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Original Article

Ophthalmology Section

Measurement and Morphology of Corneal Endothelial Cell Density in Myopic Eyes of Adult Females Using a Non Contact Specular Microscope: A Cross-sectional Study

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ABSTRACT

Introduction: A non contact specular microscope has been used to acquire the morphology of the corneal endothelial cell layer. This analysis provides measurements of the Coefficient of Variation (CV), the hexagonal appearance of the cells, and the mean corneal Endothelial Cell Density (ECD). These parameters are used to assess the function of the corneal endothelial layer.

Aim: To evaluate the average corneal ECD, Hexagonality (HEX), CV, and their relationship with myopia in young females.

Materials and Methods: The present cross-sectional study was conducted over a period of five months, from November 2016 to March 2017 at the clinics of the Department of Optometry and Vision Science, College of Applied Medical Science, King Saud University, Riyadh, Saudi Arabia and involved 107 eyes of healthy subjects aged 18-25 years. The participants' refraction was estimated using an automatic refractor. Non contact specular microscopy was performed on emmetropic and myopic subjects. The average of three successive estimations of ECD, hexagonal appearance, and CV in cell size were recorded. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (version 22.0), and the independent student's t-test was used to compare the means of various variables between low and moderate myopia.

Results: The study included the right eyes of 107 healthy Saudi female subjects aged 18-25 years, who were divided into three groups based on their refractive errors. Non contact specular microscopy was performed on emmetropic (n=36, 20.58±1.20 years and mean Spherical Equivalent (SE) of -0.01±0.23 D), low myopic (n=49, 20.63±1.51 years and SE of -1.19±0.68 D), and moderate myopic (n=22, 20.64±1.26 years with mean SE of -4.15±0.90 D) subjects. The mean ECD in low myopic eyes was 2799.59±184.39 per square millimetre, the mean CV was 26.67±4.03 percent, and the mean hexagonal appearance of the cells was 67.69±4.82%. The average ECD, mean CV, and hexagonal appearance in moderate myopic eyes were found to be 2722.36±143/mm^2, 26.68±3.57%, and 68.73±5.87%, respectively. The results showed no statistically significant differences in ECD ($p>0.05$) and hexagonal appearance ($p>0.05$) in the cells of moderate and low myopic eyes.

Conclusion: The present study found no significant relationship between corneal endothelial parameters and mild to moderate myopic eyes.

Keywords: Cornea, Microscopy, Myopia, Refractive error

INTRODUCTION

The cornea of the eye consists of five layers: the epithelium, stroma, Bowman's layer, Descemet's membrane, and endothelium [1]. The corneal endothelial cell forms a single layer that lines the internal surface and performs the important barrier function of preventing water and nutrients from entering the corneal stroma from the anterior chamber. It also maintains visual acuity through the pumping action of Sodium-potassium pump (Na-K-ATPase) [2]. Several factors can damage corneal endothelial cells, including ophthalmologic surgery [3], trauma [4], contact lens use [5], ultraviolet radiation [6], uveitis [7], and aging [8]. Decreased corneal ECD resulting from these factors can lead to blurred vision or decreased visual acuity, sometimes necessitating corneal transplantation to restore function [9].

Endothelial cells have a hexagonal shape, do not regenerate, and play a significant role in maintaining corneal transparency by pumping water from the stroma into the aqueous humour. They provide 70% of the water that the dehydrating stroma requires [10,11]. Therefore, it is clinically crucial to monitor the condition of corneal endothelial cells. However, the average ECD range varies among sexes and races [12]. The clarity of corneal cells depends on ECD and visual acuity. Severe cases with decreased ECD experience a deterioration in corneal clarity. Multiple factors such as aging [13,14], trauma, inflammation, and intraocular surgery [15-17] can affect ECD. Hence, there is an urgent need to clinically protect corneal endothelial cells to prevent visual aberrations. The use of a non contact specular microscope to study the morphology of the corneal endothelial cell layer and assess the function of the corneal endothelial layer plays a vital role [18,19]. Refractive errors are the leading cause of visual impairment globally, both in developed and developing countries. Their prevalence has been reported to be 70-90% in some Asian populations [20]. Myopia, also known as near-sightedness, is a major cause of various diseases depending on its severity. It is increasing among teenagers and young people worldwide due to reduced outdoor activity and genetic inheritance [21-23].

