ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Assessment of Corneal Endothelial Cell Status in Aging Eye

Yaşlanmanın Kornea Endotel Hücre Özellikleri Üzerine Etkisinin Değerlendirilmesi

Zeynep KAYAARASI ÖZTÜRKER[a],
Serpil AKAR[a],
Serhat İMAMOĞLU[b],
Aslı ÖZBEK FIGUEROA[c],
Serpil YAZGAN[d],
Ömer Faruk YILMAZ[c]

[a]Department of Eye Diseases, Başkent University Faculty of Medicine,
[b]Clinic of Eye Diseases, Haydarpaşa Numune Training and Research Hospital,
[c]Department of Eye Diseases, Yeni Yüzyıl University Faculty of Medicine, Gaziosmanpaşa Hospital, İstanbul, TURKEY
[d]Department of Eye Diseases, Zonguldak Bülent Ecevit University, Faculty of Medicine, Zonguldak, TURKEY

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Correspondence:
Zeynep KAYAARASI ÖZTÜRKER
Başkent University Faculty of Medicine, Department of Eye Diseases, İstanbul, TURKEY/TÜRKİYE
zeynepkayaa@yahoo.com

ABSTRACT Objective: To describe the corneal endothelial cell density and morphology in normal adult eyes, and determine the influence of aging on these parameters. **Material and Methods:** In this cross-sectional study, specular microscopy was performed in 547 eyes of 547 healthy volunteers aged 20 to 85 years. Of these, 279 were female, and 268 were male. The study parameters included endothelial cell density (ECD), cell area (CA), the coefficient of variation (CoV) and percentage of hexagonal cells (Hex%). The influence of age and gender on these parameters were evaluated. **Results:** The mean ECD and mean CA in the study population were 2607±323 cells/mm^2 and 389.6±51.2 μm^2, respectively. In the correlation analysis, there was a decrease in mean ECD (r= -0.305, p<0.001), and an increase in mean CA (r=0.295, p<0.001) with increasing age. The mean CoV in cell size and the mean Hex% were 35.7±6.06% and 56.8±9.1%, respectively. There was a weak correlation between increasing age and CoV in cell size (r=0.075, p=0.021), and the percentage of hexagonal was not correlated with age (p=0.088). There were no statistically significant differences in mean ECD, mean CoV in cell size, or hexagonality between genders (all p values>0.05). **Conclusion:** Our data for the corneal endothelial structure in Turkish population showed that age but not gender, has a negative impact on endothelial cell density. Age was not a determinant in the pleomorphic changes of the endothelium.

Keywords: Corneal endothelium; corneal endothelial cell loss; specular microscopy

ÖZET Amaç: Sağlıklı ve yetişkin gözlerde kornea endotel hücre yoğunluğunu ve morfolojisini tanımlamak ve yaşlanmanın bu parametreler üzerindeki etkisini incelemek. **Gereç ve Yöntemler:** Bu kesitsel çalışmada, 20-85 yaş arasındaki 547 sağlıklı olgunun 547 gözü çalışmaya dahil edildi. Olguların 279'u kadın, 268'i erkek idi. Speküler mikroskopi ile santral korneada yapılan analizlerde endotel hücre yoğunluğu (EHY), endotel hücre alanı (HA), endotel hücre varyasyon katsayısı (VK) ve altıgen hücrelerin yüzdesi (%Hex) değerlendirildi. Yaş ve cinsiyetin bu parametreler üzerindeki etkisi araştırıldı. **Bulgular:** Çalışma grubundaki ortalama EHY ve ortalama endotel HA, sırasıyla 2607±323 hücre/mm^2 ve 389,6±51,2 μm^2 idi. Korelasyon analizinde yaşın artması ile ortalama EHY'da (r=-0,305, p<0,001) ve ortalama endotel HA'nda (r=0,295, p<0,001) azalma tespit edildi. Endotel hücre varyasyon katsayısı ve altıgen hücre yüzdesi sırasıyla %35,7±6,06 ve %56,8±9,1 idi. Yaşın ilerlemesi ile endotel hücre VK arasında zayıf korelasyon bulundu (r=0,075, p=0,021). Altıgen hücrelerin yüzdesi (%Hex) yaş ile korele bulunmadı (p=0,088). Her iki cinsiyet arasında ortalama EHY, endotel hücre varyasyon katsayısı ve altıgen hücre yüzdesi açısından anlamlı fark bulunmadı (tüm parametreler için p>0,05). **Sonuç:** Türk popülasyonundaki kornea endotel hücre sayısı, yaşlanma ile olumsuz yönde etkilenmekle birlikte, pleomorfik değişikliklerin yaşla ilgisi bulunmamıştır. Her iki cins arasında ortalama endotel hücre yoğunluğu ve hücre morfolojisi açısından tüm yaş gruplarında bir fark saptanmamıştır.

