Original Article

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Corneal Endothelial Cell Characteristics, Central Corneal Thickness and Its Relationship to Age in Normal South Indian Eyes

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Abstract

Aim: The aim of this study was to discuss the corneal endothelial cell characteristics, central corneal thickness and determine the relationship with age. *Methods:* 200 eyes of 100 patients underwent specular microscopy. Corneal Endothelial Cell Density (CECD), Mean Cell Area (MCA), percentage of hexagonal cells, Coefficient of variation (CV) and Central Corneal Thickness (CCT) were the parameters collected. *Results:* Mean CECD was 2675.8±384.4 cells/mm^2. MCA (Mean cell area) was 387.79 μm^2±91.27 μm^2, Coefficient of variation (CV) in cell size was 37.440 %±8.395% and percentage of regular hexagonal cells was 49.870%±7.921%. There was a statistically significant inverse correlation between age and CECD. A positive correlation was observed between CV, MCA and age. *Conclusion:* CECD and CCT decreases with age. MCA and CV increase with age. Percentage of regular hexagonal cells is not dependent on age.

Keywords: Central Corneal Thickness; Corneal Endothelial Cell; Specular Microscopy.

Introduction

Corneal endothelial cells have been the key factor in maintaining the corneal transparency. Factors such as genetics, race, and age [1,2] or factors such as trauma, intraocular surgery, ultraviolet radiation and infection [3,4,5] are responsible for maintaining the structural and functional integrity of the corneal endothelium.

The ability of the corneal endothelium to pump water out of the corneal stroma against an osmotic gradient helps in maintaining corneal transparency [6,7]. The corneal endothelium is metabolically active and utilizes the Na^+–K^+ pump for keeping the stroma at its usual hydrated state of 70% water to prevent stromal edema [8]. Metabolic function of these cells play a key role as decreased number of healthy endothelial cells may maintain corneal deturgescence better than a similar number of poorly functioning cells [2]. Due to the absence of a proliferative response any loss of endothelial cells results in the increase in the cell size, to cover of the posterior corneal surface leading to increased cellular pleomorphism and a decrease in the percentage of hexagonal cells with age [9,10].

Specular microscopy enables a direct view of the endothelial cells. It helps in predicting corneas which are more likely to decompensate when they are subjected to stress like trauma or surgery. With the advent of corneal endothelial surgeries for variety of endothelial diseases the knowledge of CECD plays a significant role. Development of new techniques of cataract surgeries, learning curve involved and its impact on the endothelial count have been studied. Studies on CECD in the normal population help in identifying patients at risk for decompensation following cataract extraction. The purpose of this study was to correlate CCT and CECD with age.

Methods

This prospective, non-randomized, single arm design study protocol was approved by the University's Institutional Ethical Committee. This study included 200 eyes of 100 patients in

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the age group, 8 to 80 years, attending ophthalmic outpatient department of tertiary care centre, over 3-month period (August 2017 to October 2017) were enrolled This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to their enrollment in this study. Exclusion criteria included history of intraocular surgery, ocular trauma, contact lens wear, glaucoma, uveitis, evidence of any corneal pathology on slit-lamp biomicroscopy, high refractive errors, and systemic disease like diabetes mellitus.

All the patients were examined using a non-contact specular microscope, Tomey EM -4000 (Tomey Corporation, Japan) with an autoshot option by fixed frame method. The patient was positioned on a chair in front of specular microscope and made to fix inside the device till instrument automatically took a clean image of corneal endothelium and measured the parameters. Parameters that were recorded included corneal endothelial cell density, average cell area, coefficient of variation (CV) of cell area, percentage of hexagonal cells and central corneal thickness. Cell density was recorded as number of cells per square milli-meter. An average of 3 reading was taken for the analysis. CV of hexagonal cell in the analyzed area was used as an index of variation in cell shape (polymegathism). The percentage of hexagonal cells indicated pleomorphism. CCT varies over the day. The cornea is thickest in the morning and gradually becomes thinner [11]. Specular microscopy and CCT was measured at the same time of the day 10 PM to 12 PM, to reduce errors related to diurnal variation.

The primary outcome variable studied is correlation of endothelial cell count with respect to the age. i.e. to study the influence of age on endothelial cell count. The secondary outcome measures included the influence of age on other endothelial cell characteristics like CV, hexagonality, Mean cell area and central corneal thickness.

