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Original Research Article

Study of changes in corneal endothelial cell characteristics in dry eye disease

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ABSTRACT

Background: Corneal endothelial cells, vital for maintaining transparency and unable to regenerate, compensate for cell loss by enlarging adjacent cells, which leads to increased size and varying morphology with age. This study emphasizes how dry eye affects these cells, stressing the need to address this frequently neglected condition.

Materials and Methods: This cross-sectional study explored alterations in corneal endothelial cell characteristics among 33 individuals with dry eye disease (DED) compared to 33 age- and gender-matched controls, aged between 18 and 78 years. Participants underwent comprehensive ophthalmic evaluations, and various grades of DED were diagnosed using the Ocular Surface Disease Index questionnaire, Tear Meniscus Height measurement, Tear Film Break-Up Time test, Schirmer's I test, and Lissamine Green staining and endothelium cells were assessed for endothelial cell density (ECD), cell morphology and central corneal thickness (CCT) by specular microscopy.

Results: Compared to the control group (57.73±8), the average cell morphology showed significant changes in individuals with moderate DED (52±9) with p=0.0497, and in those with severe DED (49±7) with p=0.004. Additionally, Central Corneal Thickness (CCT) was notably lower in the severe DED group (485±32 μm) with p=0.002 as compared to control group (533±34 μm). The mean ECD was lower in severe DED patients compared to controls, but not statistically significantly.

Conclusion: The research found a correlation between DED severity and corneal endothelial cell characteristics. Severe DED leads to significant morphological alterations and reduced CCT. These findings highlight DED's impact on corneal cells, emphasizing early detection and intervention for preserving corneal health and improving intraocular surgery outcomes.

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1. Introduction

Corneal endothelium is essential for maintaining corneal clarity and regulating fluid content. As people age, endothelial cell density (ECD) decreases from about 6000 cells/mm^2 at birth to approximately 2500-3000 cells/mm^2 in adults. Since endothelial cells cannot regenerate, surviving cells enlarge to fill gaps left by cell loss, leading to increased cell size and morphological variability.[1]

Dry eye disease (DED) is a complex condition affecting the tears and the ocular surface, resulting in discomfort, visual disturbances, and tear film instability, which may lead to ocular surface damage. It is marked by increased tear film osmolarity and inflammation of the ocular surface.[2] The lacrimal and meibomian glands, together with the corneal and conjunctival surfaces, work as a cohesive unit to maintain the stability of the tear film. DED disrupts this unit, impacting both tear production and composition.[3] This condition is among the most prevalent eye disorders, with its occurrence varying from 5% to over 35% across different

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adult age groups.[4] There is no single gold-standard test for evaluating dry eye, which results in varying sensitivity and specificity among the different diagnostic techniques used. In research studies investigating dry eye under various conditions, surveys focusing on the ocular surface are often employed.[5]

This research aims to examine the effects of DED on the characteristics of corneal endothelial cells by comparing individuals with DED to age- and gender-matched control groups using specular microscopy.

2. Aim

1. To study changes in corneal endothelial cell characteristics in patients with dry eye as compared to age and gender-matched control groups.

3. Objectives

1. To assess the severity of dry eye according to OSDI score and by objective methods- Tear meniscus height, TBUT, Schirmer's I test, and Lissamine Green B staining.
2. To study the corneal endothelial cell density, cell morphology and central corneal thickness in dry eye subjects and age and gender-matched healthy control group using specular microscopy.
3. To establish a correlation between different grades of dry eye and corneal endothelial cell density, cell morphology and central corneal thickness.
4. To compare with age and gender-matched control groups.

4. Materials and Methods

4.1. Study design

This observational cross-sectional comparative study was conducted after obtaining Ethical approval from the Institutional Ethics Committee and informed consent was obtained from all participants.

4.2. Study population

A sample size of 33 for each group was calculated based on a DED prevalence of 9%, with a 95% confidence level and a 10% margin of error.