Anahtar Kelimeler: Kornea endoteli; kornea endotel hücre kaybı; speküler mikroskopi

The corneal endothelium plays an important role in preserving the homeostasis and transparency of the cornea.[1,2] Prior to the 1970s, the study of the corneal endothelium was limited to biomicroscopic evaluation for guttata, fold and keratic precipitates.[3] The development of the clinical specular microscope has permitted a detailed examination of the human corneal endothelium in vivo.[4] Quantitative analysis of corneal endothelium provides information about the functional status by evaluating endothelial cell count, polymegathism (cell size variability) and pleomorphism (cell shape variation).[5]

Endothelial cells in the cornea are constantly diminished throughout life, but normal thickness and transparency are maintained. The reasons that trigger this process are not well understood, but metabolic changes in the medium, mechanical stress, endotoxins, the loss of survival factors, and nutrient deprivation may be involved.[6] Because the endothelial reserve is important in anterior segment surgery, age-related changes in the cornea are useful in assessing the endothelial reserve of an individual. Normative data regarding endothelial cell density (ECD) and morphology are thus important in decisions regarding corneal health and function. Studies supporting this idea in the literature reveal differences in Indian, American, Iranian, Filipino, Pakistani, Chinese and Japanese populations.[3,7-11] Data regarding corneal morphological parameters in Turkish population are limited.

In this comprehensive study, we evaluated the three key aspects of corneal endothelial morphology: ECD, polymegathism, and percentage of hexagonal cells in normal Turkish adult eyes and the effect of age on these parameters.

MATERIAL AND METHODS

Approval was obtained from the Ethics Committee and the Institutional Review Board at the Prof. Dr. N. Reşat Belger Beyoglu Training and Research Eye Hospital, Istanbul. The study and data collection confirmed to all local laws and complied with the principles of the Declaration of Helsinki.

A total of 547 volunteers between 20 to 80 years of age participated in the study for the examination. All volunteers were randomly selected from the center's staff and outpatients. The ethnic mix of the subjects was not different from that of the general Turkish population. Informed consent was obtained from each participant before being included in the study. Exclusion criteria included a history of ocular trauma or intraocular surgery, corneal opacity, endothelial dystrophy, contact lens wear, high intraocular pressure, uveitis, chronic ocular disease, and diabetes mellitus. Specular microscopic images showing any gross irregularities, blebs or guttae were also excluded from the analysis.

After routine ophthalmic evaluation, specular microscopic images of the central corneal endothelium of one eye of each subject were taken using the non-contact specular microscope with an autofocus device (KONAN Cellcheck CC-7000; KONAN Medical, Inc., Hyogo, Japan). A single examiner (SA) performed all measurements. After a clear image of the central endothelium captured on the screen, three images from central cornea were taken and a minimum of 100 contiguous cells was marked with center method by using the software available in the system. To eliminate variability due to image quality, images with easily identifiable cell edges were analyzed.

