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Original Article

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

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ABSTRACT

Objective: To compare cell density of corneal endothelial cells in patients with dry eye disease versus controls.
Study Design: Case-control study.
Place and Duration of Study: Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from Jul to Dec 2024.
Methodology: After permission of the ethical committee, the corneal topographic measurements of thirty-five patients with dry eyes (Group-A) were compared to age and gender matched controls (Group-B). The diagnosis of dry eye was established on tear break-up time (TBUT), Schirmer test and ocular surface disease index (OSDI) as part of comprehensive ophthalmic evaluation. Density of corneal endothelium, central corneal thickness and hexagonality of cells was recorded and compared between groups along with demographics.
Results: The primary outcome was endothelial cell density of cornea. The corneal endothelial cell density in Group-A patients was 2294.94±242.03 cells per mm2 and corneal endothelial density in Group-B patients was 2949.62±375.8 cells per mm^2 (p<0.001). The central corneal thickness was 517.80±6.30 um in Group-A and 544.88±16.76 um in Group-B (p<0.001). The epithelial cell hexagonality was 64.17±9.90 in Group-A and 68.77±4.80 in Group-B (p=0.017).
Conclusion: We concluded that patients with dry eyes have reduced density of corneal endothelial cells along with reduced central thickness of cornea, cell hexagonality and best corrected visual acuity.

Keywords: Cornea, Dry Eye Disease, Eye, Tear Film, Xerophthalmia.

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INTRODUCTION

Xerophthalmia, or dry eye disease, affects people globally with a prevalence of 5 to 50%.[1] It has been associated with aging, autoimmune diseases, hormonal imbalance, environmental influences, allergies and female gender in the past, but is now largely attributed to the extended use of digital display terminals including tablets, computers and smart phones for work, education and entertainment. In Pakistan the prevalence of dry eye disease is around 18%.[2] The percentage is even higher in computer programmers, that is 47%, according to a cross-sectional study from Dera Ghazi Khan.[3]

Irrespective of cause, dry eye affects endothelial cells and causes instability of protective tear film. The inflammation encroaches sub-basal nerve density and affects trophic function resulting in loss of function and survival of endothelial cells which can be measured by corneal endothelial cell density (ECD).[4]

Although corneal endothelial cell density decreases progressively over the course of time with an average of six percent reduction per year but a minimum of 400 to 500 cells is required to maintain the pump function. The reduction of density below this critical level results in loss of corneal transparency and decrease in visual acuity.[5] The premature damage to the cornea is debilitating due to its limited regenerative potential.[6]

Creation of a suitable plan to protect the corneal endothelium and avert potential corneal blindness is essential due to the limited regeneration of the corneal endothelium and its significance for visual function.[7] According to reports, a variety of conditions, including systemic problems, eye diseases, and ophthalmological procedures, might harm corneal endothelial cells, which are particularly susceptible.[8] The rationale of our study was to measure different corneal topographic parameters in patients with dry eyes and normal eyes in our demographic population.

METHODOLOGY

This case-control study was conducted at the Armed Forces Institute of Ophthalmology Rawalpindi, Pakistan, between July and December 2024. Ethical approval from the hospital's Ethical Review Committee was obtained ((IERB approval number 328/ERC/AFIO).

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Inclusion Criteria: Patients of either gender, and any age group, diagnosed with dry eyes were included.

Exclusion Criteria: Patients with corneal perforation, increased intraocular pressure, history of contact lens use, thick corneal scars, previous surgery of cornea, opacity, herpetic keratitis and diabetes mellitus were excluded.

Sample size was calculated using OpenEpi calculator, Sample size was calculated keeping the mean cell density of corneal endothelium in dry eyes to be 2428±282 cells[9] /mm^2 and the mean density of corneal endothelium in healthy eyes to be 2731±2559 cells /mm^2.[9] The sample came out to be 34 for each group. We included 35 participants with dry eye disease in Group-A, and 35 controls in Group-B (Figure).

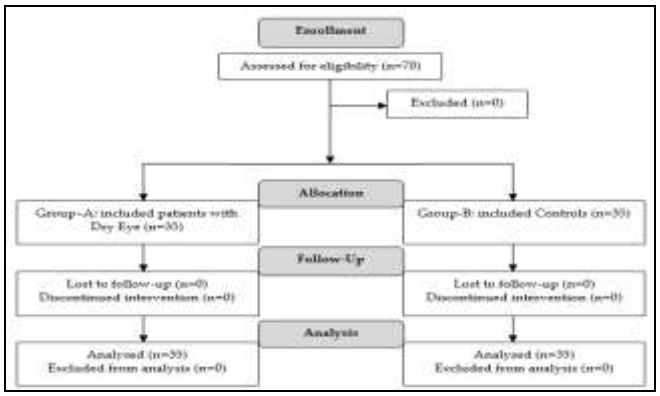

Figure: Patient Flow Diagram (n=70)

Non-probability consecutive sampling was used to include all patients who presented to OPD in six months who fulfilled our inclusion criteria. Informed consent was obtained prior to data collection.