Sample size calculation

Our study sampling was based on previous study by Mohammed Salih PA [12]. The mean of the corneal endothelial cell density observed by above referred study was 2648 cells per cubic mm and the standard deviation (SD) was 310. With alpha error of 0.05 and power of the study 90% (β= 0.10). The null hypothesis value was considered based on the standard observational value based on matched cohorts as 2500 cells per cubic mm, the minimum number of sample required in each group would be 61. We included a larger number 30% additionally as the different studies varied significantly among values and their measures of dispersion. Furthermore, we believed a minimum of 100 subjects of inclusion was necessary considering dropouts of additional 10%. Irrespective of above sampling, a data when presented in percentages, need sample of 100. Sample size was calculated using MedCalc Version 14.8,1993-2014, Medcalc Software, bvba.

All data distribution analysis was checked using Anderson Darling test. Data were evaluated using descriptive statistics which were presented using mean and standard deviation (SD). Statistical comparisons were done between left and right eye using appropriate parametric or nonparametric test. Associations between the various parameters were studied using Pearson Coefficient of Correlation (r) or Spearman's rho. Multivariable regression analysis was used for identifying the influencing factors for cell density with respect to different predictors. These predictors were checked for the modal fit by adding or removing the predictors at each level. The predictors included age, sex, CCT, Percentage of hexagonal cell in the final modal. All the data values of beta coefficients and its standard errors were analysed. A multi-collinearity was checked analysing variance inflation factors (VIF). If a high VIF was observed, then the predictor would not be considered in the model. R^2 and adjusted-R^2 was additionally noted for acceptance of the model. For the regression model, the P-value 0.25 was (adjusted P-value of 0.05, adj-P, P/number of predictors in model) considered for statistical significance.

Statistical analysis was performed using Minitab ® 17.1.0, © 2013 Minitab Inc.

Results

Mean (±SD) age of the patient was 39.8 (±15.2) years, with a near equal sex ratio of 49: 51 (male: female). Table 1 includes the corneal endothelial cell characteristics of both eyes as recorded by specular microscope. No statistical difference was observed between the parameters of right and left eye.

We observed no relationship between sex of the individuals and CECD in our study. A negative correlation was observed between age and CECD ($r = -0.369$, $P < 0.001$) as shown in the scatter plot (Figure 1) which means the CECD decreases with ageing. There was a positive correlation between age and CV ($r = 0.256$, $p < 0.01$), age and average cell area ($r = 0.426$, $p < 0.01$). Correlation analysis between age and percentage of regular hexagonal cells ($r = 0.067$, $p = 0.507$) was not significant. A negative correlation between CCT and age ($r = -0.198$, $p < 0.01$) was observed, (Figure 2). No correlation was observed between CECD and CCT. ($r = 0.103$, $p = 0.306$). The comprehensive list of correlation coefficients is presented in Table 2.

Regression analysis of cell density with age, percentage of regular hexagonal cells (6A), coefficient of variation, CCT and sex found only predictor factor 'age' to influence the CECD as depicted in Table 3. The model was adequate with R^2 and R^2-adjusted nearly 70%. There was no multi-collinearity in model observed with VIF 1-2.

Table 1: Corneal endothelial cell characteristics of both eyes as recorded by specular microscope. the values are presented Mean ± SD with 95% confidence intervals (CI) of mean differences. P-value <0.05 is considered as statistically significant.

Variable	Right eye	Left eye	95% CI	P-value
Endothelial cell density	2675.8 ± 384.4	2680.7 ± 376.5	-96 to 80	0.766
Average cell area	387.79 ± 91.27	391.21 ± 87.50	-14 to 9.99	0.807
Coefficient of Variation	37.440 ± 8.395	36,670 ± 6.479	-1.00to 2.00	0.523
Percentage of regular hexagonal cells				
Central corneal	49.870 ± 7.921	49.820 ± 8.234	-1.99 to 1.99	0.9057
thickness	527.14 ± 33.80	521.94 ± 35.86	-4.52to 14.92	0.293

Table 2: Correlation coefficients between the predictors and age:

Pearsons correlation	Age	R	P
Cell density		−0.369	<0.001
CV in cell size		0.2555	0.000
Hexagonality		-0.067	0.507
Cell area		0.426	0.000
CCT		-0.198	0.000

Table 3: Regression analysis of CECD with predictor variables.