The computer performed an automated analysis of the cell parameters including ECD, mean cell area (CA) (μm^2), coefficient of variations (CoV) of cell size, and percentage of hexagonal cells. Cell density was recorded as the number of cells per square millimeter (cells/mm^2). The mean CA and CoV of cell size (standard deviation divided by mean CA) were used as an index of the extent of variation in CA (polymegathism). The percentage of hexagonal cells in the area was used as an index of variation in cell shape (pleomorphism).[3,6]

Statistical analysis of endothelial cell characteristics was performed using data from one eye of each subject. SPSS for Windows 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software were used for the analysis. The mean values,

standard deviations of numeric variables and correlation coefficients (r) were calculated. Endothelial cell parameters in the age groups and genders were compared. Analysis of variance (ANOVA) and linear regression analysis were used to determine the changes in endothelial cell characteristics with age, and the paired and unpaired t-test was used to compare cell densities in men and women. P values less than 0.05 was considered statistically significant.

RESULTS

The study population consisted of 547 healthy Turkish volunteers. The mean age was 45.09±14.6 years (20-80 years old), of whom 279 were female, and 268 were male. The mean ECD of the study population was 2607±323 cells/mm^2 (range 1587-3623 cells/mm^2). The mean CA was 389.6±51.2 μm^2 (range 276-630 μm^2). The mean CoV in cell size was 35.7±6.06% (range 21-65%), and the mean hexagonality was 56.8±9.1% (range 29-83%).

The endothelial cell characteristics of the study population in different age groups are listed in Table 1. There was a statistically significant difference between the 20-30 year age group and subsequent age groups in terms of mean ECD and mean CA (for each comparison, p<0.001). The mean ECD in the 20-30 age group was higher than in the other decades of age. There was a statistically significant difference between the 31-40 years age group and subsequent age groups in terms of mean ECD and mean CA (for each comparison p<0.05). No significant difference in CoV in cell size or hexagonality was noted among the different age groups. There were no statistically significant differences in mean ECD, mean CA, CoV in cell size, or hexagonality between genders (all p values > 0.05) (Table 2).

The regression equation that was obtained using regression analysis was ECD=2925,6-7,3 age + 8,9 sex. According to this model, a unit increase in the age caused an average of 7,3 unit decrease in ECD. Within each decade of age, a higher cell loss rate was noted in the 31-40 and 41-50 year age groups (Table 3). Correlation analysis showed that, with increasing age, there was a general decrease in mean ECD (r= -0.305, p<0.001) and conversely, an increase in mean CA (r=0.295, p<0.001). However, the respective overall correlation coefficients for all ages were quite low (Figure 1A, B). The results of correlation analysis for polymegathism showed that there was a weak correlation between increasing age and CoV in cell size (r=0.075, p=0.021) (Figure 2A). There was no correlation between age and percentage of hexagonal cells (p=0.088) (Figure 2B).

DISCUSSION

Our study found that there is a consistent decrease in the corneal endothelial cell density with aging, particularly accelerating in the fourth and fifth decades of life. While there was a gradual increase in the CA, polymegathism and pleomorphism remained fairly constant across all age groups.

TABLE 1: Endothelial cell characteristics of the study population in different age groups.

Age group (years)	Age mean (SD)	No of eyes (n)	Cell density (cells/mm²) mean (SD)	Cell area (μm²) mean (SD)	CoV in cell size mean (SD)	Hexagonality (%) mean (SD)
20-30	25 (3)	119	2773 (271)	364 (38)	35 (9)	58 (9)
31-40	35 (3)	107	2660 (323)	381 (47)	36 (5)	58 (8)
41-50	46(2)	104	2560 (311)	397 (53)	36 (6)	57 (8)
51-60	55 (3)	113	2506 (326)	406 (54)	37 (6)	56 (8)
61-70	65 (2)	77	2502 (332)	408 (58)	38 (5)	55 (8)
>71	76 (4)	27	2499 (249)	411 (49)	39 (9)	55(10)
Total	45(15)	547	2607 (323)	390 (51)	36 (6)	57 (9)

SD: standart deviation; CoV: coefficient of variation.

TABLE 2: Endothelial cell characteristics of the study population in relation to gender.

