Research Article

Changes in Corneal Endothelial Cells Characteristics in Dry Eye Disease

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ABSTRACT

Purpose: To correlate corneal endothelium cell density with dry eye disease compared to an age-matched control group.

Materials and Methods: A total of 150 eyes of 75 subjects aged 19-25 years who did not have any history of eye injuries or eye disease affecting the corneal endothelium cell density, were recruited in this cross-sectional study. They were divided into groups based on their dry eye disease severity. All subjects underwent full ophthalmic examinations assessing their endothelium cell count by specular microscope and dryness level by Non-invasive Break up Time (NIBUT) of Keratograph 4.

Results: The mean endothelial cell density was significantly lower in subjects with severe dryness (2620.3 ± 252.2 cell/mm²) and moderate dryness (2801 ± 221.6 cell/mm²) than normal subjects (3067 ± 196.7 cell/mm²), $p < 0.01$.In addition, the mean cell area was lower in normal subjects (327.4 ± 21.5 µm²) and increased with severity of dryness, in subjects with moderate dryness (358.9 ± 27.1 µm²) and in subjects with severe dryness (384.8 ± 33.7 µm²), $p < 0.01$.There was variation in the mean cell volume, in normal subjects was (25 ± 3.6) and (27.2 ± 4.3) in moderate dryness and (25.5 ± 3.6) in severe dryness, p=.009.

Conclusion: Corneal endothelial cell density is significantly reduced in moderate to severe dryness.

INTRODUCTION

Endothelium of the cornea is formed of one layer of hexagonal cells (Figure 1), representing the posterior corneal surface. It accomplishes a crucial role in preserving the corneal transparency, thickness and hydration [1].The distinguished organization of this cell layer gives a significant corneal clinical aspect , with scans being easily imaged with a specular microscope [2]. It is around 5 µm thick. The endothelial cell count decreases normally with aging because of cell decomposition, ranging from 3000 to 4000 cells/mm² in children to1000 to 2000 cells/mm2 at age of 80years [3]. The minimum cell density required for optimum corneal endothelium function must be ranging from 400 to 700 cells/mm². Endothelial cell abnormalities can include an increase in the variation of cell size (polymegathism), or shape (pleomorphism) and endothelial cell death [4]. Added to the normal aging process, the endothelium can be damaged by trauma and diseases [5].Some disorders can injure the corneal endothelium, such as Fuchs' endothelial dystrophy (Figure 2) and corneal edema

[2]. This can lead to endothelial cell loss. A significant alteration in corneal endothelial cell characteristics was proved in eyes suffering from moderate to severe dry eye disease [6].The tear film is a pre-ocular, thin, complex and moist structure composed of four layers from anterior to posterior (lipid layer 0.1µm, aqueous layer 7µm, mucous layer 3–30 µm and glycocalyx 0.01–0.02µm from anterior to posterior) covering the cornea, and conjunctiva [7-9]. Any irregularities to its structure will have an impact on ocular surface and may change corneal clarity [10].It has optical, nutritional, mechanical, and defensive functions [11] (Figure 3). Volume of tear film is 7–10 µl. Normal basal tear secretion rate is 1–2 µl/min; while the reflex tear rate is >100 µl/min [12]. Replacement of tear volume takes place every 5–7 min [9].Tear Film Thickness (TFT) is around 5.35 µm and the central TFT value was 5.122 ± 0.034 µm [13].

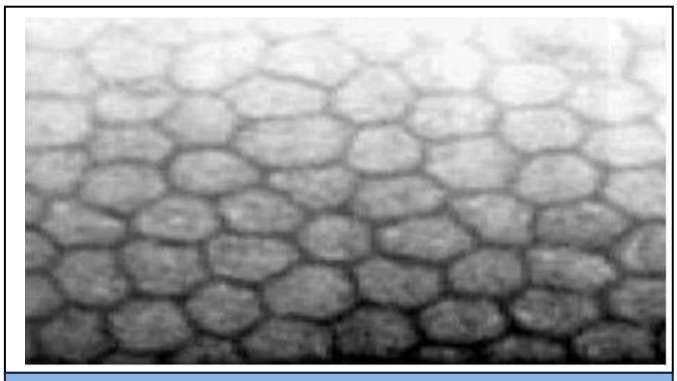

Figure 1: Specular micrograph of normal corneal endothelium cell.

