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Assessment of Age Changes in Corneal Endothelial Cell Count among Emmetropes Using Non-Contact Specular Microscope in Suez Canal University, Ophthalmology Outpatient Clinic

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ABSTRACT

Background: The human corneal endothelial cells are non-regenerating cells that are primarily hexagonal in shape. Corneal endothelium is metabolically active and serves an essential function in the maintenance of corneal transparency. It is able to accomplish this by pumping water out of the stroma and into the aqueous humor.

Objective: This study aimed to evaluate corneal endothelial cell density and morphology among different age groups of emmetropic individuals.

Patients and Methods: This study involved eighty eyes of forty subjects classified into 5 age groups. Evaluation of corneal endothelium in emmetropic participants was performed by specular microscopy.

Results: When comparing endothelial cell density (ECD), there was a highly significant age disparity. There was a statistically significant distinction in HEX% among the age ranges surveyed, while we found an increasing coefficient of variation (CV) percentage with increasing age.

Conclusion: We discovered a statistically significant difference in ECD and morphology across the various age groups, which led us to conclude that age has a substantial impact on corneal endothelial cell density and morphology.

Keywords: Cornea, Age Changes, Endothelial cell.

INTRODUCTION

The human corneal endothelial cells are non-regenerating cells that are primarily hexagonal in shape. These cells line the back of Descemet's membrane and are aligned such that their pointed ends face the front of the eye [(1)].

Corneal endothelium is metabolically active and serves an essential function in the maintenance of corneal transparency. It is able to accomplish this by pumping water out of the stroma and into the aqueous humor, so ensuring that the stroma continues to be dehydrated at a level of water content of 70% or less [(2)].

Utilizing a specular microscope, one is able to determine the density and shape of corneal endothelial cells. It has been shown that the specular microscope is highly reliable and reproducible when subjected to the necessary calibration steps [(3)].

Non-contact the corneal endothelial cell layer can be analyzed for its morphology using a spherical microscope, which is a method that does not involve any invasive procedures. It measures both the mean cell density (MCD) and the coefficient of variation (CV) in the cell size, and it also determines whether or not the cells have a hexagonal look. An index of the corneal endothelium's functioning properly can be derived from these characteristics [(4)].

Preoperative evaluation of the corneal endothelium is crucial for predicting the likelihood of corneal problems following intra-ocular or refractive surgery, as these procedures might result in substantial morphological change to the corneal endothelium. Examining the quantity and shape of corneal endothelial cells across multiple age groups in emmetropic patients is the focus of this research.

PATIENTS AND METHODS

This prospective observational study included eighty (80) eyes of forty (40) emmetropic participants. They were recruited from the Ophthalmology Department, Faculty of Medicine, Suez Canal University. We studied endothelial corneal cell changes by ageing.

Inclusion criteria: Emmetropes of both sexes aged more than 5 years.

Exclusion criteria: Eyes with previous ocular surgeries or trauma, previous contact lens wear, corneal diseases, ocular pathology, glaucoma as well as systemic diseases that could affect the eye (e.g. collagen disease and DM) were excluded.

The eighty eyes were classified into 5 age groups: (1) aged 5-14 years, (2) aged 15-29 years, (3) 30-44, (4) 45-60, (5) more than 60 years.

A thorough ophthalmic examination was conducted, including a review of the patient's medical history, measurement of visual acuity, refraction, external eye, intraocular pressure, and slit-lamp biomicroscopy with direct and indirect inspection.

Non-contact specular microscope: (NIDEK CEM-530): To count ocular endothelial cells and examine their shape, a noncontact specular microscope was utilized. The device illuminates the cornea and then records the reflection of light from the optical contact between the corneal endothelium and the aqueous humor. The device examines the reflected picture and renders a specular photomicrograph of it. The number of endothelial cells, cell density, size variation (polymegathism), and form variation (pleomorphism)

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can all be measured when looking at the cells using a specular microscope.