The diagnosis of dry eye was made if patients had positive tear break-up time (TBUT) and Schirmer test and an Ocular Surface Disease Index (OSDI) greater than 13.[10] All patients had normal intraocular pressure.[11] Comprehensive ophthalmic evaluation was done in all patients. Each patient was scored based on Ocular Surface Disease Index (OSDI) including 12 questions based on ocular symptoms (OSDI-A), vision-related functioning (OSDI-B) and environmental elements acting as triggers (OSDI-C). Response to each question was noted and overall OSDI-D score was computed. The final score was on a scale between 0 to 100 where <13 correlated with normal eyes and >13 correlated with dry eyes. The OSDI score is mentioned in Table-I. All the patients were subjected to Schirmer test and wet-area length of less than 15 showed dry eyes. In Tear film break-up time test, a wet fluorescein-impregnated strip (Fluorescein strips, 1 mg) was inserted in inferior fornix. To guarantee sufficient mixing and uniform distribution of fluorescein on the ocular surface, the patients were then told to blink their eyes multiple times. Beam of a slit-lamp microscope was used to observe the time gap amongst a full blink and the onset of the first area of break-up on tear film of the cornea to determine the TBUT. The occurrence of dry patches on the tear film was indicated by the appearance of black dots. The average of three readings was recorded after repeating the process three times. The TBUT of less than 5 seconds was considered abnormal and recruited for study. A set of healthy controls with negative Schirmer and TBUT test and OSDI-D score less than 13 were also recruited for comparison. The central cornea in each of the individuals' eyes was assessed by Specular microscopy (Topcon Specular Microscope SP. 3000P) to quantify the density of corneal endothelium, central corneal thickness and hexagonality of cells. The following parameters were recorded for all patients. Age, eye involved, gender and best corrected visual acuity.

Table-I: Ocular Surface Disease Index (OSDI) Questionnaire[11]

OSDI-A	**All of time**	**Most of time**	**Half the time**	**Some of time**	**None of the time**
Are your eyes sensitive to light?					
Do your eyes feel gritty?					
Are your eyes painful?					
Do you have blurred vision?					
Do you have poor vision?					
OSDI-B	**All of time**	**Most of time**	**Half the time**	**Some of time**	**None of the time**
Is Reading difficult?					
Is night driving difficult?					
Is working with computer or ATM difficult?					
Is watching TV difficult?					
OSDI-C	**All of time**	**Most of time**	**Half the time**	**Some of time**	**None of the time**
Do you feel uncomfortable in windy weather?					
Do you feel uncomfortable in dry areas?					
Do you feel uncomfortable in areas with air conditioner?					
OSDI-D = OSDI-A+ OSDI-B+OSDI-C OSDI-D >13 Dry Eyes					

Statistical Package for Social Sciences (SPSS) version 26 was employed for statistical analysis. Quantitative variables (age, BCVA, central corneal thickness, endothelial cell density and Epithelial cell hexagonality) were analyzed by computing means and standard deviation and qualitative variables (gender and eye involved) were analyzed by computing

frequency and percentage. An independent sample t-test was used to compare means and Chi-square analysis was applied to compare frequencies. A *p*-value of less than 0.05 was considered significant.

RESULTS

A total of seventy patients were recruited in the study, 35 patients with dry eyes and 35 patients as controls. Both study groups had similar demographics including age and gender, but the best corrected visual acuity was significantly different between groups. The mean age of patients in Group-A was 47.45±7.35 years and mean age in Group-B was 46.40±8.75 years. There were 14(40.0%) males and 21(60.0%) females in Group-A. There were 23(65.7%) males and 12(34.3%) females in Group-B. The best corrected visual acuity (BCVA) in Group-A was 0.36±0.04 and in Group-B was 0.59±0.05, which was statistically significant (*p*=0.001). Demographics and BCVA of study participants can be seen in Table-II.

