Saudi Journal of Ophthalmology (2017) 31, 86–90

Original article

Age related changes in corneal morphological characteristics of healthy Pakistani eyes

Qamar Ul Islam[a,*]; Muhammad Kamran Saeed[b]; Mohammad Asim Mehboob[a]

Abstract

Purpose: To determine the age related changes in corneal morphological characteristics in normal healthy adult Pakistani population.
Methods: Four hundred and sixty-four eyes of 232 healthy volunteers with ages between 10 and 80 years of either gender were included. Corneal endothelial cell density (CED), morphology and central corneal thickness (CCT) were evaluated in each subject with non-contact specular microscope (SP-3000 P, Topcon Corporation, Japan) and average of three readings per eye was used for final analysis. All the findings including demographic data, and corneal parameters were endorsed on a pre-devised proforma.
Results: Mean age of study population was 39.52 ± 18.09 years with 123 (53%) males and 109 (47%) females. Mean CED of study population was 2722.67 ± 349.67 cells/mm^2, while mean CCT was 505.72 ± 32.82 μm. Corneal morphological parameters among various age groups showed statistically significant difference in all parameters ($p < 0.01$). Correlation statistics revealed that CED ($r = -0.497$, $p < 0.01$), CCT ($r = -0.216$, $p < 0.01$) and hexagonality ($r = -0.397$, $p < 0.01$) decreased significantly with increasing age, while average cell size ($r = 0.492$, $p < 0.01$) and CV of size ($r = 0.454$, $p < 0.01$) increased with age.
Conclusion: This study showed that CED in Pakistani eyes was less than that reported in Chinese eyes, higher than Portuguese, Iranian and Indian eyes and comparable to the values in Turkish, Nigerian and Thai eyes.

Keywords: Specular microscopy, Corneal endothelium, Endothelial cell density, Central corneal thickness

http://dx.doi.org/10.1016/j.sjopt.2017.02.009

Introduction

A healthy cornea is of paramount importance in maintaining clarity of vision. Central corneal thickness (CCT) and corneal endothelial cell morphology are the two vital parameters in functional and morphological evaluation of cornea for diagnostic purposes and before any intraocular surgery. Corneal endothelium has a limited capacity for repair and damage to corneal endothelial cells is compensated by a combination of cell enlargement and cell spread to cover up for lost cells, resulting in a gradual decrease in endothelial cell density, increase in size of cells with increased cellular pleomorphism and decrease in hexagonality.[1–3]

Normal corneal endothelial cell density (CED) at birth ranges between 4000 and 5000 (cells/mm^2) that declines with aging at a rate of 0.3–0.6% per year with an approximate value of 2000–3000 cells/mm^2 in a normal adult eye.[1,4,5] It is now well established that CED decreases with age, trauma, refractive surgery, intraocular surgery, glaucoma, corneal dystrophies and diabetes mellitus.[1,5,6] CCT is another important parameter for corneal health as the intraocular pressure (IOP) depends on corneal thickness and CCT must be taken into consideration in evaluating glaucoma patients or suspects.

Various studies have confirmed that CED, CCT and morphology vary with age, gender, race and ethnicity.[2–5]

Received 22 November 2016; received in revised form 9 February 2017; accepted 28 February 2017; available online 9 March 2017.

[a] Department of Ophthalmology, PNS Shifa Hospital, Karachi, Pakistan
[b] Department of Ophthalmology, Combined Military Hospital, Quetta, Pakistan

* Corresponding author.
e-mail addresses: qamarulislam71@gmail.com (Q.U. Islam), m_kamransaeed@yahoo.com (M.K. Saeed), asimmehboob@gmail.com (M.A. Mehboob).

Peer review under responsibility of Saudi Ophthalmological Society, King Saud University

Production and hosting by Elsevier

Access this article online:
www.saudiophthaljournal.com
www.sciencedirect.com

Normative data regarding corneal morphological parameters in Pakistani population are limited. Ashraf et al. evaluated 450 eyes of 225 healthy Pakistani volunteers showed a mean CED of 2654 ± 341 cells/mm^2, with a decreasing cell counts as age increased.[7] Due to difference in endothelial morphological parameters among various population, races and ethnic groups, it is important to know the normative data of our population and effect of various factors on corneal morphology. The objective of this study was to determine the effect of age on CCT, CED, average cell size, coefficient of variation in cell size (CV), and percentage of regular hexagonal cells in normal healthy adult Pakistani population and to find out the relationship between endothelial cell parameters and other factors.