During the specular microscopy procedure, the patient was advised to look at the built-in fixation target, while they were seated with their chin supported by the chin rest and their forehead lightly pressed against the headband. After making the necessary adjustments to the head position and activating the automated focusing system, the image of the pupil that was displayed on the monitor was brought into sharp focus and positioned within the targeting circle that was displayed on the monitor. The center method was used to take the measurements. Measurements of corneal thickness and specular microscopy thickness were taken by the instrument when it was properly aligned and focused.

In this investigation, we utilized a specular microscope (NIDEK CEM-530), which was equipped with an in-built pachymetry to measure the central corneal thickness (CCT) at each central, paracentral, and peripheral location. The CEM-530 is one-of-a-kind function that can take paracentral images, in addition to the standard central and peripheral specular microscopy.

The paracentral images are taken at eight places with a viewing angle of five degrees inside a field that is 0.25 millimeters by 0.55 millimeters. These photos offer increased examination of the area surrounding the corneal image as illustrated in figure (1).

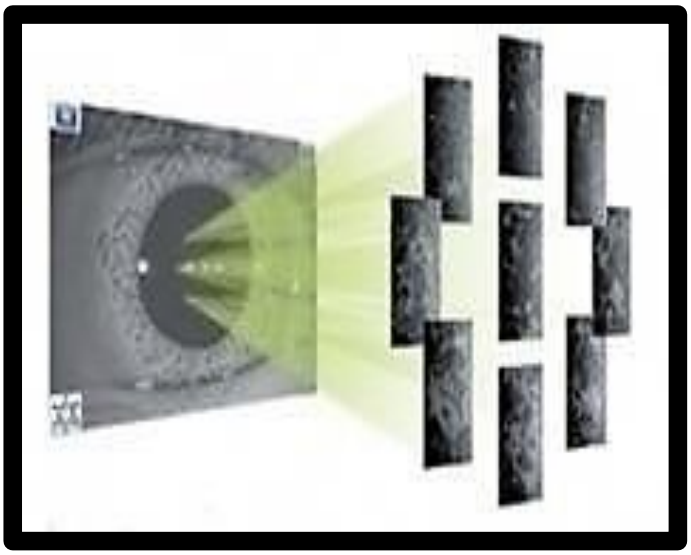

Fig. (1): The paracentral images are captured at eight points surrounding the central image.

With the diagnostic information provided by the paracentral images, the central endothelial images can be evaluated with more accuracy. CEM-530 specular microscope captures (16) images that are ranked automatically founded on quality, with the top image being highlighted in orange for examination as shown in figure (2).

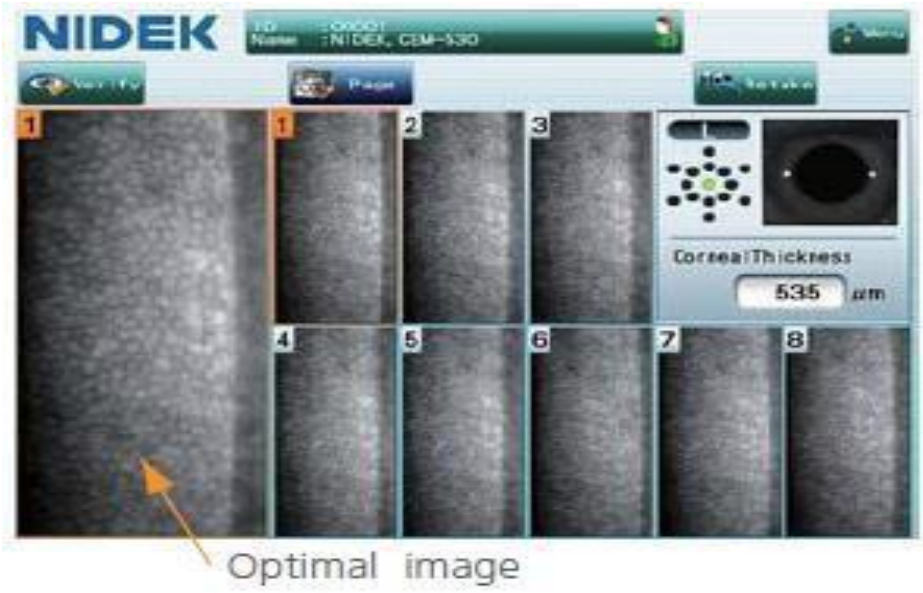

Fig. (2): CEM-530 specular microscope captures 16 images auto indication of the optimal image with orange highlight.