Age groups (years)	Female mean (SD)	Male mean (SD)	P value
20-30			
Cell density (cells/mm²)	2772(292)	2775(243)	0.940
Cell area (µm²)	365(43)	363(31)	0.749
CoV in cell size (%)	36(11)	35(5)	0.653
Hexagonality (%)	57(9)	59(8)	0.142
31-40			
Cell density (cells/mm²)	2696(295)	2620(348)	0.224
Cell area (µm²)	375 (42)	388 (51)	0.160
CoV in cell size (%)	37 (6)	35 (4)	0.066
Hexagonality (%)	56 (8)	59 (8)	0.057
41-50			
Cell density (cells/mm²)	2550 (315)	2571 (310)	0.733
Cell area (µm²)	399 (55)	395 (50)	0.692
CoV in cell size (%)	36 (6)	36 (6)	0.903
Hexagonality (%	56 (8)	58 (9)	0.345
51-60			
Cell density (cells/mm²)	2479 (363)	2530 (289)	0.409
Cell area (µm²)	412 (61)	400 (47)	0.260
CoV in cell size (%)	37 (6)	36 (7)	0.805
Hexagonality (%)	57(9)	55 (8)	0.383
61-70			
Cell density (cells/mm²)	2476 (352)	2547(305)	0.354
Cell area (µm²)	413 (64)	398 (49)	0.268
CoV in cell size (%)	37 (6)	35 (5)	0.209
Hexagonality (%)	56 (8)	56 (9)	0.905
>71			
Cell density (cells/mm²)	2477 (284)	2509(250)	0.836
Cell area (µm²)	407 (44)	412 (53)	0.875
CoV in cell size (%)	32 (9)	37 (10)	0.364
Hexagonality (%)	60 (9)	56 (8)	0.670
Total			
Cell density (cells/mm²)	2607 (341)	2607(309)	0.982
Cell area (µm²)	391 (56)	389 (48)	0.760
CoV in cell size (%)	36 (9)	36 (6)	0.306
Hexagonality (%)	56 (9)	57 (8)	0.165

SD: standart deviation; CoV: coefficient of variation.

Age, gender, and ethnicity related changes in corneal endothelial cell density and structure have been previously reported.[3,5,7-11] Different features were determined in corneal endothelium among different races.[3,7-9] In our study, the mean endothelial cell density, cell area, the percentage of pleomorphism, and hexagonality was within the range described for normal corneas in the adult population.[3,7,9,11-14] We found that one unit increase in age caused an average of 7,3 unit decrease in cell

TABLE 3: Endothelial cell loss rate in each decade of age of the study population.

Age groups (years)	Cell density (cells/mm²) mean (SD)	Cell loss rate (%)
20-30	2773 (271)	-
31-40	2660 (323)	4.1
41-50	2560 (311)	3.8
51-60	2506 (326)	2.1
61-70	2502 (332)	0.2
>71	2499 (249)	0.1