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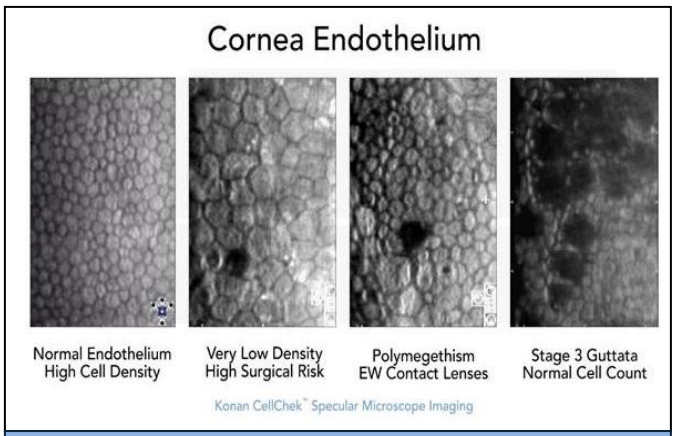

Figure 2: Specular micrograph of diseased corneal endothelium.

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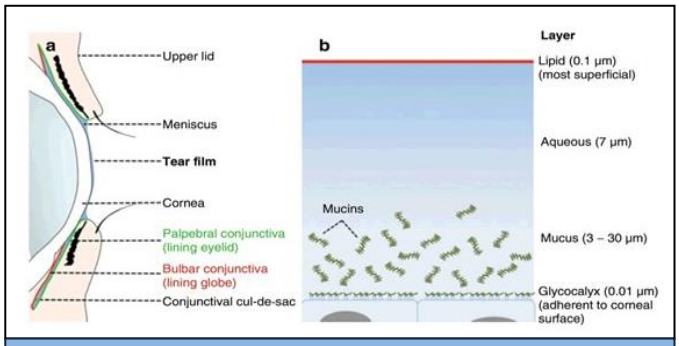

Figure 3: The tear film (a) distribution; (b) Structure (9).

International Dry Eye Workshop's (DEWS2007) provided the following definition: "Dry eye is a multifactorial disease of the tear sand ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface" [14]. Any breaking off to the lacrimal secretory and excretory units (a system composed of the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands), lids, and the sensory and motor nerves that supplies them [15] can disrupt the tear film osmolarity and subsequently ocular surface diseases [14]. Dry eye disease is either aqueous deficient or evaporative dry eye. Both can lead to tear hyperosmolarity [16]. Aqueous deficient dry eye is caused by decreased tear production and volume raising tear osmolarity followed by inflammation [17,18]. It is caused by disorders in the lacrimal gland, homeostatic disturbance induced by blockage of the afferent pathway, obstruction to lacrimal gland outflow, and by blockage of efferent pathway [19]. It also can be caused by systemic intake of drugs [20]. Aqueous deficient dry eye is classified to Sjögren and non-Sjögren Dry Eye [16]. Evaporative dry eye is hastened by increased tear evaporation rate associated with normal function of the lacrimal gland [16].

Dry eyes can be diagnosed by non-invasive tear film break up time (NITBUT) and tear meniscus assessment. NITBUT is defined as the time between the last blink and the breakup of a reflected image of a target on the tear film (Figure 4). Tear meniscus assessment carries 75% to 90% of the total tear film volume. Thus, it is used to diagnose aqueous tear deficiency. Tear meniscus parameters used for tear film volume are tear meniscus height TMH (the commonest) and tear meniscus radius

of curvature.TMH is measured from the eyelid to the top of the meniscus, the cut-off value is < 0.1mm [21] (Figure 5). Clinical examination involves the use of specular microscope and keratograph 4, as non- invasive procedures, to study changes in corneal endothelial cell characteristics with eye dryness.