Parameters	Coefficient	SE Coefficient	T-Value	P-value	VIF
Constant	1645	281	5.86		
Age	-3.5	1.09	-3.22	0.002	1.25
6A	-1.29	2.11	-0.61	0.542	1.33
Coefficient of variation	3.61	2.28	1.58	0.115	1.35
CCT (US)	-0.877	0.44	-1.99	0.048	1.09
Sex	-28.7	30.3	-0.95	0.344	1.06

Foot note: coefficient, S = 207.71, R^2= 70.95%, adjusted-R^2 = 70.05% for the final model described. SE,standard error, VIF, variance inflation factor. Adjusted P-value of 0.05 was considered for statistical significance.

Table 4: Corneal endothelial cell characteristics as described in various studies with respect to ethnics. The values are presented as Mean ± SD.

	ECD (cells/mm²)	MCA (µm²)	CV (%)	% of hexagonal cells (%)
Turkish (Arici et al)[6]	2671 ± 356	381.2 ± 51.9	34.3± 5.3	54.9± 10
Iranian ((Hashemian et al)[13]	1961± 457	537 ± 137.4	24.1± 7.1	
Chinese (Yunliang et al)[14]	2932 ± 363	347 ± 46	34± 5	59± 9
Caucasian (DumanR et al)[15]	2732 ± 305	368 ± 41	34± 7	46± 8
Malaysian (Mohammed Salih PA)[12]	2648 ± 310	382.8 ± 47.7	58.1 ± 22.6	44.3 ± 11.5
Nigerian (EweteT et al)[16]	2610.26±371.87	392.22±68.03	43.95±9.50	46.52±8.83
Present study	2675.8 ± 384.4	387.79 ±91.27	37.44 ± 8.4	49.87 ± 7.9