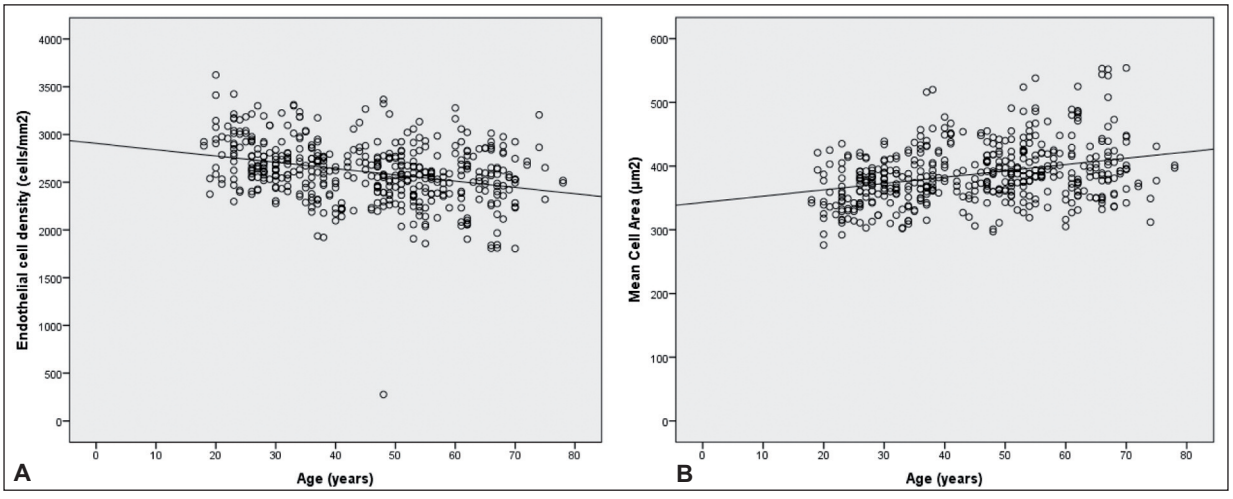

FIGURE 1: Correlation analysis showed that, with increasing age, there was a general decrease in mean endothelial cell density (r= -0.305, p<0.001) **(A)** and an increase in mean cell area (r=0.295, p<0.001) **(B)**.

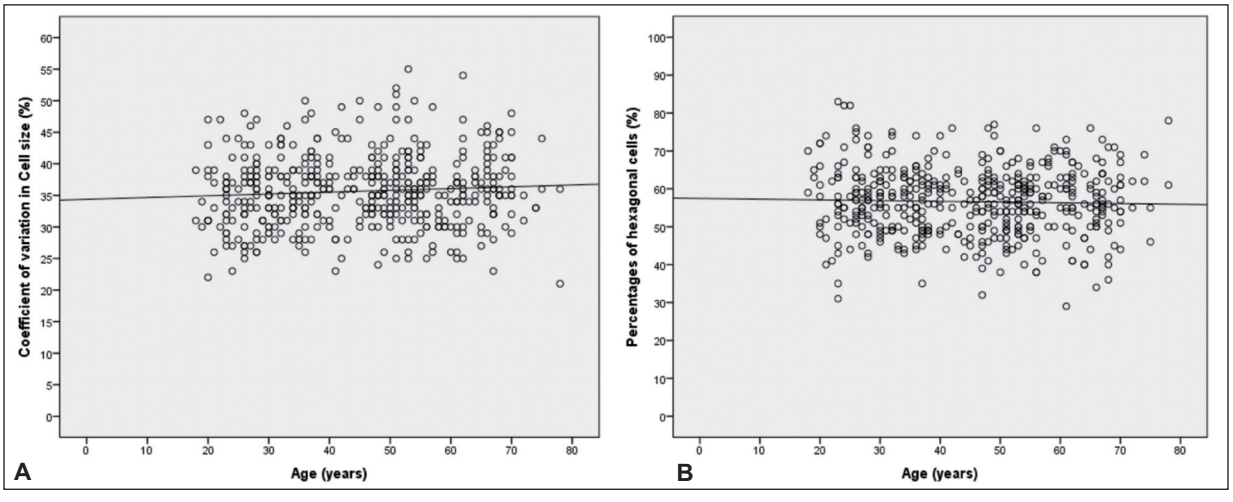

FIGURE 2: The correlation analysis for polymegathism showed that there was a weak correlation between increasing age and CoV in cell size (r=0.075, p=0.021) **(A)** There was no correlation between age and percentage of hexagonal cells (p=0.088) **(B)**.

density. The decrease in mean cell density was more pronounced after the 4th decade, and the mean cell area was found to be higher starting from the fifth decade of age. These results are consistent with the studies suggesting that as endothelial cell density decreases, cells need to enlarge to fill the space for the integrity of the corneal endothelium.[15] To the best of our knowledge, data of two studies are available for the Turkish population.[16,17] In a study by Ceyhun et al., endothelial cells were manually marked by the examiner for analysis, and they found decreased cell density and hexagonality and increased cell area and pleomorphism especially in the third and fifth decades of age.[16] On the contrary, Duman et al. did not find any correlation between pleomorphism and age in their study in which automatic cell count strategy was applied.[17] In these two studies, different quantitative techniques were used and both eyes of each subject were included in the analysis which might have influenced the independence of observations.

