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


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/mm², 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 endothelium cells—Senile cataract—South Asia.


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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 pre-operatively 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. Patients with acute or chronic corneal disease were excluded. All centers use the same non contact specular microscopy and digital image and analysis techniques. Ophthalmic assistants were specially trained by the same instructor visiting all three participating centers. Small field (0.08 mm²) 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. 90 % of the analysis included 60 cells or more (mean = 92; SD = 20; range
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 mm²;
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 \( cv = 100 \times s/x \) 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/mm² (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 2 presents the distribution of cell density, cell size variability and cell hexagonality by gender and ethnic group.

**DISCUSSION**

Hirst et al showed in their quantitative analysis using precision sampling from the central corneal endothelium 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.
It has also been shown that the accuracy of the findings decreases with increasing polymegathism and the confidence intervals widen, 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 evaluate agreement and reproducibility of the computerized method. Sensitivity and specificity were calculated for two different threshold 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 intervals19) 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 study12 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.

### REFERENCES


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

<table>
<thead>
<tr>
<th>Age group</th>
<th>Density (mm²)</th>
<th>Size variability (%)</th>
<th>Hexagonality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>40–49 y</td>
<td>70</td>
<td>2780 (340)</td>
<td>1713–3517</td>
</tr>
<tr>
<td>50–59 y</td>
<td>345</td>
<td>2715 (342)</td>
<td>1478–3887</td>
</tr>
<tr>
<td>60–69 y</td>
<td>600</td>
<td>2729 (380)</td>
<td>1144–3780</td>
</tr>
<tr>
<td>70–75 y</td>
<td>220</td>
<td>2684 (361)</td>
<td>1537–3469</td>
</tr>
</tbody>
</table>

* p value* * p = 0.12

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

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

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>Correlation coefficient</th>
<th>Correlation coefficient</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (95% CI)</td>
<td>Z</td>
<td>p value</td>
<td>r (95% CI)</td>
</tr>
<tr>
<td>Cell density and CV</td>
<td>−0.06 (−0.12,−0.01)</td>
<td>−2.25</td>
<td>0.02539</td>
</tr>
<tr>
<td>Cell density and hexagonality</td>
<td>0.11 (0.05,0.16)</td>
<td>3.89</td>
<td>0.00013</td>
</tr>
<tr>
<td>Hexagonality and CV</td>
<td>−0.20 (−0.25,−0.14)</td>
<td>−7.09</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>