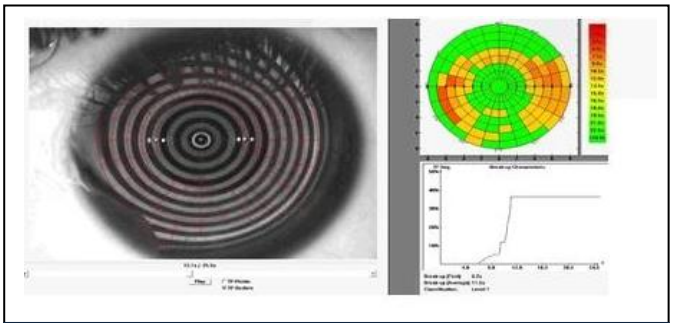

Figure 4: Non-invasive Tear Film Break Up Time (NITBUT) - Keratograph 4.

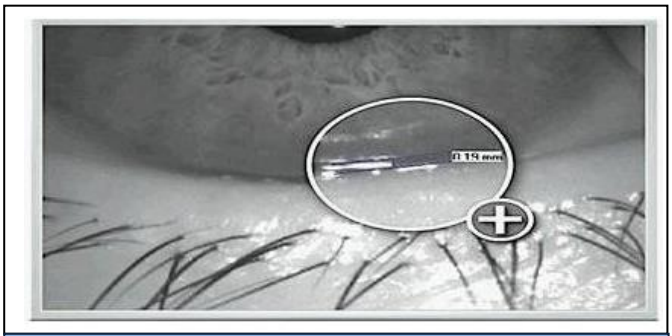

Figure 5: Tear meniscus height (TMH)- Keratograph 4.

METHODS

Study population and Examination

A total of 150 eyes of 75 subjects aged 19-25 years were enrolled in this cross sectional study. They were divided into groups based on their dry eye disease DED severity:

Group 1: comprised 40 normal eyes of 20 subjects

Group 2: comprised 64 eyes of 32 subjects with moderate dryness

Group 3: comprised 46 eyes of 23 subjects with severe dryness

All subjects underwent full ophthalmological examinations including the following: 1. Measurement of refractive error using Auto Refractometer. 2. Visual acuity using Snellen chart. 3. Anterior segment examination using Slitlamp. 4. Intraocular pressure measurement using Goldman applanation tonometry. 5. Fundus examination using indirect ophthalmoscope . 6. Non-invasive Breakup Time (NITBUT) using Keratograph 4 (to measure dryness level). 7. Endothelium cell characteristics using specular microscope. Subjects with break up time ≤ 10 seconds were considered to have dryness. Subjects with ocular allergy, ocular surface diseases, contact lens wear, glaucoma, previous ocular surgery or injury or subjects with systemic or ocular treatment were excluded from this study.

Statistical analysis

Statistical analysis was performed using SPSS version 21.0. All variables were expressed as Mean ± Standard deviation. The normality of the data was first assessed using the Shapiro-Wilk test. Levene's test was used to determine homogeneity of the data. Owing to the normal distribution and homogeneous of the data, one-way ANOVA test was used to compare the means of endothelium cell characteristics between control group and DED groups. To assess the statistical significance of differences between means using a set of confidence intervals 95% multiple comparis on post hoc Scheffe was used. The Pears on correlation analysis was used to estimate the correlations between the means of endothelium cell characteristics and the level of the NITBUT. The probability values of <0.05were considered statically significant.

Ethical consideration

The protocol of the study was explained to each participant at the time of recruitment and informed consent was obtained according to the Declaration of Helsinki.