The sample size was calculated by using prevalence:

Margin of error is 10%

The sample size was calculated by using the following formula:

$$n = \frac{(Z_\alpha)^2\, p_1\,(1 - p_1)}{(d)^2}$$

where Z_α is the critical value of the normal distribution at α (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96, p1 is the expected prevalence and d is the margin of error. Taking, p_1 = 0.09 (9%),and d = 0.1, the minimum sample size calculated was 33.

$$n = \frac{(1.96)^2 \times 0.09 \times (1 - 0.09)}{(0.1)^2}$$

$$n = 33$$

4.3. Inclusion criteria

Consenting individuals diagnosed with DED, as confirmed by the OSDI questionnaire and Objective dry eye tests (TMH, TBUT, Schirmer's I and Lissamine Green). For control group, consenting age- and gender-matched individuals without symptoms of DED were included.

4.4. Exclusion criteria

Contact lens users, patients with diabetes mellitus, glaucoma, pre-existing ocular allergies, ocular surface disorders, lid and adnexal diseases and patients who underwent previous ocular surgeries were excluded.

4.5. Methodology

Each subject underwent a comprehensive ophthalmic examination- visual acuity testing and refractive error measurement followed by detailed anterior segment evaluation on slit lamp

The OSDI questionnaire,[6] consisting of 12 items, assessed ocular symptoms over the past 2 to 4 weeks. Scores were determined and classified into the following categories: normal (0-12 points), mild (13-22 points), moderate (23-32 points), and severe (33-100 points).

DED was confirmed when two or more of following objective tests came positive.

1. TMH: A slit-lamp equipped with micrometre capabilities provides accurate measurements of the tear meniscus. A TMH ≥0.5 mm was considered normal; <0.5 mm indicated dry eye.
2. TBUT: The corneal surface is seen under slit lamp with low magnification using a cobalt blue filtered light. Breakup time refers to the interval between a complete blink and the initial appearance of a randomly occurring dry spot. Classified as normal (11-30 seconds), mild-to-moderate dry eye (5-10 seconds), or severe dry eye (≤5 seconds)..
3. Schirmer's I Test: Whatman filter paper (no. 41) strips were used to measure tear production over a 5-minute period. A wetting measurement of less than 15 mm indicated dry eye. The results were categorized as follows: normal (>15 mm), mild dry eye (11-15 mm), moderate dry eye (5-10 mm), and severe dry eye (≤5 mm).

4. Lissamine Green Test: Staining intensity was assessed according to Van Bijsterveld[7] scoring on a scale of 0 to 3 across the nasal conjunctiva, temporal conjunctiva, and cornea. Scores up to 3 were considered normal; scores above 3 indicated dry eye.

Specular microscopy was done to evaluate corneal endothelial cell characteristics- ECD, Cell Morphology and CCT in both the groups.

Data was analyzed using SPSS V 23.0 software. Continuous variables were expressed as mean and standard deviation, while categorical variables were presented as frequency and percentage. Comparative analyses were performed using the t-test, with a significance level set at $p<0.05$.

5. Results

5.1. Demographics

The average age of participants in both the DED and control groups was 46.27 ± 17.48 years, with no significant difference between the groups ($p>0.05$). Gender distribution was balanced, with 51.52% male and 48.48% female in each group.

5.2. OSDI scores and objective tests

1. OSDI scores in the DED group- 36.36% had mild symptoms, 27.27% moderate, and 27.27% severe symptomsAll control subjects had normal OSDI scores.
2. Tear meniscus height was <0.5 mm in 72.72% of DED subjects, while all controls had TMH ≥0.5 mm.
3. Tear Film Break-Up Time was <5 seconds in 27.27% of DED subjects, 5-10 seconds in 33.33% of DED subjects, and >10 seconds in all controls.
4. Schirmer's I test values were <5 mm in 27.27%, 5-10 mm in 33.33%, 11-15mm in 39.39% of DED subjects and >15 mm in all controls.
5. Lissamine Green Staining score was ≥4 in 54.54% of DED subjects and ≤ 3 in all controls.