Once the image is selected the computer automatically evaluates, calculates and displays the mean cell density (cell/mm^2), coefficient of variation (CV%) in cell size and percentage of hexagonal cells (HEX%).

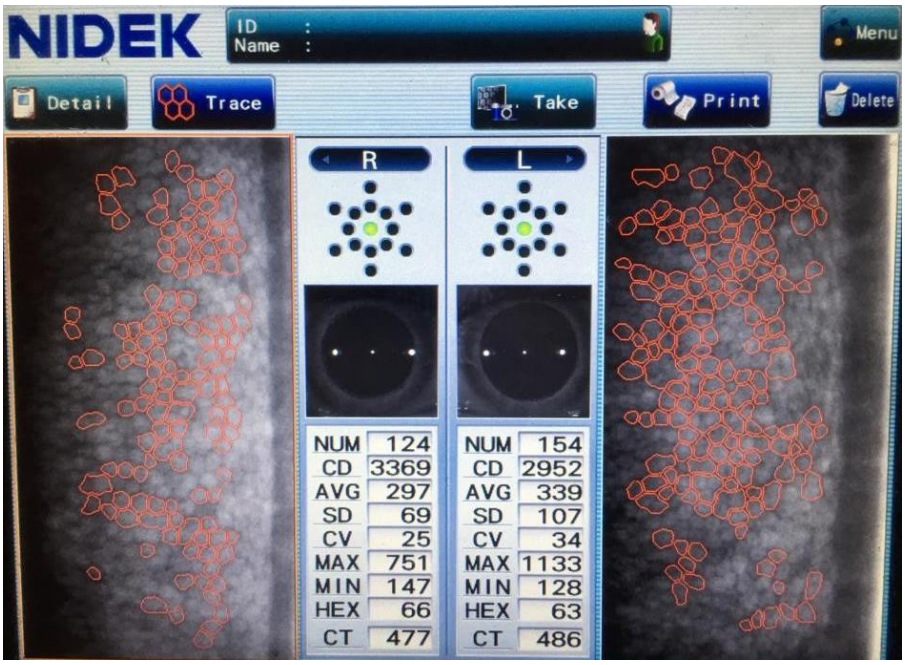

Fig. (3): A specular microscopic photo for a subject taken by NIDEK CEM -530 specular microscope.

Ethical Approval: The Institutional Review Board (IRB) approval was obtained from Suez Canal University Hospital for this study, and the patients were given all the information they required about the trial. All study subjects provided signed consent forms before taking part. The Declaration of Helsinki, a code of ethics for medical research involving humans, was followed throughout the course of this study.

Statistical analysis

SPSS v23 (SPSS Inc., Chicago, Illinois) was used for the statistical analyses. Means, standard deviations, and correlation coefficients were determined to provide descriptive statistics for quantitative variables. For parametric data, we used two-tailed Chi-square and student's t tests. For non-parametric data, we used Mann-Whitney U and Kruskall-Wallis tests. Calculating the significance level, we found that Statistical significance was indicated when the P value was ≤ 0.05, while non-significance was shown when the P value > 0.05.

RESULTS

According to Table (1), the average age of the participants in the study was 35.05 ± 0.77 years old, more than half (55%) of them were female.

Different age groups differed significantly ($P < 0.001$) in the study regarding corneal morphological parameters as shown in table (2). Correlation of ECD showed statistically negative correlation with age in groups 2, 4 and 5. Also, correlation showed statistically negative correlation of CV% in group (1) only and the other groups showed insignificant difference. In addition, HEX% showed statistically insignificant difference ($p > 0.05$) as shown in table (3).

