Cornea 20(1): 55–58, 2001.

Quantitative and Morphological Characteristics of the Human Corneal Endothelium in Relation to Age, Gender, and Ethnicity in Cataract Populations of South Asia

T. Snellingen, M.D., M.P.H., D.C.E.H., Ph.D., Gullapalli N. Rao, M.D., Jeevan K. Shrestha, F.R.C.S., F.C.Ophth., Fazlul Huq, D.O., M.P.H., and Hung Cheng, F.R.C.S., F.C.Ophth.

Purpose. To describe the differences of corneal endothelial cell densities, cell size variability and cell hexagonality in cataract populations of south Asia between sexes and ethnic groups. **Methods.** 1,235 eyes of 1,235 male and female patients 40–75 years of age with senile cataract were examined with non contact specular microscopy with semi-automated analysis technique. The cell data of the study population was analyzed in relation to age, sex, and ethnic groups. Mean arithmetic differences and the coefficient of variation of repeated observations were calculated to estimate precision of the technique utilized. The main outcome measures were corneal endothelial cell density, cell size variability and cell hexagonality. **Results.** The mean corneal endothelial cell density was 2,720 cells/mm^2, mean cell size variability was 37.8% and percent cell hexagonality 40%. We found statistical significant difference between the three ethnic populations in all the corneal endothelial cell measurements ($p < 0.0001$). Females had a 2.9% greater cell density than males ($p = 0.0001$). There was no significant difference in mean cell density according to age. Variability of cell size, however, increased with age ($p < 0.001$). These findings were consistent across the three ethnic groups. **Conclusions.** In a total sample of 1,235 eyes distributed evenly in three cataract patient populations of south Asia, we found statistically significant differences of corneal endothelial cell densities of cell size variability and cell hexagonality between sexes and ethnic groups.

Key Words: Corneal endtohelium cells—Senile cataract—South Asia.

Vogt[1] first observed the corneal endothelial cells through the specular reflection of the biomicroscope in 1920. From 1975, with the introduction the specular microscope for clinical use by Laing et al.[2] and subsequently by Bourne and Kaufman,[3] numerous studies using both small and wide field specular microscopy have described the normal corneal endothelium.[4] Hirst and coworkers found in their quantitative analysis of wide and small field specular microscopy[5] that both techniques provides an accurate representation of the true mean of cell size in about 80% of the time but as endothelium becomes more irregular the accuracy of samples diminishes greatly. Previous studies have suggested that there is a continuous cell loss from the third to fourth decades of life.[6–11] Due to the increasing variability of the individual cell populations of central corneas after the third decade of life, increasing sample sizes are needed to detect true differences in subgroups of the population. To our knowledge none of the previous studies that have looked at age and corneal endothelial cells included a sample larger than 150 eyes and only one study has described the corneal endothelium across different population groups.[12] In this article we describe the pre-operative central corneal endothelium cells according to age, sex and ethnic group of 1,235 eyes as part of a baseline examination for a clinical trial on cataract surgery.

Submitted February 29, 2000. Revision received June 2, 2000. Accepted June 5, 2000.

From the Department of Ophthalmology (T.S.), Institute of Clinical Medicine, University of Tromso, Norway; the L.V. Prasad Eye Institute (N.G.R.), Hyderabad, India; the B.P. Koirala Lions Centre for Ophthalmic Studies (K.J.S.), Institute of Medicine, Tribhuvan University Kathmandu, Nepal; the Institute of Community Ophthalmology (F.H.), Chittagong University, Bangladesh; and the Oxford Eye Hospital (H.C.), Honorary Senior lecturer, University of Oxford, U.K.

Financial support: The study has been funded by a Norwegian Government Grant: Norwegian Universities' Council for Development Research and Education-NUFU grant # 17/91.

Address correspondence and reprint requests to Dr. T. Snellingen, Institute of Community Medicine, 9037 University of Tromso, Norway. E-mail: torkel.snellingen@ism.uit.no

PATIENTS AND METHODS

From January 1993 to December 1995, 1,235 eyes of 1,235 cataract patients between 40–75 years of age were examined preoperatively with non contact specular microscopy. The patients were from three ethnically distinct regions of south Asia: Andhra Pradesh, south India, Chittagong, Bangladesh and south western Nepal.[13] Patients with acute or chronic corneal disease were excluded.[14,15] All centers use the same non contact specular microscopy and digital image and analysis techniques. 2 ophthalmic assistants were specially trained by the same instructor visiting all three participating centers. Small field (0.08 mm^2) specular microscopy was taken of one eye using a SL-7F slitlamp with a non contact specular attachment set at 25X magnification. The images were captured and digitized using the IMAGEnet Cell Soft (version 3.5; Topcon Corporation, Tokyo, Japan). The best image was saved on optical cartridges. Image analysis was done using the *semiautomatic analysis* option of the software.[16] 90 % of the analysis included 60 cells or more (mean = 92; SD = 20; range

= 22–261). The quality of images was systematically reviewed and a visual assessment was cross checked with the data from the semiautomatic analysis by the same study monitor visiting all three centers. When errors of analysis were suspected the analysis were repeated by both the study monitor and the local examiner and an agreement was reached.