5.3. Severity of dry eye disease (DED)

In the case group, 39.40% (n=13) had mild DED, 33.33% (n=11) had moderate DED, and 27.27% (n=9) had severe DED. (Table 1)

5.4. Endothelial cell characteristics

5.4.1. Mean endothelial cell density (in cells/mm^2)

The mean endothelial cell density in subjects without DED (control group) was 2856±318 cells/mm^2. In mild DED, the mean cell density was 2740±318 cells/mm^2, compared to 2772±311 cells/mm^2 in controls (P=0.397). In moderate DED, the mean cell density was 2871±352 cells/mm^2 versus 2885±349 cells/mm^2 in controls (P=0.463). For severe DED, the mean cell density was 2753±285 cells/mm^2 compared to 2943±284 cells/mm^2 in controls (P=0.087). Although cell density tended to be lower in DED subjects, these differences were not statistically significant. (Tables 2 and 3)

5.4.2. Mean cell morphology (Hexagonality in %)

In subjects without DED (control group), the mean cell morphology was 57.73±8 %. No significant difference was observed in subjects with mild dry eye disease (DED), with a mean morphology of 56±8%, compared to 56±8% in age- and gender-matched controls (P=0.486). However, in moderate DED, cell morphology was significantly reduced to 52±9% compared to 58±9% in controls (P=0.0497). In severe DED, cell morphology was also significantly decreased to 49±7%, while controls had 59±8% (P=0.004). (Tables 4 and 5)

5.4.3. Mean central corneal thickness

In subjects without DED (control group), the mean CCT was 533±34 μm.

Corneal thickness (CCT) varied by DED severity, with no significant difference in mild DED (mean CCT of 519±32 μm) compared to controls (524±33 μm, P=0.351). In moderate DED, the mean CCT was 517±36 μm, slightly lower than controls (536±37 μm, P=0.117), but this difference was not statistically significant. In severe DED, the mean CCT was significantly reduced to 485±38 μm compared to 542±30 μm in controls (P=0.002). (Tables 6 and 7)

Table 1: Distribution of subjects according to severity of DED

Severity of DED	Case	
	Frequency (n)	Percentage (%)
Mild	13	39.40
Moderate	11	33.33
Severe	9	27.27
Total	33	100

Table 2: Distribution of subjects according to mean cell density in DED severity and control groups

Severity of DED	Mean Cell density (in cells/mm^2)	
	Mean	SD
Normal	2856	318
Mild	2740	352
Moderate	2871	316
Severe	2753	285

6. Discussion

All cases meeting the inclusion and exclusion criteria were included in our study. A total of 66 subjects were assessed

Table 3: Comparison of mean cell density according to severity

Severity	Mean Cell density (mean±SD)(in cells/mm^2)		P value
	Case	Control	
Mild	2740±318	2772±311	0.397
Moderate	2871±352	2885±349	0.463
Severe	2753±285	2943±284	0.087

Table 4: Distribution of subjects according to mean cell morphology in DED severity and control groups

Severity of DED	Mean Cell morphology (Hexagonality in %)	
	Mean	SD
Normal	57.73	8
Mild	56	8
Moderate	52	9
Severe	49	7

Table 5: Comparison of mean cell morphology according to severity

Severity	Mean Cell morphology (mean±SD) (Hexagonality in %)		P value
	Case	Control	
Mild	56±8	56±8	0.486
Moderate	52±9	58±9	0.0497
Severe	49±7	59±8	0.004

Table 6: Distribution of subjects according to mean CCT in DED severity and control groups

Severity of DED	CCT (in μm)	
	Mean	SD
Normal	533	34
Mild	519	32
Moderate	517	36
Severe	485	38

Table 7: Comparison of mean CCT according to severity

Severity	Mean CCT (mean±SD) (in μm)		P value
	Case	Control	
Mild	519±32	524±33	0.351
Moderate	517±36	536±37	0.117
Severe	485±38	542±30	0.002

for dry eye disease and underwent specular microscopy to examine corneal endothelial cell characteristics.

The study aimed to evaluate the characteristics of corneal endothelial cells in patients with DED compared to a control group. The primary parameters examined were endothelial cell density, cell morphology, and central corneal thickness (CCT). The goal was to investigate the impact of DED severity on these corneal features and to determine any significant differences between the DED group and the control group.