Material and methods

After approval of hospital ethical review committee, this prospective cross-sectional study was conducted at the Department of Ophthalmology, PNS Shifa Naval hospital Karachi, from August 2015 to November 2016. Four hundred and sixty-four eyes of 232 healthy volunteers with ages between 10 and 80 years of either gender were included in the study through non-probability convenience sampling. Subjects with refractive error of ≥± 1.00 diopters, history of intraocular surgery or trauma, corneal opacity or dystrophy, glaucoma, uveitis, use of contact lens, use of topical eye drops and diabetes mellitus were excluded. Calculated sample size was 218 based on the power (90%) to detect a difference in cell density of 75 cell/mm^2 using mean CED for normal population of 2654 ± 341 cell/mm^2 and $\alpha = 0.05$.[7] Written informed consent was obtained from each subject before enrollment and study was conducted in accordance with the Declaration of Helsinki. All the subjects were stratified into six groups on the basis of age that included <20 years, 21–30 years, 31–40 years, 41–50 years, 51–60 years and >60 years. All the participants underwent complete ocular examination including visual acuity assessment, auto refraction, slit lamp bio microscopic examination of anterior and posterior segment and non-contact IOP measurement. CED, morphology and CCT were evaluated in each subject with non- contact specular microscope (SP-3000 P, Topcon Corporation, Japan) by a single experienced examiner between 09:00 and 11:00 AM. Three images from central cornea of each eye were captured and 100 contiguous cells per image were included for analysis by built- in software. An average of three readings per eye was used for final analysis. All the findings including demographic data, and corneal parameters (CED, CCT, mean cell area (MCA), CV of cell size, percentage of hexagonal cells) were endorsed on a pre-devised proforma.

Statistical analysis of the data was done using SPSS version 13.0. All the data were tested for normality before analysis. Descriptive statistics i.e. means ± standard deviation (SD) for quantitative variables and frequencies and percentages for qualitative variables were used. The mean differences between independent samples for the two groups were assessed using the Student's two-sided *t*-test, and the paired Student's *t*-test was used to compare means of dependent samples. Means of more than two groups were compared using one-way analysis of variance (ANOVA). Pearson's correlation coefficient was used to establish correlations between age, CED, CCT, CV and hexagonality. A p value of ≤0.05 was considered significant.

Results

Data of 464 eyes of 232 healthy subjects were evaluated. Mean age of study population was 39.52 ± 18.09 years (range: 12–80 years). There were 123 (53%) males and 109 (47%) females. Mean CED of study population was 2722.67 ± 349.67 cells/mm^2 (range: 1700.9–3756.7 cells/mm^2), while mean CCT was 505.72 ± 32.82 µm (range: 409–606 µm). Mean average cell size, CV of cell size and hexagonality of study population are given in Table 1. The endothelial characteristics did not show significant difference between males and females or between right and left eyes except the CCT values that were significantly higher in females ($p < 0.01$) (Table 2). Corneal morphological parameters among various age groups showed statistically significant difference in all parameters ($p < 0.01$) (Table 3). Average corneal endothelial cells loss per decade was 87 cells/mm^2 that equals to approximately 0.28% cells loss per year (Table 3). The highest rate of loss was noted in the third decade of life in this study population (6.04%). Correlation statistics revealed that CED ($r = -0.497$, $p < 0.01$), CCT ($r = -0.216$, $p < 0.01$) and hexagonality ($r = -0.397$, $p < 0.01$) decreased significantly with increasing age, while average cell size ($r = 0.492$, $p < 0.01$) and CV of size ($r = 0.454$, $p < 0.01$) increased with age (Table 1). CED and CCT showed positive correlation ($r = 0.175$, $p < 0.01$) indicating high CED with thicker corneas.

Table 1. Corneal morphological parameters among various age groups.

