Characteristics of the Corneal Endothelium Across the Primary Angle Closure Disease Spectrum

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PURPOSE. To evaluate the corneal endothelial characteristics across the primary angle closure (PAC) disease spectrum amongst patients diagnosed as PAC suspects (PACS), PAC, PAC glaucoma (PACG), and previous acute PAC (APAC).

METHODS. We analyzed a total of 529 subjects (51 PACS, 170 PAC, 234 PACG, and 74 with previous APAC). All subjects had undergone laser peripheral iridotomy prior to study recruitment. Corneal endothelial parameters were measured using a noncontact specular microscope and the following parameters were obtained: mean central endothelial cell density (ECD; cells/mm²), coefficient of variation (CV) in cell area, and percentage of hexagonal cells.

RESULTS. The mean age of the subjects was 65.1 ± 8.2 years, and 55.2% were females. The mean central ECD was 2582.0 ± 472.8 cells/mm² in PACS, 2566.0 ± 408.3 cells/mm² in PAC, 2523.8 ± 406.8 cells/mm² in PACG, and 2504.0 ± 558.1 cells/mm² in APAC, with no significant differences in ECD across the subgroups (P = 0.61). The CV was lowest in PACS (34.38 ± 6.05 µm²/cell), and highest in APAC (37.61 ± 7.98 µm²/cell), but the differences were not significant (P = 0.07). Likewise, the percentage of hexagonality was not significantly different between the groups. A subgroup analysis on the eyes with previous APAC with their fellow eye also showed no significant differences in the corneal endothelial characteristics.

CONCLUSIONS. The corneal ECD and morphological characteristics such as CV and hexagonality are not significantly different across the PAC disease spectrum. This may reflect the lack of a sustained and/or dramatic IOP insult and/or an insignificant deleterious effect from medications, age, and chronicity on corneal endothelial parameters.

Keywords: corneal endothelial cell density, coefficient of variation, hexagonality, primary angle closure disease spectrum

The corneal endothelium, a single layer of hexagonal-shaped cells, plays an important role in maintaining clear vision. Due to the lack of regenerative ability, preservation of these cells is crucial. Studies have shown that corneal endothelial cells could be irreversibly lost if the cornea is subjected to insults, such as intraocular surgery1-4 or inflammation.5 Glaucoma per se, and its management including medications, surgery, and laser procedures are presumed to have deleterious effects on the corneal endothelium. Endothelial cell loss has been reported in primary open angle glaucoma (POAG),6,7 pigmentary,8 pseudo exfoliation,9 inflammatory glaucoma,10 following an acute angle-closure attack11-15 and primary angle closure glaucoma (PACG).15,16; however, a few contradictory studies did not find such association of endothelial cell loss in glaucoma.17,18

Many of the previous studies that reported lower endothelial cell density (ECD) in angle closure glaucoma have compared them with normal controls that had not undergone any laser procedures. In a comparative study, Varadaraj et al.19 compared the ECD in eyes with open angles and those with various stages of untreated angle closure disease. They found that primary angle closure suspects (PACS) eyes had lower ECD compared to eyes with open angles; however, there were no difference between primary angle closure (PAC) or PACG eyes and those with open angles.

In addition to the conflicting associations of corneal endothelial cell loss with glaucoma subtypes and elevated IOP, there are also differing associations of corneal endothelial loss with chronicity or duration of glaucoma and the effect of glaucoma medications. There is paucity of published data on the corneal endothelial characteristic across the PAC disease spectrum.

The purpose of this study was to evaluate the corneal ECD and corneal morphological characteristics across the angle closure disease spectrum amongst patients who had undergone sequential argon–Nd: YAG laser peripheral iridotomy (LPI). We hypothesized that the eyes with previous acute PAC (APAC) and PACG have reduced ECD compared to PACS eyes.

MATERIALS AND METHODS

This was a cross-sectional study of subjects with PAC disease spectrum including PACS, PAC, PACG, and those with history of...
previous APAC, who were recruited from the glaucoma clinics of the Singapore National Eye Centre. Approval for the study was granted by the hospital’s institutional review board and the study was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all study subjects.

