excluded criteria.

Conclusion

The study group showed a decrease in the corneal endothelial cell density. There was no effect on central corneal thickness, coefficient variant (polymegathism), or hexagonality (pleomorphism). Severe dry eyes showed less CCT and CD than eyes with mild and moderate degrees of dryness. They showed higher HEX than that of mid and moderate dryness. However, there was no difference in CV between the three degrees of dry eye disease.

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