Age group (years)	Age (years) mean ± SD	No of eyes	CCT (µm) mean ± SD	CED (cells/mm^2) mean ± SD	Avg cell size (µm^2) mean ± SD	CV of size (%) mean ± SD	Hexa (%) mean ± SD
<20	18 ± 2.09	84	518.20 ± 25.81	3021.24 ± 312.24	335.23 ± 35.67	29.86 ± 4.68	61.12 ± 10.26
21–30	23.70 ± 2.85	92	509.18 ± 37.56	2838.48 ± 264.59	355.76 ± 34.41	31.31 ± 3.68	59.09 ± 7.60
31–40	35.11 ± 3.59	74	495.08 ± 30.48	2706.80 ± 280.24	373.35 ± 40.33	34.60 ± 4.93	55.03 ± 7.75
41–50	44.09 ± 2.75	68	517.68 ± 21.51	2626.42 ± 280.31	385.78 ± 44.02	36.06 ± 4.07	52.46 ± 6.88
51–60	55.80 ± 2.96	82	498.99 ± 32.51	2555.16 ± 359.88	400.28 ± 58.46	35.75 ± 4.52	52.41 ± 7.64
>60	69.91 ± 6.29	64	492.61 ± 36.42	2499.59 ± 303.52	406.60 ± 50.81	35.78 ± 4.41	53.16 ± 8.55
Total	39.52 ± 18.09	464	505.72 ± 32.82	2722.67 ± 349.67	374.13 ± 50.75	33.67 ± 5.01	55.84 ± 8.55
p Value[a]	<0.01		<0.01	<0.01	<0.01	<0.01	<0.01
Correlation[b] *r* (*p* value)	–	–	−0.216 (<0.01)	−0.497 (<0.01)	0.492 (<0.01)	0.454 (<0.01)	−0.397 (<0.01)

[a] ANOVA.
[b] Pearson's correlation.

Table 2. Endothelial cell loss per decade of age.

Age group (years)	CED (cells/mm^2) mean ± SD	Cell loss, no (%)
<20	3021.24 ± 312.24	–
21–30	2838.48 ± 264.59	182 (6.04)
31–40	2706.80 ± 280.24	131 (4.63)
41–50	2626.42 ± 280.31	80 (2.96)
51–60	2555.16 ± 359.88	71 (2.71)
>60	2499.59 ± 303.52	55 (2.17)

Discussion

Growing insight into the morphology of corneal endothelium with the advent of better diagnostic tools has led to better understanding of its role in maintaining vision. Corneal endothelium consists of a monolayer of predominantly hexagonal cells that play a vital role in maintaining clear vision by virtue of its barrier and ionic pump function. The critical number of CED to maintain corneal transparency is 500 cells/mm^2 and any further deterioration in cell count leads to corneal decompensation.[4,5] Significant differences in corneal endothelial morphology do exist among various races and ethnic groups and these parameters are affected by age, measurement protocol and ocular/systemic co-morbidities. Endothelial health and function in an individual should be assessed on the basis of normative data for that population. This study provides normative data on various corneal morphological parameters in the normal Pakistani population. In our study mean CED was 2722.67 cells/mm^2 with an average decrease of 0.28% cells per year. Studies from various regions of the world showed variable results in terms of CED, CV, and hexagonality (Table 4).[2,6,3,8–11] The most plausible explanation for these variations could be difference in ethnicity, population demography, and methods of measurement.

Mean CCT in our study was 505.72 μm using SP-300 specular microscope. CCT values differ among various ethnic groups and races and it also depends on the method of measurement. Islam et al. in their study on Pakistani population with a mean age of 31 years found mean CCT values of 536.48 μm, 498.62 μm and 526 μm using Dual Scheimpflug Analyzer, Specular microscope and Ultrasonic pachymeter respectively.[12] CCT values ranging from 513 μm to 567 μm had been reported in various international studies using non-contact specular microscopes.[2,6,13–15] Relationship of corneal morphological parameters with age, gender and ethnicity had been studied extensively worldwide and it has been established that significant difference in corneal morphology does exist among races and ethnic groups. Corneal morphological parameters in right and left eyes and according to gender were similar in our study except for CCT values that were significantly higher in females ($p < 0.01$). Xu et al. in their study reported that corneas were thicker in men than in women.[16] Tayyab et al. in their study in Pakistani population found no statistically significant difference in CCT values between males and females.[17] Torres et al. reported that CCT was greater in American Indian/Alaskan Natives females than males.[18] Studies had confirmed a negative correlation between CCT and age i.e. CCT decreases with advancing age. Similar negative correlation was found in our study that was statistically significant. It was found that CCT declines about 4 μm (in male corneas) to 5 μm (in female corneas) every 10 years.[19] In our study average decline in CCT per decade was approximately 4 μm, but the pattern was quite variable among various decades. Galgauskas et al. also reported a weak inverse correlation between age and CCT ($r = -0.156$, $P < 0.01$).[20]