The following three variables were measured:

1) central endothelial cell density defined as *the number of cells per mm^2;*
2) central corneal endothelial cell size variability or the coefficient of variation defined as *sd/x* where *sd* is the standard deviation of the cell size expressed as a percentage and *x* is the mean cell size;
3) central corneal endothelial hexagonality defined as the *percentage of cells having six bordering cells.*

A sampling error study was conducted by taking serial images of the central cornea of 10 consecutive cases both of the operated eye and the non operated contra-lateral eye. Every case had a age and sex matched control. A total of 6 images were taken of each eye. Two examiners made three examinations at three consecutive sittings.

Three images were taken at each sitting with the best image saved for further analysis. A total of 180 digital images were available for analysis. Mean arithmetic differences between observers and within observers were calculated to estimate reproducibility of corneal endothelial cell measurements. The formula used for calculating the coefficient of variation of differences was cv = 100 x s/*x* where s is the measurement error and *x* is the mean value of the corneal endothelial cell measurement. The cell data of the study population was analyzed in relation to age, sex and ethnic groups and adjustments were made with analysis of co-variance. Statistical Analysis System (SAS, Cary, NC, U.S.A.) version 6.11 for windows was used for the statistical computations.

RESULTS

The patient population consists of 610 (49.4%) males and 625 (50.6%) females with a mean age of 61.5 (SD = 7.6) for males and 59.5 (SD = 7.4) for females.

The precision estimates of the measurements of corneal endothelium cell data is presented in Table 1. The mean number of cell counted for this analysis was 77 cells/mm^2 (SD = 23.5). The coefficient of variation (cv) of mean difference between repeated measurements for the cell density was for between and within observers was 5.2% and 6.4% respectively. For the cell size variability the cv was 14.2% and 11.7% and for cell hexagonality 25.5% and 29.8%.

TABLE 1. *Reproducibility of corneal endothelial cell measurements*

	No. of pairs	Mean	Mean difference (SD)	CV*
Cell density				
Between observers	90	2460.7	9.8 (181.1)	5.2
Within observers	60	2450.7	−79.1 (222.5)	6.4
Cell size variability				
Between observers	90	42.79	3.53 (8.57)	14.2
Within observers	60	42.51	0.55 (7.05)	11.7
Cell hexagonality				
Between observers	90	43.47	−0.76 (15.7)	25.5
Within observers	60	43.80	−1.63 (18.5)	29.8

* CV of differences = 100 × *s/x*: *s* = standard deviation of arithmetic difference between measurements/square root of 2 and *x* = mean value of corneal endothelial cell measurement.

TABLE 2. *Distribution of cell density, cell size variability, and cell hexagonality by gender and ethnic group*

	n	Cell density (mm^2) Mean (SD)	Cell size variability (%) Mean (SD)	Cell hexagonality (%) Mean (SD)
Nepali	302	2634 (386)	39.3 (7.4)	34.4 (7.0)
Male	127	2586 (379)	40.0 (7.6)	33.6 (6.9)
Female	175	2670 (388)	39.8 (7.2)	34.9 (7.0)
Between genders*		$p = 0.0617$	$p = 0.17$	$p = 0.10$
Bangladeshi	464	2782 (342)	33.2 (5.7)	37.8 (5.8)
Male	295	2754 (337)	34.1 (6.0)	36.9 (5.6)
Female	169	2830 (345)	31.6 (4.8)	39.4 (5.6)
Between genders*		$p = 0.02$	$p < 0.01$	$p = 0.000005$
South Indian	469	2714 (360)	41.3 (6.4)	45.2 (8.9)
Male	188	2628 (370)	41.9 (6.6)	43.5 (8.8)
Female	281	2773 (342)	41.0 (6.3)	46.4 (8.7)
Between genders*		$p = 0.000016$	$p = 0.11$	$p < 0.000471$
All	1235	2720 (364)	37.8 (7.4)	40.0 (8.6)
Male	610	2680 (364)	37.7 (7.4)	38.0 (8.0)
Female	625	2760 (361)	37.8 (7.3)	41.0 (9.0)
Between genders†		$p = 0.0001$	$p = 0.8$	$p < 0.000001$
Between ethnic groups‡		$p < 0.00001$	$p < 0.00001$	$p < 0.00001$

* Test between sexes in each population group adjusted for age.
† Test between sexes in total population adjusted for age and ethnic groups.
‡ Test between ethnic groups adjusted for age and gender.

