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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

Introduction: The majority of the cells that line the back of Descemet's membrane and the inside of the anterior chamber of the human eye are hexagonal, non-regenerating cells that make up the endothelium of the cornea. These cells are found in the cornea. In order to preserve the clarity of the cornea, the metabolically active endothelium pumps water out of the stroma and into the aqueous humour, maintaining the stroma at a degree of dehydration that is 70% water.
Objective: The purpose of the study is to compare the number and shape of corneal endothelial cells in emmetropic people of varying ages. **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: Among the five age groups studied, there was a statistically significant variation in endothelial cell density (ECD). The HEX% varies significantly between the five age groups, and we found that the CV% rises with age.
Conclusion: We identified statistically significant differences in ECD and shape between age groups, suggesting that age has a major impact on corneal endothelial cells.
Keywords: Cornea, Age Changes, Corneal Endothelial Cell, Emmetropes, Non-Contact Specular Microscope

INTRODUCTION

The majority of human corneal endothelial cells are hexagonal, non-regenerating cells that line the rear of Descemet's membrane and the interior of the anterior chamber of the eye. These cells are found in the cornea [(1)]. In order to preserve the clarity of the cornea, the metabolically active endothelium pumps water out of the stroma and into the aqueous humour, maintaining the stroma at a degree of dehydration that is 70% water [(2)].

It is possible to examine the number and shape of corneal endothelial cells with a specular microscope. After proper calibration [(3)], the specular microscope has proven to be dependable and reproducible. The corneal endothelial cell layer can be analysed morphologically with a slit-lamp specular microscope without causing any damage to the eye [(4)]. Mean cell density (MCD) is calculated, as is the coefficient of variation (CV) in cell size and the cell's apparent hexagonal shape. The health of the endothelium layer of the cornea can be gauged from these measurements [(5)]. The corneal endothelium can undergo significant morphological change during intra-ocular and refractive surgeries [(6)] ;therefore, assessing the endothelium prior to these procedures is critical for predicting the likelihood of corneal problems. The goal of the study is to examine the differences in corneal ECD and morphology that occur across age groups in patients who are emmetropic.

PATIENTS AND METHODS

In this prospective, observational study, there were eighty (80) eyes of forty (40) emmetropic participants. They were recruited from the Ophthalmology Department, Faculty of Medicine, Suez Canal University. In this study, we studied endothelial corneal cell changes by ageing. This study's inclusion criteria were: emmetropes of both sexes aged more than 5 years. 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, 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.

Slit-lamp biomicroscopy with direct and indirect inspection, as well as measurements of visual acuity, refraction, external eye, and intraocular pressure, were part of the comprehensive ophthalmic examination.

A non-contact specular microscope: (NIDEK CEM-530): To determine the number of corneal endothelial cells and their morphology, a non-contact specular microscope was used. The apparatus shines light onto the cornea and records the picture reflected from the endothelium of the cornea and the aqueous humour. A specular photomicrograph is produced by analysing the reflected image. Endothelial cell number, cell density, size variation (polymegathism), and form variation (pleomorphism) can all be assessed by seeing the cells with specular microscopy. Specular microscopy required the patient to sit with their chin supported by the chin rest and their forehead lightly placed on the headband. Automatic focusing and adjusting the head position were used to bring the pupil into sharp focus within the monitor's aiming circle. The centre method was used to take the measurements. Specular microscope and corneal thickness measurements were taken when the equipment was properly focused and aligned.

In this study we used (NIDEK CEM-530) specular microscope, it is provided with a built-in pachymetry to measure central corneal thickness (CCT) at each central, paracentral and peripheral points. Paracentral imaging is a special feature of the CEM-530 that complements the standard central and peripheral specular microscopy. As can be seen in figure (1), paracentral images are recorded at eight places, 5^{o} viewing angle, inside a 0.25 mm × 0.55 mm field, allowing for improved assessment in the immediate periphery of the central image.