High levels of myopia are the most common pathological eye changes and can lead to irreversible vision loss, including blindness [24,25]. Major disorders associated with higher levels of myopia include retinal detachment, cataracts, posterior vitreous detachment, retinal tears, an increased risk of choroidal neovascularisation, and myopic macular degeneration [25,26]. The current study aimed to evaluate the density and morphology of corneal endothelial cells in young adult Saudi females with low and moderate myopia. Additionally, it sought to compare corneal endothelial features based on the degree of myopia. Understanding the epidemiological connection between myopia and

ECD, CV, and HEX is an essential parameter for illustrating the influence of increasing myopia. To the best of authors knowledge, this is the first report on an epidemiological study of Saudi adult females, providing reliable information on normal ECD, CV, and HEX, which may differ from other races. The study aimed to investigate the relationship between SE Refraction (SER) and the prevalence of abnormal values of ECD, CV, and HEX in Saudi young females, in order to examine the impact of myopia on ECD and endothelial morphology.

MATERIALS AND METHODS

The present cross-sectional study was conducted over a five-month period from November 2016 to March 2017 at the clinics of the Department of Optometry and Vision Science, College of Applied Medical Science, King Saud University, Riyadh, Saudi Arabia. Ethical approval was obtained from the Research Ethics Committee of King Saud University with code number (CAMS 017-37/38) to conduct the study.

A convenient sample of 107 females was included in the study. Participants were recruited from the female student population of King Saud University through email announcements. The entire procedure was explained to the participants, and written consent was obtained from them.

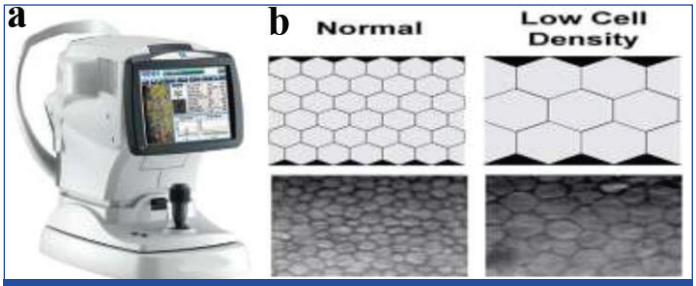
Inclusion criteria: Healthy female subjects aged 18 to 25 years with no refractive error, or with mild or moderate myopia.

Exclusion criteria: Subjects with astigmatism of more than 1.00 DC, regular contact lens wearers, and subjects with any ocular diseases or who had undergone ocular refractive surgeries were excluded.

Study Procedure

The present study involved the right eyes of 107 healthy female students aged 18 to 25 years. The subjects were divided into three groups based on their SER: mild myopia, moderate myopia, and emmetropes with SER values between -0.5 D to -3.0 D, -3.0 D to -6.0 D, and -0.25 D to +0.50 D, respectively [27]. There were 49 mild myopic eyes, 22 moderate myopic eyes, and 36 emmetropic eyes. The participants' refraction was measured using an Automatic Refractor, and non contact specular microscopy (Nidek 530-CEM-SET, Japan) was performed on all subjects. All measurements were taken between 9:00 AM and 12:00 PM by a single examiner using the same autorefractor and non contact specular microscope in the same room setting. There was no corneal contact, thus avoiding the risk of corneal lesions or eye infections.