Table 2 presents the distribution of cell density, cell size variability and hexagonality between the different ethnic groups and between sexes. There were statistically significant differences between ethnic groups in cell density, cell size variability and cell hexagonality. Between the three patient populations mean cell density varied from 2634 cells/mm^2 in western Nepal to 2782 cells/mm^2 in southern Bangladesh ($p = 0.0001$), while mean cell size variability varied from 33.2% in south Bangladesh to 41.2% in south India ($p < 0.0001$).

Between sexes females had a 2.9% greater cell density and a 7.8% higher percentage of hexagonal cells than males. There was, however, no significant difference between sexes in cell size variability. Table 3 shows that the cell density did not differ significantly between age groups ($p = 0.12$), however, there was a significant increase in cell size variability with increasing age ($p < 0.001$). These findings were consistent across the three ethnic groups (data not shown). In the overall study population, as was expected, there was a slight but statistically significant inverse correlation between cell density and cell size variability, a significant positive relationship between cell density and percentage of hexagonal cells, and a inverse relationship between the percentage of hexagonal cells and cell size variability (Table 4).

DISCUSSION

Hirst et al showed in their quantitative analysis using precision sampling from the central corneal endothelium[5] that to be able to generate accurate data using small or wide-field specular microscopy large enough groups of patients are needed to compensate for the high variability demonstrated by current sampling techniques.

TABLE 3. *Mean cell density, cell size variability, and cell hexagonality by age*

	Density (mm^2)			Size variability (%)		Hexagonality (%)	
Age group	n	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
40–49 y	70	2780 (340)	1713–3517	34.3 (6.3)	22–55	37.4 (6.3)	23–50
50–59 y	345	2715 (342)	1478–3887	37.5 (7.0)	23–62	40.2 (8.6)	17–71
60–69 y	600	2729 (380)	1144–3780	37.8 (7.4)	20–67	40.0 (8.5)	15–80
70–75 y	220	2684 (361)	1537–3469	39.3 (7.7)	25–80	39.3 (9.3)	18–78
p value*		$p = 0.12$		$p < 0.001$		$p < 0.4$	

* Test between age groups adjusted for gender and ethnic group.

It has also been shown that the accuracy of the findings decreases with increasing polymegathism and the confidence intervals widens, indicating that the sample may be as far off as much as 30–60% from the true mean. The studies that have described the corneal endothelium to date have included small samples giving unacceptably large population sampling errors.

The precision of the semiautomatic analysis software programme for the corneal endothelial cell density measurements analysis was tested by Vecchi et al.[16] Confidence limits and standard errors of mean differences between values obtained by different methods were used to to evaluate agreement and reproducibility of the computerized method. Sensitivity and specificity were calculted for two different treshold limits of endothelial cell density. The semiautomated Image-NET system, in half the analysis time required by the manual method (digitilized cell tracings), provided endothelial cell count estimates that were not clinically different from those obtained from manual counting. As was shown by our study, the study described above and other studies,[8,17,18] non contact specular microscopy gives excellent precision for the corneal endothelial cell measurements and adequate precision for cells size variability and hexagonality in large population samples.

In this study population of 1,235 eyes of 1,235 subjects we have for the first time been able to describe differences of corneal endothelial cell density, cell size variability and cell hexagonality in the human corneal endothelium between sexes and between ethnic groups in a large cataract population of three different regions of south Asia. Based on our sample size we are confident that the analysis of cell data in respect to the three variables reflects real differences in the sub-groups both in relation to age, sex and the different population groups. The results of analysis of cell data between sexes at center level, although not statistically significant for all three variables, showed remarkable consistency across the three participating centres (Table 2).

Several authors have observed significant corneal endothelial cell loss with age until the fourth decade.[6,7,9,11] Smaller cohort studies have shown evidence of significant differences in corneal endothelial cell density of eyes measured at two different point in time (Bourne et al: examinations at 10 years intervals[19]) however no significant correlation between cell loss rate and age have been found likely due to the significant increase of polymegathism from the fourth decade. In our study population we found a highly significant increase in polymegathism but no significant increase in corneal endothelial cell density for the age group 40 and above.

