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A Descriptive Study on Age-Related Changes in Corneal Endothelial Cell Density.

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Abstract

Background: The corneal endothelium plays a vital role in maintaining corneal transparency by regulating stromal hydration. Human corneal endothelial cells have minimal regenerative capacity, resulting in a gradual decline in ECD (Endothelial Cell Density) with advancing age. Assessment of ECD is important before intraocular surgeries and corneal transplantation. This study was undertaken to evaluate age-related changes in corneal endothelial cell density and to determine the influence of gender and laterality on ECD. ***Methods:*** A descriptive study was conducted in the Ophthalmology Outpatient Department of Government Medical College, Kottayam, from March 2012 to March 2013. A total of 120 healthy volunteers were enrolled after informed consent. Subjects with previous intraocular surgery, ocular disease, or systemic disease were excluded. Corneal endothelial cell density was measured in both eyes using a non-contact specular microscope (Topcon SP3000P). Statistical analysis was performed using SPSS version 16.0. Independent t-test, paired t-test, ANOVA, Pearson correlation, and linear regression analyses were used, with $p<0.05$ considered statistically significant. ***Results:*** The mean age of participants was 45.25 years; 58 (48%) were males and 62 (52%) were females. Corneal endothelial cell density showed a significant decline with increasing age in both eyes ($p<0.001$). A strong negative correlation was observed between age and ECD (right eye: $r = -0.849$; left eye: $r = -0.825$). The annual endothelial cell loss was 13.70 cells/year (0.57%) in the right eye and 13.97 cells/year (0.58%) in the left eye. No statistically significant differences were found between males and females or between the right and left eyes. ***Conclusion:*** Corneal endothelial cell density decreases significantly with advancing age, while gender and eye laterality have no significant influence. The endothelial cell density values observed were comparable with reported normative Indian data. Routine endothelial assessment using specular microscopy is valuable in the preoperative evaluation of patients undergoing intraocular procedures.

Keywords: Corneal Endothelial Cell Density, Ageing, Specular Microscopy, Corneal Endothelium, Endothelial Cell Loss.

INTRODUCTION

Corneal blindness is a major cause of visual impairment in India, with millions of individuals affected and a substantial number requiring corneal transplantation. However, the shortage of donor corneas, largely due to the limited regenerative capacity of human corneal endothelial cells, remains a major challenge in restoring vision.

The cornea is the transparent anterior part of the eye and consists of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. The corneal endothelium is a single layer of predominantly hexagonal cells that maintains corneal transparency by actively pumping excess fluid from the stroma, thereby preserving its state of relative dehydration. Since human corneal endothelial cells have minimal proliferative capacity, endothelial cell density (ECD) decreases progressively with age and following ocular trauma or surgery. In healthy infants, ECD ranges from 3500–4000 cells/mm², whereas adults typically have 2400–3200 cells/mm², with further decline occurring in older individuals. Reduced endothelial reserve predisposes elderly patients to corneal decompensation following intraocular surgery.[1,2]

Damage to the corneal endothelium caused by disease or trauma results in stromal edema and loss of corneal transparency. Endothelial wound healing occurs primarily through cell enlargement and migration rather than cell division. Therefore, assessment of endothelial health is of great clinical importance. Specular microscopy provides a non-invasive method for evaluating endothelial cell density and morphology before the onset of clinical decompensation, making it valuable in the preoperative assessment of patients undergoing cataract surgery, keratoplasty, vitrectomy, refractive procedures, and donor corneal evaluation.[3]

Several ocular and systemic conditions, including glaucoma, diabetes mellitus, previous intraocular surgeries, and prolonged contact lens wear, have been shown to alter endothelial cell density and morphology.[4] Although numerous studies have demonstrated an age-related decline in ECD, the magnitude of this decline varies among different populations, and the minimum endothelial cell density required to maintain corneal transparency remains uncertain.[5] Therefore, establishing age-related normative data for corneal endothelial cell density is essential for clinical practice and future research.

AIMS AND OBJECTIVES

The present study was undertaken to evaluate the age-related changes in corneal endothelial cell density in a healthy population using non-contact specular microscopy. It also aimed to assess whether there is any significant difference in corneal endothelial cell density between males and females and to determine the presence of intra-individual variations in endothelial cell density between the right and left eyes.

MATERIALS AND METHODS

Study Design

This descriptive study was conducted in the Ophthalmology Outpatient Department of Government Medical College, Kottayam, over a period of one year from March 2012 to March 2013. The study was approved by the Scientific Review Committee and the Institutional Ethics Committee.