Table-II: Demographic Characteristics and Best Corrected Visual Acuity across Groups (n=70)

		Group-A n=35 Mean±SD	Group-B n=35 Mean±SD	*p*-value
Age (years)		47.45±7.35	46.40±8.75	0.503
Best corrected visual acuity (BCVA)		0.36±0.04	0.59±0.05	0.001
		n(%)	n(%)	
Gender	Male	14(40.0)	23(65.7)	0.309
	Female	21(60.0)	12(34.3)	
Eye involved	Right	18(51.4)	11(31.4)	0.166
	Left	17(48.6)	24(68.6)	

The mean corneal endothelial cell density in Group-A patients was 2294.94±242.03 cell per mm^2 and mean corneal endothelial density in Group-B patients was 2949.62±375.8 cell per mm^2 with *p*-value of <0.001. The mean central corneal thickness was 517.80±6.30 um in Group-A and 544.88±16.76 um in Group-B with *p*-value <0.001. The mean epithelial cell hexagonality was 64.51±9.82 in Group-A and it was 68.57±5.18 in Group-B with *p*-value of 0.017. The data is presented in Table-III.

Table-III: Corneal Topographic Parameters across Groups (n=70)

	Group-A n=35 Mean±SD	Group-B n=35 Mean±SD	*p*-value
Central corneal thickness (CCT) um	517.80±6.30	544.88±16.76	0.001
Endothelial cell density (ECD) cell per mm^2	2294.94±242.03	2949.62±375.8	0.001
Epithelial cell hexagonality (HEX)	64.51±9.90	68.57±4.80	0.017

DISCUSSION

The corneal topographic measurements were found to be significantly different in both study groups. The endothelial density is reduced in dry eyes as shown by our study. According to a study done on African population, the prevalence of low cell density of corneal endothelium was 63% in patients with dry eyes.[12] The mean endothelial density was 2763 cells per mm^2. In our study, the central corneal thickness and cell hexagonality showed significant difference in patients with dry eyes versus normal eyes. The findings of our study were not in agreement with Brott *et al.,* who advocated that dry eye disease was associated with decreased corneal endothelial density, but it did not show significant association with decrease in central corneal thickness and cell hexagonality.[13]

Karadayi *et al.,* conducted a study to evaluate the impact of artificial tear usage on central corneal thickness (CCT) in individuals with dry eye.[14] Their findings indicated that CCT was reduced in dry eye patients but showed an increase following the application of artificial tears. In the dry eye group, the average CCT was approximately 530 before treatment and increased to 560 approximately, after one week of therapy. In the control group, the central corneal thickness was almost 549 initially and 552 approximately after one week of treatment. The increase in CCT after therapy was statistically significant (*p*=0.0001). This means that central corneal thickness is a reliable measurement for diagnosis of dry eye and helps to identify recovery of disease.

In our opinion, the scope of their research was not entirely comparable with ours, as their study primarily focused on evaluating the effects of artificial tear application rather than directly addressing the underlying condition of dry eye disease. While artificial tears are commonly used as a treatment for dry eye, their study emphasized the impact of this specific intervention on certain parameters, rather than exploring the broader aspects or pathophysiology of dry eye disease itself. This distinction makes it less comparable to our research, which may have had a different focus or scope.

Corneal endothelial cells lack the ability to regenerate, which means that any loss of these cells cannot be replaced through cell division. To compensate for this loss, the remaining endothelial cells spread out to cover the affected area. This compensatory mechanism leads to an increase in cell

size and a change in cell shape, resulting in greater cellular pleomorphism.[15] Additionally, the percentage of hexagonal cells, which is a key indicator of a healthy and stable endothelial layer, decreases.[16] These changes can compromise the structural integrity and functionality of the corneal endothelium, potentially affecting the cornea's ability to maintain its transparency and proper hydration. Over time, such alterations may contribute to corneal decompensation and visual impairment if the endothelial cell density falls below a critical threshold.[17,18]

One study revealed a gradual decline in corneal endothelial cell density over time. They observed that, on average, there was a reduction of 87 cells/mm² per decade.[19] This decline translates to an approximate annual loss of 0.28% of the total endothelial cell population. Such a consistent reduction in cell density is a natural part of the aging process and highlights the progressive nature of endothelial cell loss. Over the years, this gradual decrease can have significant implications for corneal health, as the endothelium plays a critical role in maintaining corneal transparency and hydration by regulating fluid balance. If the cell density falls below a critical threshold, the cornea may lose its ability to function properly, potentially leading to conditions such as corneal edema or decompensation.[20] This finding underscores the importance of monitoring endothelial cell density, particularly in older individuals or those undergoing procedures that may impact the corneal endothelium.

LIMITATION OF STUDY

A small sample size and the fact that it was a single-centre study limit the generalizability of our findings.

CONCLUSION

We found that patients with dry eyes exhibit a marked reduction in corneal endothelial cell density, central corneal thickness, and cell hexagonality, coupled with a significant decline in best corrected visual acuity. These changes strongly highlight the profound structural and functional impairments caused by dry eye disease.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MS & WM: Data acquisition, data analysis, critical review, approval of the final version to be published.

SP & MWS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MBB & MZH: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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