Progressive decline in CED with advancing age is well documented and it is advisable to assess CED values before any intraocular procedure. The most probable reason for this decline could be the role of apoptosis and/or necrosis caused by light-induced oxidative damage.[21] In our study, cell loss was 87 cells/mm^2 per decade (0.28% per year) with greatest decline occurred in third decade of life. CED loss with advancing age showed significant negative correlation in our study. Various international studies documented endothelial cell loss ranging from 71 to 145 cells/mm^2 per decade i.e. 0.24–0.57% per year.[2,6,3,8–11] Reparative process of damaged human corneal endothelium involves combination of cellular enlargement and cell spread along with increase in the variation of individual cell areas i.e. polymegethism or coefficient of variation (CV). Hexagonality (Six sided cells) is another index of healthy corneal endothelium which is expected to be around 60% in normal corneas.[1,3] The CED is less with greater variation in cell shape and size in diseased or aging cornea. Our data showed a significant positive correlation of age with average cell area or CV of cell size and a significant negative correlation was observed between age and percentage of hexagonal cells. The results of various other studies have shown that with increasing age there is a general trend toward decreased CED and percentage of hexagonal cells along with increased average cell area, and increased CV in cell size.[2,6,9–11] The strength of this study was the appropriate sample size with apparently healthy corneas, prospective data collection, and evaluation of various corneal parameters (CCT, CV, Avg cell size, and Hexagonality) for the first time in Pakistani population. Limitations of the study include lack of multivariate analysis and not taking into account possible confounding factors such as smoking, IOP and corneal diameter.

Table 3. Corneal morphological parameters according to laterality and gender.

Parameter	Laterality			Gender		
	Right eye ($n = 232$)	Left eye ($n = 232$)	p Value[a]	Male ($n = 123$)	Female ($n = 109$)	P value[b]
Age (yrs) mean ± SD	–	–	–	39 ± 17.91	40.11 ± 18.37	0.642
CCT (μm) mean ± SD	506.09 ± 33.07	505.35 ± 32.63	0.199	500.39 ± 30.88	511.73 ± 33.95	**0.000**
CED (cells/mm^2) mean ± SD	2721.92 ± 342.17	2723.43 ± 357.75	0.882	2736.10 ± 345.39	2707.52 ± 354.63	0.380
Avg cell size (μm^2)mean ± SD	374.20 ± 48.96	374.06 ± 52.58	0.936	372.09 ± 50.03	376.44 ± 51.56	0.357
CV of size (%) mean ± SD	33.83 ± 4.97	33.51 ± 5.06	0.209	33.88 ± 4.65	33.43 ± 5.40	0.335
Hexa (%) mean ± SD	55.88 ± 8.77	55.80 ± 8.99	0.896	55.70 ± 8.52	55.99 ± 9.27	0.726

[a] Paired sample '*t*' test.
[b] Independent sample '*t*' test.

Table 4. Comparison of corneal parameters among various study groups.

	Portuguese	Turkish	Nigerian	Thai	Chinese	Iranian	Indian
No of eyes	256	252	359	404	1329	525	537
CCT (µm)	567.5 ± 43.2	521 ± 33					
CED (cells/mm^2)	2526.4 ± 362.5	2671 ± 356	2610.2 ± 371.8	2623.4 ± 325	2932 ± 363	1961 ± 457	2525 ± 337
CV	33 ± 3	34.3 ± 5.3	43.9 ± 9.5	39.4 ± 8.2	33 ± 5	24.1 ± 7.1	
Hexa (%)	53.8 ± 6.8	54.9 ± 10	46.5 ± 8.8	51.5 ± 10.9	59 ± 9		
Cell loss (cells/mm^2)	145 cells/mm^2 per decade or 0.54% per year	82 cells/mm^2 per decade or 0.28% per year	71 cells/mm^2 per decade or 0.24% per year	84 cells/mm^2 per decade or 0.30% per year	81 cells/mm^2 per decade or 0.24% per year	139 cells/mm^2 per decade or 0.57% per year	75 cells/mm^2 per decade or 0.3% per year

Results of this study provide a greater insight into the understanding of corneal morphology in Pakistani population especially in the context of pre-operative evaluation before intra ocular surgeries.

Conclusion

Apart from providing normative data on corneal morphological parameters, results of this study also confirmed an age related progressive decrease in CED, CCT and hexagonality of corneal endothelial cells along with increase in CV and average cell size in normal healthy adult Pakistani population.

Conflicts of interest

The authors declared that there is no conflict of interest.

Grant support & financial disclosures

None.