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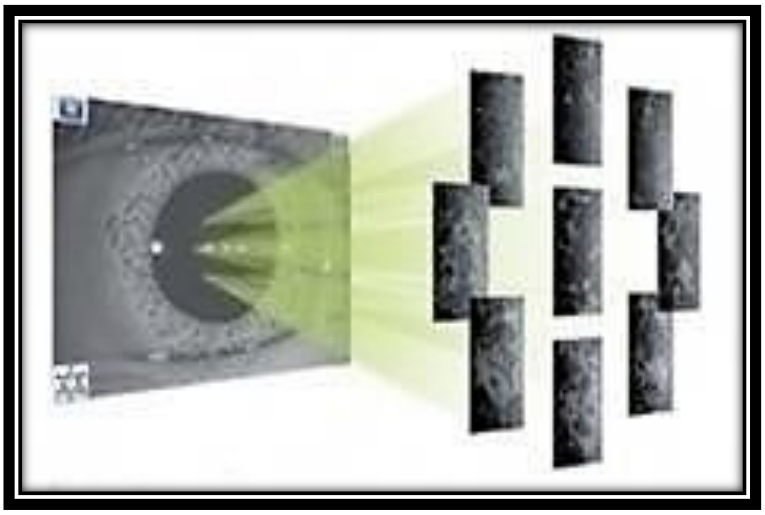

Fig. (1): Paracentral images are obtained from eight locations in close proximity to the central image.

The diagnostic information provided by the paracentral images aids in the precise evaluation of endothelial cells using the central images. As can be seen in figure (2), the CEM-530 specular microscope takes sixteen photos, automatically sorts them depending on quality, and highlights the best image for examination in orange.

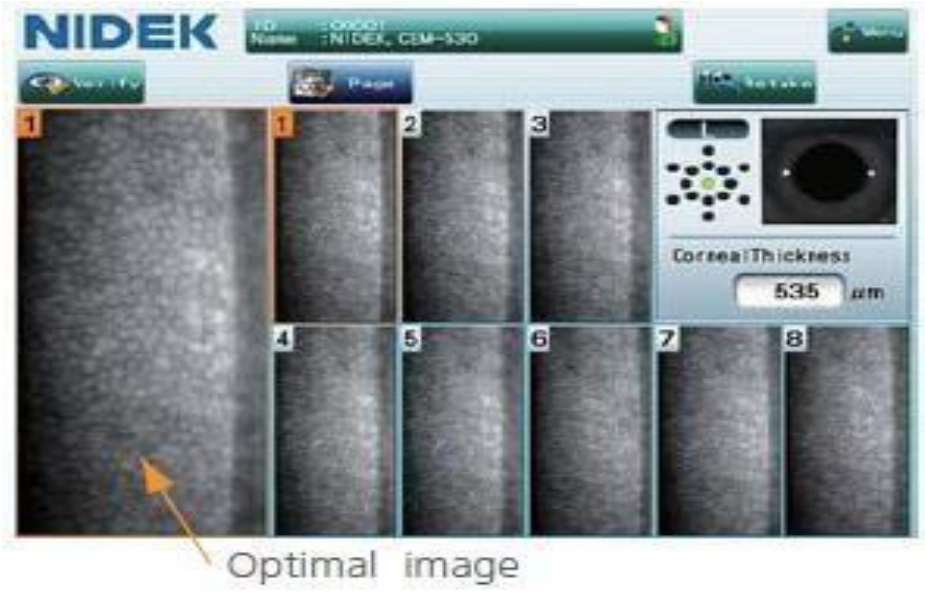

Fig. (2): CEM-530 specular microscope captures 16 images auto indication of the optimal image with orange highlight.

After selecting a picture, the software immediately evaluates it, calculates the mean cell density (cell/mm^2), the (CV%) in cell size, and the percentage of hexagonal cells (HEX%) (Figure 3).