A total of 120 healthy volunteers, selected from the bystanders of patients attending the ophthalmology outpatient department, were enrolled after obtaining written informed consent. Demographic details and relevant clinical history were recorded using a structured proforma. Following a routine ophthalmological examination, corneal endothelial cell density was assessed in both eyes using a non-contact specular microscope (SP3000P, Topcon Corporation).

Inclusion and Exclusion Criteria

The study included healthy individuals of different age groups attending the Ophthalmology Outpatient Department who were willing to participate and provided informed consent. Subjects were excluded if they were unwilling to participate, had a previous history of intraocular surgery, had any ocular disease, or had systemic diseases that could influence corneal endothelial cell density.

Data Collection Procedure

All participants underwent corneal endothelial evaluation using a non-contact specular microscope (SP3000P, Topcon Corporation). Each subject was seated comfortably with the chin placed on the chin rest and the forehead against the forehead band, and was instructed to fixate on the target light. The working distance was maintained at 25 mm, and the central cornea was aligned using the joystick.

The instrument's automatic tracking, focusing, and image capture system obtained an image of the corneal endothelium. If the captured image was not of adequate quality, the participant was asked to blink, and the examination was repeated. The captured image was automatically analyzed by the software to determine the corneal endothelial cell density. Measurements were obtained for both eyes, and only corneal endothelial cell density was recorded for analysis. To minimize observer-related variability, all measurements were performed by the same examiner using the standardized automated procedure.

Statistical Analysis

The collected data were entered into Microsoft Excel 2003 and analyzed using SPSS software version 16.0. The study population comprised 120 subjects, including 58 (48%) males and 62 (52%) females, who were categorized into four age groups for analysis. Mean corneal endothelial cell density was calculated for both eyes across different age groups and between genders.

Descriptive statistics were expressed as mean and standard deviation. The independent t-test was used to compare the mean corneal endothelial cell density between two groups, including comparisons between males and females. One-way Analysis of Variance (ANOVA) was used to compare mean endothelial cell density among the four age groups.

The paired t-test was employed to compare endothelial cell density between the right and left eyes of the same individual. Pearson's correlation coefficient (r) was used to assess the relationship between age and corneal endothelial cell density, while linear regression analysis was performed to estimate the rate of endothelial cell loss with advancing age. A p value of less than 0.05 was considered statistically significant.

RESULTS

Table 1. Demographic Characteristics of the Study Population

Variable	Category	Number (n=120)	Percentage (%)
Sex	Male	58	48.3
	Female	62	51.7
Age Group (years)	<25	33	27.5
	25–45	28	23.3
	46–65	31	25.8
	>65	28	23.3

Table 1 illustrates the demographic distribution of the study population. A total of 120 subjects were included, with females (51.7%) slightly outnumbering males (48.3%). The highest proportion of participants belonged to the age group <25 years (27.5%), while the remaining age groups were almost equally represented.

Table 2. Distribution of Subjects According to Corneal Endothelial Cell Density

Variable	<2000 cells/mm²	2000–3000 cells/mm²	>3000 cells/mm²
Male	5	51	2
Female	7	54	1
<25 years	0	30	3
25–45 years	0	28	0
46–65 years	2	29	0
>65 years	10	18	0

Table 2 shows the distribution of corneal endothelial cell density according to sex and age. The majority of subjects in both sexes and across all age groups had endothelial cell densities between 2000 and 3000 cells/mm². Cell counts >3000 cells/mm² were observed only in subjects younger than 25 years, whereas counts <2000 cells/mm² were predominantly seen in older age groups, especially those above 65 years.

Table 3. Mean Corneal Endothelial Cell Density in Different Age Groups

Age Group	n	Right Eye Mean±SD	Left Eye Mean±SD
<25 years	33	2761.79 ±194.41	2775.21 ±224.76
25–45 years	28	2506.11 ±167.05	2505.18 ±228.50
46–65 years	31	2235.52 ±158.33	2266.84 ±222.64
>65 years	28	1986.54 ±233.13	1986.75 ±200.18
ANOVA			
Eye		**F value**	**p value**
Right		94.615	<0.001
Left		71.080	<0.001

Table 3 demonstrates a progressive decline in mean corneal endothelial cell density with advancing age in both eyes. ANOVA revealed a highly significant difference among the four age groups (p<0.001), indicating a strong association between increasing age and decreasing endothelial cell density.

Table 4. Pairwise Comparison of Mean Corneal Endothelial Cell Density between Age Groups

Comparison	Right Eye t value	Left Eye t value	p value
<25 vs 25–45	5.456	4.640	<0.001
<25 vs 46–65	11.829	9.084	<0.001
<25 vs >65	14.165	14.349	<0.001
25–45 vs 46–65	6.386	4.055	<0.001
25–45 vs >65	9.586	9.030	<0.001
46–65 vs >65	4.840	5.060	<0.001

Table 4 compares the endothelial cell densities between individual age groups. All pairwise comparisons showed statistically significant differences (p<0.001), confirming a continuous reduction in endothelial cell density with increasing age in both eyes.