RESULTS

The endothelial cell characteristics including Cell Density (CD), Cellarea (CA), Coefficient of Variation (CV), Hexagonality (HEX) and Center Cornea Thickness(CCT) of the three groups were studied and compared (Table 1-2). Mean ECD was significantly lower in subjects with severe DED (2620.3 ± 252.2 cell/mm²) and moderate DED (2801 ± 221.6 cell/mm²) than normal subjects (3067 ± 196.7 cell/mm²) (Figure 6), **P <** 0.01.Moreover, the mean cell area was lower in normal subjects (327.4 ± 21.5 µm²) and increased with severity of the DED, in subjects with moderate DED (358.9 ± 27.1µm²) and in subjects with severe DED (384.8 ± 33.7 µm²) (Figure 7), **P <** 0.01. Variations in mean CV was noted as follows: in normal subjects was (25 ± 3.6), (27.2 ± 4.3) in moderate DED and (25.5 ± 3.6) in severe DED (Figure 8), *p* =.009. Mean HEX was lower in subjects with severe DED (65.3 ± 6.9%) and moderate DED (66 ± 5.2 %) than normal subjects (68.1 ± 3.5 %) (Figure 9), *p*=.045. Mean CCT in normal subjects was (569.8 ± 38.22µm),

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and (561 ± 32.7 µm), (563 ± 23 µm) in moderate and severe DED respectively (Figure 10), *p* =.41. CD showed higher statistically significant difference between normal to severe DED with mean difference (446.67cell/mm²) **P<** 0.01 than between normal to moderate DED with mean difference (265.48 cell/mm²) **P <** 0.01 and between moderate to severe DED with mean difference(181.18 cell/mm²) **P<** 0.01 CA showed higher statistically significant difference between normal to severe DED with mean difference(-57.35µm²) **P<** 0.01 than between normal to moderate DED with mean difference(-31.53 µm²) **P<** 0.01 and between moderate to severe DED with mean difference (-25.82 µm) **P<** 0.01 CV showed higher statistically significant difference between normal to moderate DED with mean difference (-2.24%) (*P*=.013)than between normal to severe DED with mean difference (-1.99 %) (*P*=.049) with no statistical difference between moderate to severe DED with mean difference (0.25%) (*P*=.94). HEX showed no statistically significant difference between normal to moderate DED with mean difference(2.15%)(*P*=.15) and between normal to severe DED with mean difference (2.88%) (*P* =.05) and between moderate to severe DED with mean difference (0.73%) (*P*=.78).

Table 1: Endothelial cell characteristics of the study population in different NITBUT level groups (mean ± SD).

NITBUT level	No. of eyes	CD (cell/mm²)	CA (µm²)	CV (%)	HEX (%)	CCT µm)
Normal	40	3067 ± 196	327.4± 21.5	25 ± 3.6	68.1±3.5	569.8±38.2
Moderate	64	2801 ± 221	358.9 ±27.1	27.2±4.3	66±5.2	561±32.7
Severe	46	2620 ± 252	384.8 33.7	25.5±3.6	65.3±6.9	563±23

CCT showed no statistically significant difference between normal to moderate DED with mean difference (8.35 µm) (*P*=.43)and between normal to severe DED with mean difference (6.85µm) (*P*=.60)and between moderate to severe DED with mean difference (-1.49 µm) (*P*=.97) (Table 3). The ECD showed statistically significant negative correlation with the NITBUT level (r_S=-.6, *P*=0.000), CA showed statistically significant positive correlation with the NITBUT level (r_S=.61, *P*=0.000), CV showed weak positive correlation with the NITBUT level (r_S=.191 *P*=0.19), HEX showed weak negative correlations with the NITBUT level (r_S=-.194*P*=0. 18), andCCT showed irrelevant (very weak) negative correlations with the NITBUT level (Table 4).

Table 2: One way Anova.

		Sum of Squares	df	Mean Square	F	Sig.
CD	Between Groups	4294644.3	2	2147322.1	42.3	< 0.01**
	With in Groups	7466006.1	147	50789.2		
	Total	11760650.4	149			
	Between Groups	70407.4	2	35203.7	44.8	< 0.01**
CA	With in Groups	115578.1	147	786.2		
	Total	185985.5	149			
CV	Between Groups	149.5	2	74.7	4.8	.009**
	With in Groups	2278.7	147	15.5		
	Total	2428.2	149			
HEX	Between Groups	191.5	2	95.7	3.2	.045*
	With in Groups	4430	147	30.1		
	Total	4621.4	149			
CCT	Between Groups	1803.2	2	901.6	.9	.411
	Within Groups	146985.1	146	1006.7		
	Total	148788.2	148			

*P <0.05 significant.