Regarding the rate of cell loss in this study, it was 243 cell (7.6 %) between group 1 & group 2, it was 141 cell (4.7%) between group 2 and group 3, it was 151cell (5.3%) between group 3 and group 4, and it was 227 cell (8.5 %) between group 4 & group 5, which showed that group 5 had the greatest loss of endothelial cells (table 4).

Table (1): Distribution of the studied participants according to gender

Age group	Total	Males	Females
Age: Range (years) Mean ± SD	 5 – 65 35.05 ± 20.77		
Groups: No (%)	80 (100)	44 (55)	36 (45)
• Group 1 (5-14)	16 (20%)	8 (50)	8 (50)
• Group 2 (15-29)	16 (20%)	10 (62.5)	6 (37.5)
• Group 3 (30-44)	16 (20%)	10 (62.5)	6 (37.5)
• Group 4 (45-60)	16 (20%)	8 (50)	8 (50)
• Group 5 (> 60)	16 (20%)	8 (50)	8 (50)
P value*		0.876 (insignificant)	

* Chi square test (χ^2)

Table (2): Corneal morphological parameters among the different age groups

Age group (years)	**ECD**	**CV%**	**HEX%**
Group 1 (5-14)	3213.1 ± 104	24.38 ± 2.8	72.5 ± 3.98
Group 2 (15-29)	2970.2 ± 64.1	32.69 ± 1.85	73.88 ± 3.95
Group 3 (30-44)	2829.1 ± 51.4	28.19 ± 4.69	69.75 ± 5.9
Group 4 (45-60)	2678.3 ± 60.01	26.13 ± 6.79	62.19 ± 5.22
Group 5 (> 60)	2450.9 ± 163	24.56 ± 6.2	58.63 ± 3.09
Total mean	2830.1 ± 275.7	27.19 ± 5.65	67.39 ± 7.45
P value	<0.001*	<0.001*	<0.001*
P1 (1 & 2)	<0.001*	<0.001*	0.912
P2 (1 & 3)	<0.001*	0.184	0.344
P3 (1 & 4)	<0.001*	0.846	<0.001*
P4 (1 & 5)	<0.001*	1.000	<0.001*
P5 (2 & 3)	<0.001*	0.077	0.087
P6 (2 & 4)	<0.001*	0.002*	<0.001*
P7 (2 & 5)	<0.001*	<0.001*	<0.001*
P8 (3 & 4)	<0.001*	0.751	<0.001*
P 9 (3 & 5)	<0.001*	0.277	<0.001*
P 10 (4 & 5)	<0.001*	0.892	0.184

P by One Way ANOVA with post Hoc. *: statistically significant.

Table (3): Correlation between corneal morphological parameters and age among different age groups

Age group (N)	**ECD**		**CV%**		**HEX%**	
Group 1 (16)	-0.457	0.075	-0.652	**0.006***	-0.290	0.276
Group 2 (16)	-0.679	**0.004***	0.249	0.352	-0.130	0.631
Group 3 (16)	-0.398	0.127	0.366	0.164	-0.064	0.813
Group 4 (16)	-0.626	**0.009***	-0.384	0.142	-0.110	0.685
Group 5 (16)	-0.937	**<0.001***	-0.491	0.053	-0.139	0.608

P by One Way ANOVA with post Hoc. *: statistically significant.

Table (4): Endothelial cell loss per age group among the studied participants

Age group	**ECD, Mean± SD**	**Cell loss, No (%)**
Group 1 (5-14)	3213.1 ± 104	-
Group 2 (15-29)	2970.2 ± 64.1	243 (7.6)
Group 3 (30-44)	2829.1 ± 51.4	141 (4.7)
Group 4 (45-60)	2678.3 ± 60.01	151 (5.3)
Group 5 (> 60)	2450.9 ± 163	227 (8.5)

DISCUSSION

The study analyzed the endothelial cell density (ECD) of a sample of healthy emmetropic subjects in different age groups to determine how becoming older affects ECD and endothelial morphological markers. We found that there was highly statistically significant difference between different age groups regarding ECD and endothelial morphological parameters. This study results are consistent with **Jorge *et al.*** [5] who analyzed the difference in corneal endothelial cell density in healthy emmetropic subjects.