Matsuda et al. in their study[12] comparing 73 eyes of 73 subjects of all age groups in an American population and equal number of eyes of subjects of a Japanese population suggested that there are ethnic differences in the corneal endothelial cell density between these population groups and that the higher cell count found in the Japanese population could be related to a lower incidence of aphakic bullous keratopathy. If differences in the characteristics of human corneal endothelium between population groups exist, as also is suggested by this study, what could be the possible clinical implications of these findings? Can there be possible differences in the vulnerability of corneal endothelium to surgical trauma across ethnic groups? New and improved corneal endothelial cameras using modern digital imaging technology have now been developed for routine clinical use. Further clinical studies of large populations of pre-operative and post-operative corneal endothelium across different ethnic groups should be undertaken to give further evidence that can refute or strengthen these findings.

TABLE 4. *Correlation between cell density, cell size variability, and cell hexagonality*

	Correlation coefficient r (95% CI)	Z	*p* value
Cell density and CV	−0.06 (−0.12,−0.01)	−2.25	0.02539
Cell density and hexagonality	0.11 (0.05,0.16)	3.89	0.00013
Hexagonality and CV	−0.20 (−0.25,−0.14)	−7.09	<0.00001

REFERENCES

1. Vogt A. Die sichtbarkeit des lebenden hornautendothels im lichtbueschel der gullstrandschen spaltlampe. *Klin Monatsbl Augenheilkd* 1919;63:233–4.
2. .Laing RA, Sandstrom MM, Leibowitz HM. In vivo photomicrography of the corneal endothelium. *Arch Ophthalmol* 1975;93:143–5.
3. Bourne WM, Kaufman HE. Specular microscopy of human corneal endothelium in vivo. *Am J Ophthalmol* 1976;81:319–23.
4. Committee on Ophthalmic Procedure Assessment. Corneal endothelial cell photography: three-year revision. *Ophthalmology* 1997;104: 1360–5.
5. Hirst LW, Yamauchi K, Enger C, et al. Quantitative analysis of wide field specular microscopy. II Precision of sampling from the central corneal endothelium. *Invest Ophthalmol Vis Sci* 1989;30:1972–9.
6. Yee RW, Matsuda M, Schultz RO, et al. Changes in the normal corneal endothelial cellular pattern as a function of age. *Curr Eye Res* 1985; 4:671–7.
7. Sturrock GD, Sherrard ES, Rice NSC. Specular microscopy of the corneal endothelium. *Br J Ophthalmol* 1978;62:809–14.
8. Cheng H, Jacobs PM, McPherson K, et al. Precision of cell density estimates and endothelial cell loss with age. *Arch Ophthalmol* 1985; 103:1478–81.
9. Laing RA, Sandstrom MM, Berrospi AR, et al. Changes in the corneal endothelium as a function of age. *Exp Eye Res* 1976;22:587–94.
10. Rao GN, Waldron WR, Aquavella JV. Morphology of graft endothelium and donor age. *Br J Ophthalmol* 1980;64:523–7.
11. Laule A, Cable MK, Hoffman CE, et al. Endothelial cell population changes of human cornea during life. *Arch Ophthalmol* 1978;96: 2031–5.

12. Matsuda M, Yee RW, Edelhauser HF. Comparison of the Corneal Endothelium in an American and a Japanese Population. *Arch Ophthalmol* 1985;103:68–70.
13. Snellingen T, Shrestha BR, Gharti MP, et al. Socio-economic barriers to cataract surgery in Nepal: the south Asian cataract management study. *Br J Ophthalmol* 1998;82:1424–8.
14. The South Asian Cataract Management Study Group. The South Asian Cataract Management Study. I. The first 662 cataract surgeries: a preliminary report. *Br J Opthalmol* 1995;79:1029–35.
15. Snellingen T, Shrestha JK, Huq F, et al. The South Asian Cataract Management Study: complications, vision outcomes, and corneal endothelium cell loss in a randomized multicenter clinical trial comparing intracapsular cataract extraction with and without anterior chamber intraocular lens. *Ophthalmology* 2000;107:1–10.
16. Vecchi M, Braccio L, Orsoni JG. The Topcon SP 1000 and Image-NET Systems: a comparison of four methods for evaluating corneal endothelial cell density. *Cornea* 1996;15:271–7.
17. Price NC, Cheng H. Contact and noncontact specular microscopy. *Br J Ophthalmol* 1981;65:568–74.
18. Ohno K, Nelson LR, McClaren JW, et al. Comparison of recording and analysis methods in specular microscopy. *Cornea* 1999;18:416–23.
19. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial changes over a ten year period. *Invest Ophthalmol Vis Sci* 1997;38:779–82.