PACS was defined as present in an eye in which the pigmented posterior trabecular meshwork was not visible on nonindentation gonioscopy for at least 180° in the primary position, and the IOP was less than 21 mm Hg without peripheral anterior synchiae (PAS) or glaucomatous optic neuropathy. PAC was defined as an eye with an occludable drainage angle for at least 180° in the primary position, and features indicating that trabecular obstruction by the peripheral iris had occurred, such as PAS, elevated IOP, iris whorling (distortion of the radially orientated iris fibres), “glaucofleken” lens opacities, or excessive pigment deposition on the trabecular surface. The optic disc did not have glaucomatous damage. PACG was defined as the presence of glaucomatous optic neuropathy (defined as loss of neuroretinal rim with a vertical-cup-disc ratio (CDR) of >0.7 and/or notching with nerve fibre layer defect attributable to glaucoma) with compatible visual field loss, in association with a closed angle (presence of at least 180° angle in which the posterior trabecular meshwork was not visible on gonioscopy).20 Documented evidence of APAC included subjects who previously had IOP greater than 21 mm Hg and the presence of at least three of the following signs: conjunctival injection, corneal epithelial edema, middilated unreactive pupil and a shallow anterior chamber with the presence of an occluded angle in the affected eye, verified by gonioscopy;21; and presence of at least two of the following symptoms: ocular or periorbital pain; nausea, vomiting, or both; an antecedent history of intermittent blurring of vision with haloes. Patients with history of intraocular surgery, primary corneal diseases, secondary angle closure, trauma, and ocular inflammation were not included. All subjects had undergone sequential argon–Nd: YAG LPI on an average of at least 3 years prior to recruitment into the study. Some of our subjects diagnosed as PAC or PACG at the time of recruitment may have undergone LPI when they had presented at an earlier disease stage such as PACS or PAC, and would therefore have shorter disease duration compared to the LPI duration.

All subjects underwent a standardized ophthalmic examination that included visual acuity testing, slit lamp examination, IOP measurement with Goldmann applanation tonometry, stereoscopic evaluation of the optic disc, and visual field testing. Static gonioscopy was performed using a Goldmann 2-mirror lens by an experienced examiner under dark conditions at high magnification (>×16). Indentation gonioscopy with a Sussman 4-mirror lens was used to establish the presence or absence of PAS. A noncontact specular microscope (Konan software, center method) was used to examine the central corneal endothelium and the following parameters were obtained: mean cell density (cells/mm²), coefficient of variation (CV) in cell area, and percentage of hexagonal cells. All measurements were done by single experienced technician. The Konan Noncon Robo is a noncontact specular microscope with an autofocus device. The image of the endothelium is obtained on an incorporated screen. After a clear image of the central endothelium was captured, the centers of at least 100 contiguous endothelial cells were marked using software available in the system, after which the computer performed an automated analysis of the cell parameters that were displayed on the screen and obtained as a printout. Central corneal thickness (CCT) was measured using an ultrasonic pachymeter. The mean of the three readings for each parameter was used for further analysis. In the eyes with previous APAC, all measurements were taken at least 3 weeks after the documented acute angle closure attack. Anterior chamber depth (ACD), axial length (AL), and lens thickness (LT) measurements were obtained using an A-scan ultrasonography. Data on the number of glaucoma medications of the subjects were also obtained.

Statistical Analysis

Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows, version 22.0; IBM-SPSS, Chicago, IL, USA). One eye of each subject was analyzed. If both eyes of a single subject were found to have same diagnosis, the more severe eye based on visual field mean deviation in case of PACG, and greater presenting IOP in case of PACS or PAC was chosen. If both eyes had similar diagnosis and severity, then the study eye was randomly chosen. Comparisons of ocular characteristics and corneal endothelial parameters across the angle closure subtypes were performed using 1-way ANOVA. Intergroup differences in mean values of variables were analyzed using post hoc Bonferroni tests. In addition, effects of presenting IOP total number of glaucoma medication, and CCT were adjusted and analyzed using analysis of covariance. Correlation of continuous data variables was analyzed using the Pearson correlation test (2-sided). A subanalysis was also performed after matching for age across the groups in a ratio of 1:2:2:1 for PACS: PAC: PACG: APAC. Significance was set at $P < 0.05$ for this study.