Table 5. Comparison of Corneal Endothelial Cell Density between Males and Females

Eye	Sex	n	Mean±SD	t value	p value
Right	Male	52	2406.45 ±363.02	0.643	0.522
	Female	68	2365.48 ±335.22		
Left	Male	52	2445.52 ±384.89	1.416	0.159
	Female	68	2351.42 ±342.89		

Table 5 illustrates the comparison of endothelial cell density between males and females. Although males demonstrated slightly higher mean endothelial cell counts in both eyes, the differences were not statistically significant ($p>0.05$).

Table 6. Comparison of Corneal Endothelial Cell Density between Right and Left Eyes

Eye	N	Mean±SD	Minimum	Maximum	t value	p value
Right	120	2385.28 ±348.06	1403	3343	-0.968	0.338
Left	120	2396.90 ±365.32	1429	3279		

Table 6 shows the comparison of mean endothelial cell density between the right and left eyes. Although the left eye exhibited a slightly higher mean cell density, the difference was not statistically significant ($p=0.338$).

Table 7. Correlation Between Age and Corneal Endothelial Cell Density

Eye	Pearson Correlation (r)	p value
Right Eye	-0.849	<0.001
Left Eye	-0.825	<0.001

Table 7 demonstrates a strong negative correlation between age and corneal endothelial cell density in both eyes. As age increased, endothelial cell density decreased significantly ($p<0.001$).

Table 8. Linear Regression Analysis Showing Annual Endothelial Cell Loss

Eye	Regression Equation	R^2	Estimated Cell Loss/Year
Right Eye	$y = -13.70x + 3005$	0.720	13.70 cells/year (0.57%)
Left Eye	$y = -13.97x + 3029$	0.680	13.97 cells/year (0.58%)

Table 8 illustrates the results of linear regression analysis. Corneal endothelial cell density showed a linear decline with increasing age. The estimated annual endothelial cell loss was 13.70 cells/year (0.57%) in the right eye and 13.97 cells/year (0.58%) in the left eye.

DISCUSSION

The corneal endothelium is a single layer of flattened hexagonal cells that plays a vital role in maintaining corneal transparency by regulating stromal hydration. Since these cells possess limited regenerative capacity, a gradual decline in endothelial cell density occurs with advancing age. Numerous studies have evaluated the influence of age, gender and ethnicity on corneal endothelial characteristics, demonstrating considerable variation among different populations. However, almost all studies have consistently reported an inverse relationship between age and corneal endothelial cell density.[6-10]

The present study was conducted to evaluate age-related changes in corneal endothelial cell density in a healthy South Indian population. A total of 120 healthy subjects with a mean age of 45.25 years were included in the study. There were 58 (48.3%) males and 62 (51.7%) females. Subjects with previous ocular surgery, ocular trauma, corneal diseases, diabetes mellitus and hypertension were excluded to eliminate factors that could influence endothelial cell density.

In the present study, the mean corneal endothelial cell density progressively decreased with advancing age in both eyes. In the right eye, the mean endothelial cell density decreased from 2761.79 ± 194.41 cells/mm² in subjects younger than 25 years to 1986.54 ± 233.13 cells/mm² in subjects older than 65 years. Similarly, in the left eye, the mean endothelial cell density declined from 2775.21 ± 224.76 cells/mm² in subjects younger than 25 years to 1986.75 ± 200.18 cells/mm² in subjects older than 65 years. Analysis using ANOVA demonstrated that this reduction across different age groups was highly statistically significant (right eye: $F = 94.615$, $p < 0.001$; left eye: $F = 71.08$, $p < 0.001$). Furthermore, all pairwise comparisons between age groups using the independent t-test were also statistically significant ($p < 0.001$), confirming that endothelial cell density decreases progressively with increasing age.

The findings of the present study are in agreement with those reported by Sarath et al.,[1] Sanchis-Gimeno et al.,[2] and Collin Murphy,[11] all of whom demonstrated a significant age-related decline in corneal endothelial cell density. Collin Murphy[11] reported that endothelial cell loss begins during fetal life and continues throughout life. According to his study, the rapid decline during prenatal life is related to rapid corneal growth with limited endothelial mitotic activity, whereas after early childhood the reduction follows a linear pattern due to progressive endothelial cell loss without further increase in corneal size. The present study similarly demonstrated a continuous decline in endothelial cell density throughout life, supporting these observations.