In a recent study, Jorge *et al.* [5] found that there were substantial shifts in the corneal endothelial mosaic's quantitative properties among age groups. There was a significant decline in ECD and age-related changes in cell surface counts, while correlations were less or non-significant for cell geometry counts.

In the current study the rate of cell loss was 243 cell (7.6 %) between group 1 and 2, it was 141 cell (4.7%) between group 2 and 3, it was 151cell (5.3%) between group 3 and 4, and it was 227 cell (8.5 %) between group 4 and 5 which showed that the fifth group had the greatest rate of endothelial cell loss.

This is consistent with **Rao *et al.*** [6] who studied the corneal ECD and morphology in normal Indian eyes. The rate of endothelial cell loss was reported to be 0.3% per year (roughly 75 cells per decade for an average ECD of 2500 cells/mm^2), which is in line with the findings of **Islam *et al.*** [7] who discovered a loss of 87 cells/mm^2 per decade (0.28% per year) when studying the impact of aging on the corneal morphological parameters of healthy Pakistani eyes.

We did not find any differences in ECD between women and men. However, different studies have

obtained results that differ from those presented in this study. As **Snellingen *et al.* [8]** reported that compared to males, females showed a 2.9% higher cell density (p 0.0001). Another study by **Padilla *et al.* [9]** carried out on Filipino eyes found that females had an ECD that was 7.8% higher than that of males ($p < 0.01$) indicating that there may be a greater corneal endothelium reserve in women. Cells of varied surface areas make up the human corneal endothelium. The coefficient of polymegathism describes the range of cell size [10]. It's possible that the probability of polymorphism would rise if corneal endothelial cells had to flatten to conquer the increased surface, in which case the proportion of hexagonal appearance of the cells would drop [11].

As regards CV%, we finds that differences between age groups were marginally significant (p <0.001) as the mean CV% of group 1was 24.38 ± 208, of group 2 was 32.69 ± 1.85), of group 3 was 28.19 ± 4.69, of group 4 was 26.13 ± 6.79, and of group 5 was 24.56 ± 6.22. We finds that there was a weak negative correlation between CV% and age among the studied participants (CV% slightly decrease with age). Unlike this study, the proportion of hexagonal cells declines progressively with age, as observed by **Jorge *et al.* [5]**, who used a non-contact specular microscope to analyze age-related alterations of the corneal endothelium in normal eyes. The percentage of cells with 4, 5, and 7 sides, on the other hand, increased marginally but significantly with age (increasing CV% with age). Also, unlike the present study, according to **Islam *et al.* [7],** who examined age-related changes in corneal morphological parameters of healthy Pakistani eyes and found that the average cell area or CV of cell size (increasing CV% with age) has a strong positive connection with age. After discovering a strong positive link, the researchers came to this conclusion.

Regarding the HEX (%), this study showed that the differences between the five age categories were quite significant ($p < 0.001$). There was a strong negative association between HEX% and age among the sampled population (HEX% declines with advancing years). This study is consistent with **Rao *et al.* [6]** who studied corneal ECD and morphology in normal Indian eyes. They reported that age was significantly correlated with a decline in the proportion of hexagonal cells ($p = 0.01$, correlation = -0.127). In addition, **Islam *et al.* [7],** who studied age-related alterations in corneal morphological characteristics in healthy Pakistani eyes. They found a strong negative connection between age and percentage of hexagonal cells (HEX% decreases with age). Moreover **Jorge *et al.* [5]** used a non-contact specular microscope to analyze age-related changes in corneal endothelium in normal eyes, and reported that the number of hexagonal cells declines progressively with age.

CONCLUSION

We found substantial differences in ECD and morphology among age groups, indicating that aging has a profound effect on the density and shape of endothelial cells in the cornea.

DECLARATIONS

- **Consent for publication:** All authors agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** No conflicts of interest.

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