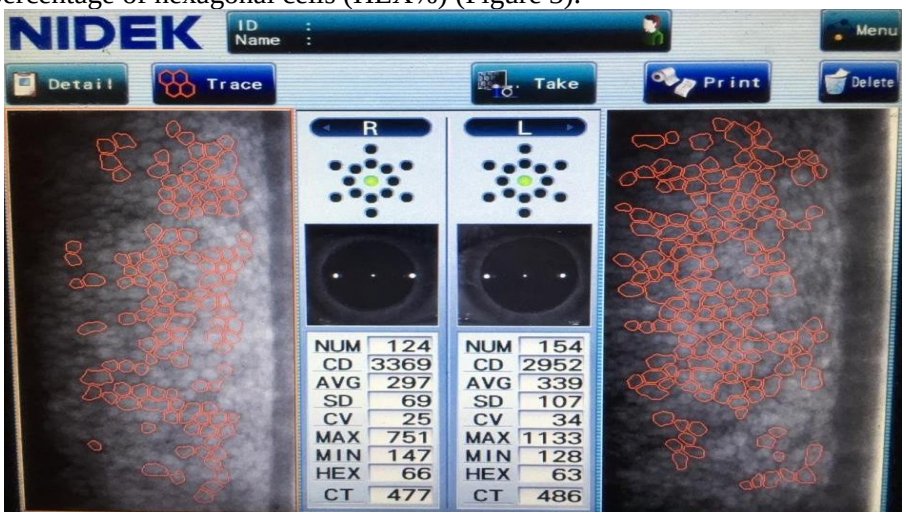

Fig. (3): An image of the subject acquired with an NIDEK CEM-530 specular microscope

Ethical consent:

The study was approved by the Institutional Review Board (IRB) at Suez Canal University Hospital. The study's quality was ensured by a research ethics committee that reviewed and approved it. Before receiving their written agreement to participate in the current investigation, all adult participants and the caregiver of child participants received a thorough and understandable explanation of the study. The trial coordinator routinely checked the quality of screening, data management, and protocol adherence.

All studies involving human volunteers were done in compliance with the ethical guidelines laid out in the World Medical Association's Declaration of Helsinki.

Statistical analysis

SPSS v23 statistical software (SPSS Inc, Chicago, Illinois) was utilized for the statistical analyses. Quantitative data were presented as means, standard deviations (SD), and range and were compared by analysis of variance test. Qualitative data were presented as frequency and percentage and were compared by chi-square test. The significance level was determined to be P< 0.05.

RESULTS

Table (1) illustrate that the mean age of the studied participants was 35.05 ± 0.77 years and more than half (55%) of them were females.

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)	

The study shows that there was high statistically significant difference among different age groups regarding corneal morphological parameters as shown in table (2).

Table (2): Corneal morphological parameters between 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 and 2)	<0.001*	<0.001*	0.912
P2 (1 and 3)	<0.001*	0.184	0.344
P3 (1 and 4)	<0.001*	0.846	<0.001*
P4 (1 and 5)	<0.001*	1.000	<0.001*
P5 (2 and 3)	<0.001*	0.077	0.087
P6 (2 and 4)	<0.001*	0.002*	<0.001*
P7 (2 and 5)	<0.001*	<0.001*	<0.001*
P8 (3 and 4)	<0.001*	0.751	<0.001*
P 9 (3 and 5)	<0.001*	0.277	<0.001*
P 10 (4 and 5)	<0.001*	0.892	0.184

*: statistically significant.

Correlation of ECD showed statistically negative correlation with age in groups (2, 4, 5), and showed statistically negative correlation of CV% in group (1) only and the other groups presented insignificant correlation, and also HEX% showed statistically insignificant correlation as presented in table (3).

Table (3): Correlation among 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

In this study the rate of cell loss was 243 cell (7.6 %) between group 1 and group 2, was 141 cell (4.7%) between group 2 and group 3, was 151 cell (5.3%) between group 3 and group 4, and was 227 cell (8.5 %) between group 4 and group 5 which shows that the

highest rate of endothelial cell loss was noted among group 5 (table 4).