6.1. Corneal endothelial cell density

The study observed that ECD decreased as the severity of DED increased. While the reductions in ECD between DED patients and controls were notable, they did not reach statistical significance. Several other studies have reported similar findings, supporting the observation of reduced ECD in patients with DED. Noma et al. found a mean cell density of 2710±308 cells/mm^2 in DED patients compared to 2945±247 cells/mm^2 in controls ($p < 0.001$). Severe DED patients showed an even lower ECD, averaging 2570±296 cells/mm^2, with significant differences compared to moderate (2780±310 cells/mm^2) and mild DED (2875±270 cells/mm^2) groups.[8]

Similarly, Fahmy et al. reported significantly lower mean ECDs in subjects with severe dryness (2620.3±252.2 cells/mm^2) and moderate dryness (2801±221.6 cells/mm^2) compared to normal subjects (3067±196.7 cells/mm^2, $p<0.01$).[9]

Kheirkhah et al. also observed reduced ECD in the DED group (2595±356.1 cells/mm^2) compared to the control group (2812±395.2 cells/mm^2, p=0.046). These findings support the current study's results, highlighting the significant impact of DED on corneal ECD, particularly in severe cases.[10]

In study conducted by Kheirkhah and et al, The mean ECD was found to be 2620 ± 386 cells/mm^2 in subjects with dry eye, compared to 2465 ± 391 cells/mm^2 in the control group, (p-values <0.05) indicating statistical significance.[11]

6.2. Endothelial cell morphology

Notable alterations in endothelial cell morphology were observed in patients with moderate and severe DED. The hexagonality of endothelial cells, which indicates cellular health and uniformity, was significantly reduced in DED patients compared to controls.

This finding is consistent with the study by Fahmy et al.[9] who found significantly lower mean endothelial cell morphology in severe (65.3±6.9%) and moderate dryness (66±5.2%) compared to normal subjects (68.1±3.5%, p=0.045).

In similar study conducted by Li et al, they found that DED severity influenced corneal endothelial cell morphology, with more severe DED associated with greater morphological changes.[12]

Similarly, Matsuda et al. highlighted a decrease in hexagonality with increasing DED severity, underscoring the importance of considering DED severity when examining corneal health, as more severe symptoms lead to more pronounced cellular alterations.[13]

6.3. Central corneal thickness

CCT was significantly reduced in patients with severe DED compared to controls. This thinning of the cornea can be

attributed to chronic inflammation and cell loss, which weaken the corneal structure.

In similar study done by Fujimoto et al, showed CCT in Non DED subjects as 553.1±27.2 μm whereas in severe DED it was 552.6±32.3 μm when assessed by AS-OCT.[14]

Research by Barbosa et al. indicated that reduced CCT is a marker of severe ocular surface disease, and our study supports this by showing a clear correlation between DED severity and decreased CCT. Thinner corneas are more susceptible to injury and can compromise visual acuity, underscoring the need for early intervention in DED management.[15]

7. Study Limitations

While this study provides valuable insights into the effects of DED on corneal endothelial cells, it has some limitations. The sample size, although calculated to be sufficient, is relatively small and may not capture the full spectrum of DED severity. Additionally, this study is cross-sectional, and longitudinal studies are needed to understand the progression of endothelial cell changes over time in DED patients. Future research should also explore the underlying mechanisms linking DED to corneal endothelial damage to develop targeted therapeutic strategies.

8. Conclusion

This study underscores the substantial impact of Dry Eye Disease (DED) on corneal endothelial cell characteristics. With increasing DED severity, there is a pronounced reduction in endothelial cell density (ECD), significant alterations in cell morphology, and a notable decrease in central corneal thickness (CCT).

These changes compromise the cornea's ability to maintain transparency and hydration, potentially impairing visual function. Chronic inflammation and hyperosmolarity in DED damage endothelial cells, reducing ECD and disrupting cell morphology. The observed decrease in hexagonality and corneal thinning in severe DED patients further indicate compromised corneal health. These findings emphasize the importance of early detection and comprehensive management of DED. Clinicians should incorporate detailed assessments of corneal endothelial health in patients with dry eye symptoms, as early intervention can mitigate the adverse effects of DED, preserving visual acuity and ocular health. The study underscores the need for further research, including longitudinal studies to understand the progression of corneal endothelial changes and investigations into the underlying mechanisms of DED-related damage. The findings have important clinical implications, aiding in better surgical planning and improving outcomes. Comprehensive ocular examinations in patients presenting with dry eye symptoms are essential, and early detection and appropriate management of DED can help preserve corneal endothelial health and visual function.

9. Source of Funding

None.

10. Conflict of Interest

None.

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