**P <0.01 highly significant.

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DISCUSSIONS

Author	No. of subjects	Instrument used	Result
Current Study 2017	Group 1:40 normal eyes of 20 subjects Group 2: 64eyes of 32 subjects with moderate dryness Group 3: 44 eyes of 22 subjects with severe dryness	Specular microscopy CEM-530 NITBUT Keratograph4	This cross-sectional study showed That in moderate to severe DED, there was a significant reduction in the corneal endothelial cell density (ECD)as compared to the age-and sex-matched control group. ECD showed significant correlation with clinical severity of the disease, as judged by the level of non-invasive tear breakup time test. In addition, in DED there is a significant reduction in percentage of hexagonal cells (Polymegathism) and an increase in endothelial cell area and coefficient of variation (pleomorphism) that correlates
Kheirkh et al., [6]	15normal subjects 45 patients with DED.	IVCM using a Heidelberg Retina Tomography3 with the Rostock Cornea Module.	Eyes with DED displayed a Significant reduction in corneal ECD in DED that correlates with clinical severity of the disease and Significant lower sub basal nerve density than did those in the control group.
Rohit Shettyet al., 2015	43 healthy control 52 DED patients	IVCM imaging using Rostock Corneal Module/Heidelberg Retina Tomographll	A significant decrease in SBNP features(corneal nerve fiber length, fiber density, fiber width, total branch density, nerve branch density, and fiber area) was observed in DED patients with OSDI score > 23(<0.05).
Ceyhun Arıcı, et al., 2014	252 eyes of 126 healthy volunteers	Specular microscopy	It has been reported that there is a negative correlation between CA and CD.
Bernardo Bercht, et al., 2014	Group 1 (2-4- month-old) Group 2 (48- month-old) Group 3	Specular microscopy	It has been reported that there is a negative correlation between endothelial cell density and an endothelial cell area and pleomorphism.

CONCLUSION

In conclusion, results show that in moderate to severe DED, there was a significant reduction in the corneal Endothelial Cell Density (ECD) as compared to the age-and sex-matched control group. ECD showed significant correlation with clinical severity of the disease, as judged by the level of non-invasive tear break uptime test. In addition, in DED there is a significant reduction in percentage of hexagonal cells (Polymegethism) (-ve) and an increase in endothelial cell area and coefficient of variation (pleomorphism) (+ve) that correlates with clinical severity of the disease.

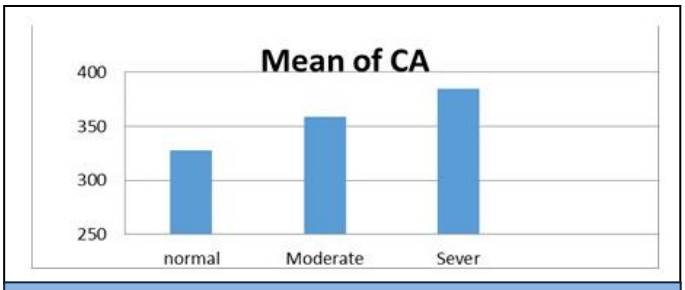

Figure 7: CA in various groups.

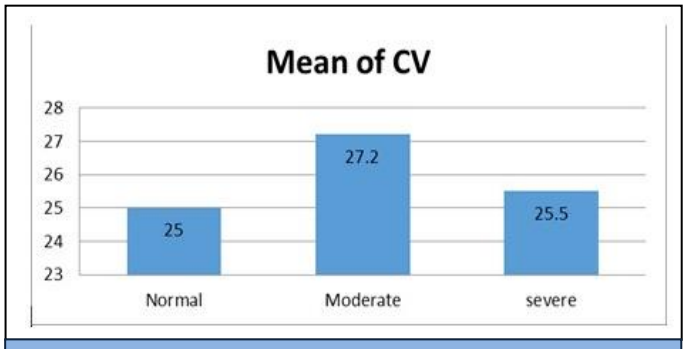

Figure 8: CV in various groups.