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 ECD of a sample of healthy emmetropic subjects in different age groups to assess the effect of age on ECD and endothelial morphological parameters. We found that there was highly statistically significant difference between different age groups concerning ECD and endothelial morphological parameters. This study results were consistent with another study, which analyzed the difference in corneal ECD in healthy emmetropic subjects. **Jorge *et al.*** [(5)] stated that there were significant differences between age groups for all quantitative aspects of the corneal endothelium mosaic. Cell surface counts and ECD were found to be significantly correlated with age, but cell geometry count associations were less or non-significant.

In the current study the rate of cell loss was 243 cell (7.6 %) between group 1 and 2, was 141 cell (4.7%) between group 2 and 3, was 151 cell (5.3%) between group 3 and 4, and was 227 cell (8.5 %) between group 4 and 5 which shows that the highest rate of endothelial cell loss was found among group 5.

Parallel results were found by **Rao *et al.*** [(6)], who examined corneal (ECD) and morphology in healthy Indian eyes, reporting a loss of 0.3% of ECD per year (roughly 75 cells per decade for an average ECD of 2500 cells/mm2) and by **Islam *et al.*** [(7)], who examined age-related changes in the morphological characteristics of healthy Pakistani eyes, reporting a loss of 87 cells/mm per decade, 0.28% per year).

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 women had a 2.9 percent greater cell density than males ($p = 0.0001$). 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 women may have a greater reserve of healthy ocular endothelial cells than men. Cells of varied surface areas make up the human corneal endothelium. The coefficient of polymegathism [(10)] describes the spread in cell size.

In order to cover a larger area, corneal endothelial cells must become flatter. This may increase the likelihood of polymorphism and cause a decline in the percentage of cells exhibiting a hexagonal shape. [(11)].

As regard CV%, we found that there was slightly significant difference between different age groups ($p <0.001$) as the mean CV% of group 1: (24.38 ± 208), group 2 (32.69±1.85), group 3 (28.19±4.69), group 4 (26.13±6.79), and group 5 (24.56±6.22). We found that there was weak negative correlation between CV% and age among the studied participants (CV% slightly decreased with age).

In contrast to the consequences of this study, **Jorge *et al.*** [(5)], who used a non-contact specular microscope to examine changes in the corneal endothelium of healthy eyes as they aged, found that the proportion of hexagonal cells decreased as people get older. The percentage of cells with 4, 5, and 7 sides, on the other hand, increased little but statistically significantly with age (increasing CV% with age). While we found no significant age correlation with average cell area or CV of cell size (increasing CV% with age). **Islam *et al.*** [(7)], who studied age related changes in corneal morphological parameters of healthy Pakistani eyes, did find a positive link between age and average cell area or CV of cell size.

Regarding the HEX (%), this study shows that there was highly statistically significant difference among the five age groups, ($p <0.001$), we showed that there was highly statistically negative association among HEX% and age among the studied participants (HEX% decreased with age). According to **Rao *et al.*** [(6)], who investigate the density and morphology of corneal endothelial cells in healthy Indian eyes, the proportion of hexagonal cells decreases with age ($p = 0.01$, correlation = -0.127). This is consistent with **Jorge *et al.*** [(5)], who study age-related changes of the corneal endothelium in normal eyes using a non-contact specular microscope,

Likewise with **Islam *et al.*** [(7)], who studied age-related changes in the corneal morphological parameters of healthy Pakistani eyes. They found that a strong negative association was identified between age and percentage of hexagonal cells (HEX% decreased with age).

CONCLUSION

We found that there was a statistically significant difference in ECD and morphology among the various age groups, indicating that age has a substantial influence on corneal ECD and morphology.

DECLARATIONS

- **Permission for publication: I attest that all authors consented to the submission of the work.**
- **Availability of information and resources: Available**
- **Competing interests: Absent**
- **Funding: No funds was available**
- **Conflicts of interest: there are no conflicts.**

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