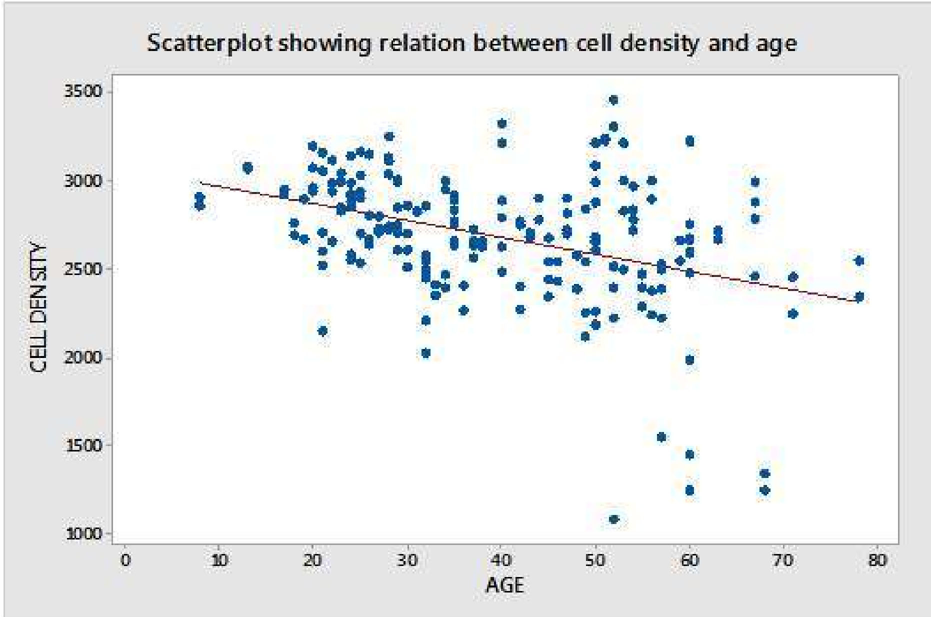

Fig. 1: Scatter plot showing the relation of CECD with age

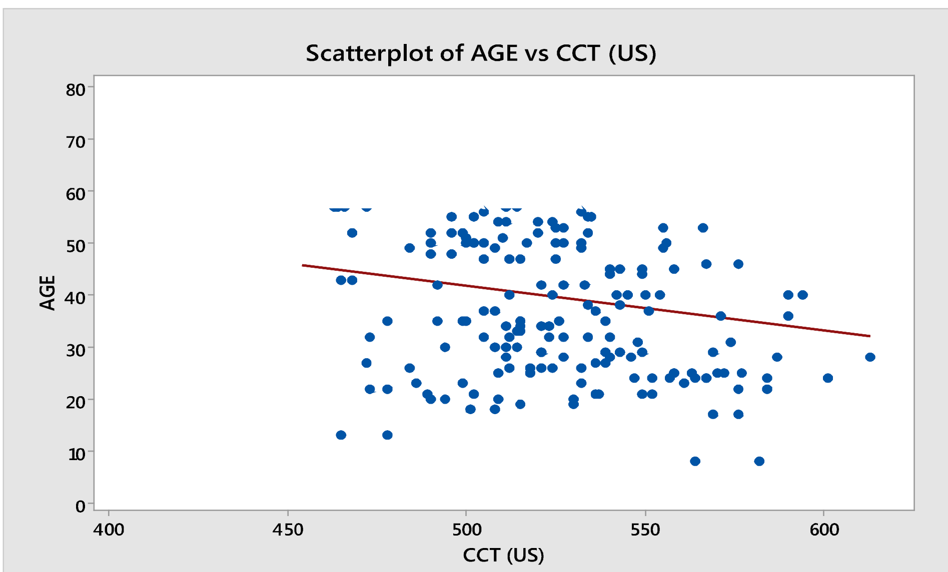

Fig. 2: Scatter plot showing the relation of CCT with age

Discussion

Endothelial cell layer that forms the innermost layer of the cornea is very crucial in maintaining the transparency of the cornea. Influence of age on functioning of the endothelium has been studied since many years. There is a large population variability of the endothelial cell counts. Clinically the endothelium can be assessed by three modalities, specular microscopy to study endothelial morphology, fluorophotometry to measure barrier function, and pachymetry to measure corneal thickness. Determination of CECD can help in planning complicated intraocular surgeries. Corneal endothelial cell characteristics observed in various studies have been shown in Table 4.

The mean value for CECD (2675.8) in our study is in agreement with other studies mentioned in the However, the Iranian study showed a very low CECD.

Influence of sex on CECD is still a debated issue. Sex did not influence CECD in our study. However, in a study conducted by Snellengen et al. found

CECD to be 2.9% higher in women [17]. A positive correlation of mean cell area and CV with age in our study is comparable to other studies. Jorge et al reported an increase of 5-6% in 10 years [18]. This suggests that the endothelial cells lost during ageing are not replaced by new cells, but the gaps are filled by enlargement of cells.

Galgauskas S et al [19] in their study on caucasiann patients found a strong inverse correlation between age and CECD as in our study. They observed a weak inverse correlation between age and CCT. Few studies have found a positive correlation between CCT and age. A negative correlation of CCT with age was observed in our study indicating thinner corneas with ageing. While some studies confirmed a relationship of CCT with age, [20] few other studies haven't found such dependence [21].

There was no correlation detected between CECD and CCT in our study. Many studies have reported no correlation between CECD and CCT [22]. Corneal thickness may thus be an independent parameter of normal cornea. A positive correlation of CV with age in our study indicating that with ageing there is increased variation in cell size have been reported in previous studies [19].

Limitation: Measurements in our study were taken in the central cornea only. Paracentral and peripheral CECD measured with the noncontact specular microscope were 5.8% ($p < .01$) and 9.6% ($p < .001$) increased compared with central ECD in a study conducted by Amann J et al. [23]. The human cornea has an increased CECD in the paracentral and peripheral regions of cornea compared with the central region. Also the superior peripheral region of the corneal endothelium has been found to have an increased ECD. As the corneal thickness increases in the periphery the correlation between the various parameters of the cornea in the central and peripheral corneas may yield more accurate results.

The findings in the present and previous studies indicate that the hexagonal cells of the endothelium that are lost with ageing result in the increase in size and change of shape of the cells.

Conclusion

Our study demonstrated an inverse relationship of CECD and CCT with age. A positive correlation was observed between CV, average cell area and age. No correlation was found between percentage of hexagonal cells, polymegathism, and age. We observed that the CECD was not influenced by CCT.

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