References

1. McCarey BE, Edelhauser HF, Lynn MJ. Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices and new intraocular drugs and solutions. *Cornea* 2008;**27**(1):1–16. http://dx.doi.org/10.1097/ICO.0b013e31815892da.
2. Arıcı C, Arslan OS, Funda Dikkaya F. Corneal endothelial cell density and morphology in healthy Turkish eyes. *J Ophthalmol* 2014. http://dx.doi.org/10.1155/2014/85262. Article ID 852624, 5 pages.
3. Ewete T, Ani EU, Alabi AS. Normal corneal endothelial cell density in Nigerians. *Clin Ophthalmol (Auckland, NZ)* 2016;**10**:497–501. http://dx.doi.org/10.2147/OPTH.S9707.
4. Mohd-Salih PAK. Corneal endothelial cell density and morphology in normal Malay eyes. *Med J Malaysia* 2011;**66**(4):300–3.
5. Joyce NC. Proliferative capacity of corneal endothelial cells. *Exp Eye Res* 2012;**95**(1):16–23. http://dx.doi.org/10.1016/j.exer.2011.08.014.
6. Jorge J, Queirós A, Peixoto-de-Matos SC, Ferrer-Blasco T, González-Méijome JM. Age-related changes of corneal endothelium in normal eyes with a non-contact specular microscope. *J Emmetropia* 2010;**1**:132–9.
7. Ashraf KM, Saeed MU, Zia R. Corneal endothelial cell density in a normal Pakistani population. *Eye* 2006;**20**:116–8. http://dx.doi.org/10.1038/sj.eye.6701798.
8. Sopapornamorn N, Lekskul M, Panichkul S. Corneal endothelial cell density and morphology in Phramongkutklao Hospital. *Clin Ophthalmol (Auckland, NZ)* 2008;**2**(1):147–51.
9. Yunliang S, Yuqiang H, Ying-Peng L, Ming-Zhi Z, Lam DS, Rao SK. Corneal endothelial cell density and morphology in healthy Chinese eyes. *Cornea* 2007;**26**(2):130–2.
10. Hashemian MN, Moghimi S, Fard MA, Fallah MR, Mansouri MR. Corneal endothelial cell density and morphology in normal Iranian eyes. *BMC Ophthalmol* 2006;**6**:9. http://dx.doi.org/10.1186/1471-2415-6-9.
11. Rao SK, Ranjan Sen P, Fogla R, Gangadharan S, Padmanabhan P, Badrinath SS. Corneal endothelial cell density and morphology in normal Indian eyes. *Cornea* 2000;**19**:820–3.
12. Islam QU, Malik S. Comparison of central corneal thickness measurement using non-contact and contact pachymetry devices in normal eyes. *Pak J Ophthalmol* 2015;**31**(1):27–31.
13. Doughty MJ. A prospective analysis of corneal endothelial polymegethism and cell density in young adult Asians. *Clin Exp Optom* 2014;**97**(3):256–63. http://dx.doi.org/10.1111/cxo.12127.
14. Bao F, Wang Q, Cheng S, Savini G, Lu W, Feng Y, et al. Comparison and evaluation of central corneal thickness using 2 new noncontact specular microscopes and conventional pachymetry devices. *Cornea* 2014;**33**(6):576–81. http://dx.doi.org/10.1097/ICO.0000000000000113.

15. Ogbuehi KC, Osuagwu UL. Repeatability and inter observer reproducibility of Artemis-2 high-frequency ultrasound in determination of human corneal thickness. *Clin Ophthalmol (Auckland, NZ)* 2012;**6**:761–9. http://dx.doi.org/10.2147/OPTH.S3169.
16. Xu L, Zhang H, Wang YX, Jonas JB. Central corneal thickness and glaucoma in adult Chinese: the Beijing eye study. *J Glaucoma* 2008;**17**(8):647–53.
17. Tayyab A, Masrur A, Afzal F, Iqbal F, Naseem K. Central corneal thickness and its relationship to intra-Ocular and epidemiological determinants. *J Coll Physicians Surg Pak* 2016;**26**(6):494–7.
18. Torres RJ, Jones E, Edmunds B, Becker T, Cioffi GA, Mansberger SL. Central corneal thickness in northwestern American Indian/Alaskan natives and comparison to white and African Americans. *Am J Ophthalmol* 2008;**146**(5):747–51. http://dx.doi.org/10.1016/j.ajo.2008.05.047.
19. Foster PJ, Baasanhu J, Alsbirk PH, Munkhbayar D, Uranchimeg D, Johnson GJ. Central corneal thickness and intraocular pressure in a Mongolian population. *Ophthalmology* 1998;**105**(6):969–73.
20. Galgauskas S, Norvydaitė D, Krasauskaitė D, Stech S, Ašoklis RS. Age-related changes in corneal thickness and endothelial characteristics. *Clin Interv Aging* 2013;**8**:1445–50. http://dx.doi.org/10.2147/CIA.S51693.
21. Abib FC, Barreto J. Behavior of corneal endothelial density over a lifetime. *J Cataract Refract Surg* 2001;**27**:1574–8.