In a study of Padilla et al., there was a gradual decrease in the endothelial cell density and an increase in cell area until the sixth decade of age.[3] Goktas et al. investigated the corneal endothelial characteristics of cataract patients, and they re-

ported that there was a statistically significant decrease in endothelial cell density and an increase in cell area with age.[18] The cause of this cell loss is unclear but may be associated with the increased metabolic breakdown of endothelial cells with advancing age.

There was no significant change in cellular pleomorphism or hexagonality among the different age groups in our study. Some authors have reported that with aging, there is a decrease in hexagonality and an increase in the cell pleomorphism.[4,7,9,13,16,19,20] However, pleomorphism and hexagonality were not found to depend on age in other studies.[17,18,21] Different results suggest that there may be regional differences in endothelial cell density distributions across the cornea, and different thickness or curvature values may affect the analysis of morphological characteristics of the endothelial cells. In addition, for the comparisons of corneal endothelial parameters with other ethnic populations, it is important to be aware of the device and technique used to compare endothelial cell parameters of the subjects. Several studies concluded that specular microscope can be reliably used to have useful information on the cell area and cell density, but it should be used cautiously for estimates of the pleomorphism.[22-25] In order to reliably estimate clinical parameters, it is suggested that at least 100 cells must be evaluated in each image and the morphometric parameters can be estimated more reliably by the fully automatic method.[26,27] Our findings are consistent with the studies showing that age is not a determinant in the polymegathism and pleomorphism of the endothelium.[18,21]

Our data revealed no statistically significant differences between the endothelial cell characteristics of women and men. Some authors have reported differences between genders, but others have found no significant difference.[3,5,7-9,13,19,28] Reports about the relationship between gender and endothelial cell density are under debate.

Changes in corneal endothelial morphology are the first signs of endothelial stress. However, the three parameters of corneal endothelial morphology; cell density, pleomorphism and hexagonality do not react in the same way.[29] Corneal endothelial cell loss is the most important change that develops with aging. The characteristics of corneal endothelium determine the postoperative success rate after several anterior segment surgeries, and a lower cell density is a harbinger of a possible corneal dysfunction.[21,30] Higher donor age is shown to be associated with lower graft survival after penetrating keratoplasty.[31] Moreover, age should be considered as an important factor in research designs in order to obtain a reliable result in comparative studies. A normative database of corneal characteristics provides a foresight for endothelial function in a population that would aid the clinical investigations.

In conclusion, our results from a large series constituted a normative database for endothelial cell parameters in normal adult Turkish eyes. These parameters provide an index of the functional capacity of the endothelium. The representative values of endothelial cell density in aging eyes may be useful in determining the risks of anterior segment surgeries and also in the treatment and follow-up of corneal diseases.

Ethics Committee Approval

Approval was obtained from the Ethics Committee and the Institutional Review Board at the Prof. Dr. N. Reşat Belger Beyoğlu Education and Research Eye Hospital, İstanbul.

Informed Consent

Informed consent was obtained from each participant before being included in the study.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Author Contributors

Opinion/Concept: *Zeynep Kayaarası Öztürker, Serpil Akar;* ***Design:*** *Zeynep Kayaarası Öztürker, Serpil Akar;* ***Supervision/ Consultancy:*** *Serpil Akar, Ömer Faruk Yılmaz;* ***Data Collection and/or Processing:*** *Serpil Yazgan, Serhat İmamoğlu, Aslı Özbek Figueroa;* ***Analysis and/or Comment:*** *Zeynep Kayaarası Öztürker, Serpil Akar;* ***Resource Scanning:*** *Zeynep Kayaarası Öztürker, Serpil Akar;* ***Combined Writing:*** *Zeynep Kayaarası Öztürker, Serpil Akar;* ***Critical Investigation:*** *Zeynep Kayaarası Öztürker;* ***Providing Resources and Funds:*** *Ömer Faruk Yılmaz;* ***Materials:*** *Serpil Akar.*

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