A non contact microscope in combination with image-analysis auto-focus programs was used for the study of the corneal endothelium [28]. The average ECD, CV in cell size, and hexagonal appearance were calculated, and a comparison between the three groups was made. Corneal ECD was recorded by a physician in the optometry department clinic. After proper positioning of the circle, alignment dot, screen bar, and pictures of the cornea centre, images were captured in automatic mode with a low level of flash intensity. Image-in-built software was used to measure the count of endothelial cells. The average of three successive estimations was taken to assess ECD and represented as the number of cells present per mm square. The analysis was conducted on one eye of each patient to eliminate the potential influence of intrasubject variability that could occur in both eyes of the same subject [29]. A digital image of the Specular Microscope CEM-530 is shown in [Table/Fig-1a], and images of corneal endothelium with normal and low density are shown in [Table/Fig-1b].



[Table/Fig-1]: (a) Specular microscope CEM-530; (b) Top images are cartoon representation of normal and low density corneal endothelium and bottom images are real images of corneal endothelium with normal and low density.

STATISTICAL ANALYSIS

Data were analysed using the SPSS statistical program (version 22.0). Mean differences, standard deviations, and correlations between observations were calculated. The normality of the data was checked using the Shapiro-Wilk test. The means of different variables between low and moderate myopia were tested using independent t-tests. A p-values less than 0.05 was considered statistically significant.

RESULTS

The study included the right eyes of 107 females with a mean age of 20.62±1.35 years and an average SE of -1.40±1.62 D. The subjects were divided into three groups based on their refractive errors. The emmetropic group consisted of 36 eyes (n=36) with a mean age of 20.58±1.2 years and a mean SE of -0.01±0.23 D. The mild myopic group had 49 eyes (n=49) with a mean age of 20.63±1.51 years and a mean SE of -1.19±0.68 D. The moderately myopic group had a sample size of 22 eyes (n=22) with a mean age of 20.64±1.26 years and a mean SE of -4.15±0.90 D. The difference in sample size among the three groups (emmetropic, mild myopic, and moderate myopic) was found to be statistically insignificant, with p-values of 0.199, 0.771, and 0.108 for ECD, CV, and HEX, respectively. The normality of the data for the moderately myopic group was checked using the Shapiro-Wilk test, which showed that the data were normal with no significant difference. The corneal endothelial characteristics in the emmetropic, mild, and moderate myopic subjects are reported in [Table/Fig-2].

When comparing corneal endothelial cell parameters among the emmetropic, mild, and moderate myopic groups, no significant differences were found (p>0.05) for ECD, mean hexagonal appearance percentage of cells, and CV. [Table/Fig-3] shows the comparative

Groups	Number (n)	Mean age (Years) (Range)	Mean spherical equivalent±SD (Range) (D)	ECD Mean±SD (Range) (cell/mm^2)	CV% Mean±SD (Range)	HEX% Mean±SD (Range)
Emmetropic	36	20.58±1.20 (19-23)	-0.01±0.23 (-0.25 to+0.5)	2827.03±229.78 (2218-3326)	26.92±3.42 (19-34)	67.31±4.38 (57-77)
Mild myopic	49	20.63±1.51 (19-24)	-1.19±0.68 (-0.5 to-3.0)	2799.59±184.39 (2342-3254)	26.67±4.03 (19-36)	67.69±4.82 (56-80)
Moderate myopic	22	20.64±1.26 (19-23)	-4.15±0.90 (-3.0 to-6.0)	2722.36±143 (2306-3114)	26.68±3.57 (21-34)	68.73±5.87 (54-79)
p-value		0.98	<0.05	0.199	0.771	0.108

[Table/Fig-2]: Corneal endothelial characteristics in emmetropic, mild and moderate myopic subjects.
Sample size (n) of each group, Spherical Equivalent (SE), means of Endothelial Cell Density (ECD), CV and Hexagonal (HEX)

Comparisom between groups	ECD (Mean difference) cell/mm^2	p-value	HEX% (Mean difference)	p-value	CV% (mean difference)	p-value
Emmetroic vs. mild myopic	27.435	0.562	1.45	0.099	0.51	0.484
Emetropic vs. moderate myopic	104.66	0.119	2.54	0.055	0.41	0.632
Mild vs. moderate myopic	77.23	0.203	1.11	0.440	0.098	0.906

[Table/Fig-3]: Pairwise comparison of corneal endothelial parameters between the three groups.

results of corneal endothelial parameters among the three groups (emmetropic, moderate myopic, and mild myopic). [Table/Fig-4] provides a summary of the correlations between corneal endothelial parameters. The present study found no correlation between mild and moderate myopia and the morphology and cell density of the corneal endothelium, as the p-values for all variables were greater than 0.05, which is considered insignificant.