In contrast, Laing et al.,[9] reported no significant association between age and endothelial cell density among subjects aged over 40 years. Padilla et al.,[7] observed a progressive decline in endothelial cell density up to the sixth decade of life, followed by a slight increase after 60 years of age. They suggested that the increase in polymegathism occurring after the fourth decade could account for this observation. However, no such increase was observed in the present study, where endothelial cell density consistently declined across all age groups.

The present study also demonstrated a strong negative correlation between age and endothelial cell density. Pearson's correlation coefficient was −0.849 for the right eye and −0.825 for the left eye ($p < 0.001$), indicating that endothelial cell density decreases significantly as age advances. Similar findings were reported by Sarath et al,[1] Sanchis Gimeno et al.,[2] - and Collin Murphy[11] who also demonstrated a significant inverse relationship between age and endothelial cell density. Linear regression analysis in the present study revealed an annual endothelial cell loss of 13.70 cells/year (0.57%) in the right eye and 13.97 cells/year (0.58%) in the left eye. These findings are comparable with those of Faragher et al.,[1] and Hashemian et al.,[12] who reported an annual endothelial cell loss of approximately 0.6%. However, Sarath et al.,[1] observed a comparatively lower annual endothelial cell loss of 0.29%, which may be attributed to ethnic differences, study population characteristics and sample size.

Gender-related differences in endothelial cell density were also evaluated in the present study. The mean endothelial cell density of the right eye was 2406.45 ± 363.02 cells/mm² in males and 2365.48 ± 335.22 cells/mm² in females ($p = 0.522$). Similarly, the left eye showed mean endothelial cell densities of 2445.52 ± 384.89 cells/mm² in males and 2351.42 ± 342.89 cells/mm² in females ($p = 0.159$). Although males demonstrated slightly higher mean values, the differences were not statistically significant. These findings are consistent with those reported by Sarath et al,[1] Hashemian et al.[12] and Shao Yunliang et al.,[13] who also found no significant gender-related differences in endothelial cell density. In contrast, Snellingen[6] Padilla et al.,[7] and Laule et al.,[9] observed significant gender differences, with Padilla et al.,[7] reporting approximately 7.8% higher endothelial cell density in females, suggesting a greater endothelial reserve among women.

The present study further demonstrated no statistically significant difference between the right and left eyes. The mean endothelial cell density was 2385.28 ± 348.06 cells/mm² in the right eye and 2396.90 ± 365.32 cells/mm² in the left eye ($p = 0.338$). These observations are comparable with the findings of Laule et al.,[9] and Rao et al.,[14] who also reported no significant interocular difference in endothelial cell density.

Several studies have demonstrated significant racial and ethnic variations in corneal endothelial characteristics. Comparisons among American, Japanese and Indian populations have shown the highest endothelial cell density in the Japanese population with comparatively lower values observed in the Indian population. Hashemian et al.,[12] reported even lower endothelial cell density values in the Iranian population compared with Indian and American populations. In the present study, the mean endothelial cell densities were generally comparable with previously reported Indian normative data but were slightly lower across most age groups. This difference may be attributed to the relatively smaller sample size and regional variations within the Indian population.

Age-related endothelial cell loss has been attributed to oxidative DNA damage and reduced proliferative capacity of endothelial cells. Increased oxidative stress induces expression of cell-cycle inhibitors such as p21cip1 and p16INK4a, resulting in progressive endothelial cell loss with advancing age.[15] The findings of the present study support this biological mechanism by demonstrating a significant age-dependent decline in endothelial cell density.

The clinical significance of endothelial cell assessment is well established. Preoperative endothelial cell density evaluation is useful before cataract surgery, refractive surgery and other intraocular procedures to identify eyes with reduced endothelial reserve that are at increased risk of postoperative corneal decompensation. Endothelial cell assessment is also valuable in contact lens practice and in monitoring corneal graft survival following keratoplasty. Therefore, the normative data generated in the present study may serve as useful baseline reference values for the South Indian population and contribute to improved clinical decision-making in ophthalmic practice.

CONCLUSION

The present study demonstrated a significant age-related decline in corneal endothelial cell density, with a strong negative correlation between age and endothelial cell count. A statistically significant difference in mean endothelial cell density was observed among all age groups, while no significant differences were found between males and females or between the right and left eyes. The annual rate of endothelial cell loss was estimated to be 0.57% in the right eye and 0.58% in the left eye. The endothelial cell density values obtained were comparable with the normative data reported for the normal Indian population. However, the critical endothelial cell density required to maintain corneal transparency has not yet been clearly established. Therefore, further studies with larger populations are needed to determine the minimum endothelial cell density required to prevent corneal edema. In addition, advances in the in vitro cultivation of human corneal endothelial cells offer promising opportunities for the development of tissue-engineered corneal endothelium in the future.

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