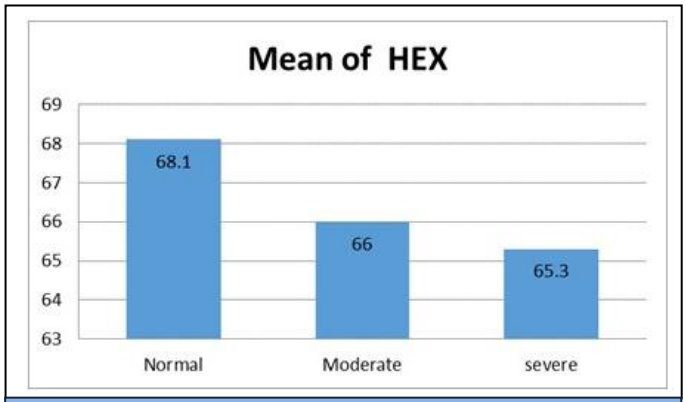

Figure 9: HEX in various groups.

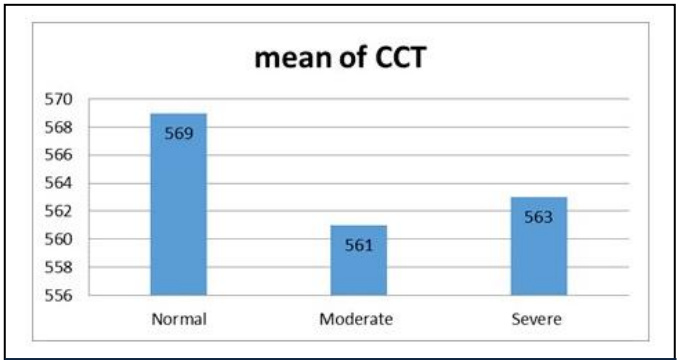

Figure 10: CCT in various groups.

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Table 3: Post hoc multiple comparisons (Scheffe).

Dependent Variable	(I) NIBUT level	(J) NIBUT level	Mean Difference (I-J)	Std. Error	Sig.	95%Confidence Interval	
						Lower Bound	Upper Bound
CD	normal	moderate	265.5*	45.4	< 0.01**	153.2	377.8
		severe	446.7*	48.7	< 0.01**	326.2	567.2
	moderate	severe	181.2*	43.6	< 0.01**	73.5	288.9
CA	normal	moderate	-31.5*	5.7	< 0.01**	-45.5	-17.6
		severe	-57.4*	6.1	< 0.01**	-72.3	-42.4
	moderate	severe	-25.8*	5.4	< 0.01**	-39.2	-12.4
CV	normal	moderate	-2.2*	.8	.013*	-4.1	-.4
		severe	-2*	.8	.049*	-4	-.01
	moderate	severe	.3	.7	.9	-1.5	2.0
HEX	normal	moderate	2.2	1.1	.3	-.6	4.9
		severe	2.9	1.2	.1	-.05	5.8
	moderate	severe	.7	1.1	.8	-1.9	3.4
CCT	normal	moderate	8.4	6.4	.4	-7.5	24.2
		severe	6.9	6.9	.6	-10.1	23.8
	moderate	severe	-1.5	6.2	1	-16.7	13.7

Table 4: Comparison between NITBUT and Endothelial cell characteristics.

Parameter (mean)	r (person correlation)	p-value
CD (cell/mm²)	-.6	< 0.01**
CA (µm²)	.6	< 0.01**
CV (%)	.1	.019*
HEX (%)	-.19	.018
CCT(µm)	-.08	.340

*P <0.05 significant.

** P < 0.01 highly significant.

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