Parameters	HEX	CV
ECD	r=0.65 p=0.003	r=-0.23 p=0.036
CV	r=-0.21 p=0.025	r=1.00
HEX	r= 1.00	r=-0.25 p=0.026

[Table/Fig-4]: Summary of correlation between corneal endothelial parameters.

DISCUSSION

Numerous studies have examined the relationship between age, ethnicity, and gender and corneal endothelial characteristics. Significant variations in corneal endothelial properties have been found among different races and ethnic groups [30-32]. Previous studies have also shown differences in corneal endothelial morphology and Endothelial Cell Density (ECD) among various populations of young healthy individuals aged 19-27 years [33-36]. For example, a study conducted on young Nigerian subjects showed an ECD of 2610±372 cells/mm^2 [37], while a similar study on Egyptian youth reported an ECD of 2648±383 cells/mm^2 [38]. Another study on Thai youth found an ECD of 2732±258 cells/mm^2 [39]. Research on Turkish adult youth reported an ECD of 2732±305 cells/mm^2 [40], and a study on Chinese young volunteers reported an ECD of 2932±363 cells/mm^2 [41].

The focus of the present study was to estimate the mean ECD for normal healthy young Saudi females aged 18 to 25 years. The results revealed a slightly higher ECD of 2827.03±229.78 cells/mm^2 compared to other countries, except China. This highlights the need to establish and maintain normative data for corneal endothelial characteristics in individuals of different racial and ethnic backgrounds, as decisions regarding endothelial function may depend on these data.

The current study also aimed to examine corneal endothelial cell characteristics in myopic eyes of adult Saudi females. Previous studies have reported on corneal endothelial cell features in myopic eyes among different age, gender, and ethnicity groups [42-44]. For example, a study on Japanese subjects found that female participants with high myopia had a significantly higher odds ratio of having abnormal values of Coefficient of Variation (CV) and Hexagonal appearance (HEX) compared to those with emmetropia [45].

It is recommended that there may be a connection between high myopia and abnormal values of CV and HEX, which can affect corneal endothelial morphology [46]. A study conducted by Chang S et al., found a correlation between longer axial length and both low ECD and steeper corneal curvature in young participants (mean age of 22.2 years) who had never worn contact lenses before [47]. Another study by Delshad S and Chun JM found that in young participants (mean age of 21.6 years) without a history of contact lens wear, mean ECD and HEX values were lower in moderate myopia compared to low myopia [48]. However, Sheng H and Bullimore MA described an association between myopia and abnormalities in both CV and HEX, without any correlation between myopia and ECD, among subjects aged 19-71 years, regardless of contact lens wear [46].

The present study revealed differences in corneal endothelial cell parameters among emmetropic, mild, and moderate myopia groups, including ECD, mean hexagonal appearance percentage of cells, and CV. However, these differences were not statistically significant. The study also found a general tendency toward lower ECD in Saudi females with increasing myopia. It is important to note that direct comparisons of the results presented in this paper with those reported by others are limited due to the use of different assessment methods and study populations with different refractive errors across various age groups [37-41].

Limitation(s)

The present study was performed on females only, and the small sample size was also considered a limiting factor. Additionally, a few females used to wear contact lenses occasionally, which is another factor that possibly affected the results.

CONCLUSION(S)

In the present cross-sectional study, the Endothelial Cell Density (ECD), Hexagonal Cell Count (HEX), and central corneal thickness were found to be higher in emmetropic eyes compared to myopic eyes. Additionally, these values decreased with the degree of myopia. However, no significant relationship was found between mild, moderate myopia, emmetropia, and corneal endothelial parameters.

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