Results

A total of 529 subjects were included in the study. The demographic characteristics of these subjects are summarized in Table 1. The majority of subjects were Chinese ($N = 486, 91.9\%$) and female ($N = 292, 55.2\%$). The mean age of the subjects was $65.1 \pm 8.2$ years (range, 40–91 years), with PACG comprising the oldest group. Of the 529 subjects, 51 (9.6\%) were diagnosed as PACS, 170 (32.1\%) PAC, 234 (44.2\%) PACG, and 74 (13.9\%) had previous APAC. The subjects in APAC subgroup had the highest presenting IOP ($54.2 \pm 11.2, P < 0.001$) and shortest AL ($22.39 \pm 0.72, P < 0.001$). There were no significant differences in the ACD or LT between the groups. The subjects in PACG group were on the greatest number of glaucoma medications.

Corneal endothelial cell parameters are compared in Table 2. We noted no significant differences in the mean central corneal ECD across the groups ($P = 0.61$). The CV was lowest in PACS ($34.38 \pm 6.05 \text{ \mu m}^2$/cell) and highest in the APAC group ($37.61 \pm 7.98 \text{ \mu m}^2$/cell); however, the differences were not significant ($P = 0.07$) across the groups. The difference reached statistical significance when adjusted for the total number of glaucoma medications. No significant difference was noted for hexagonality ($P = 0.25$). The CCT was significantly thinner in PACG subgroup ($538.83 \pm 37.45, P < 0.001$). While we noted no significant differences in any of the corneal endothelial parameters when we performed a sub-analysis in age-matched subjects across the groups (Table 3), however, the APAC group showed slightly lower ECD ($P = 0.07$).

Table 4 presents correlation of endothelial cell count and CV with IOP at presentation, total number of glaucoma medications, duration of disease, and ACD. CV was modestly correlated (Pearson correlation 0.10, $P = 0.04$) with the IOP at presentation.

We next compared the eyes with previous APAC with their fellow eye (Table 5). Only phakic fellow eyes were included,
and all of them had previously undergone LPI. A total of 30 subjects had data available for both eyes. We noted no significant differences in the corneal endothelial count (P = 0.14), CV (P = 0.96), and hexagonality (P = 0.53) between the previous APAC and fellow eyes. Of note, all the fellow-eyes were diagnosed as PACS.

**DISCUSSION**

In this cross-sectional study, the corneal endothelial cell count and morphological characteristics such as CV and hexagonality were not significantly different across the angle closure disease spectrum. Interestingly, there was no correlation between the corneal endothelial cell count with presenting IOP, disease duration, glaucoma medications, and ACD.

The relationship between glaucoma and corneal endothelial characteristics has not been established conclusively; with conflicting reports on the effect of elevated IOP and glaucoma-medications on the endothelial cell count. The Kumejima Study examined the distribution of ECD and relating factors in ophthalmologically normal Japanese in a population-based setting and found that higher IOP was significantly correlated with lower ECD.22 Gagnon et al.6 found that patients with glaucoma may have lower corneal ECD compared to those without glaucoma of same age group. Their study population mainly comprised POAG and PACG subjects. The authors hypothesized that elevated IOP, congenital alterations in corneal endothelial layer and trabecular meshwork, and/or glaucoma medications-induced toxicity could be the possible mechanisms causing corneal endothelial damage. Supporting the hypothesis of the association between increased IOP and lower ECD, Cho et al.23 reported the endothelial cell count was significantly lower in POAG than NTG. In contrast, a study by Lee et al.24 found significantly lower corneal endothelial cell count in NTG compared to POAG. They postulated that a common hypoperfusion mechanism accounted for both the progressive optic neuropathy and ECD reduction in their NTG population. Our study showed no correlation between presenting IOP and ECD across the angle closure disease spectrum that included eyes with previous APAC that presented with very elevated IOP. It is to be noted that all

**Table 2.** Corneal Parameters Across the PAC Disease Spectrum (N = 529)

<table>
<thead>
<tr>
<th>Corneal Parameters</th>
<th>PACS (N = 51)</th>
<th>PAC (N = 170)</th>
<th>PACG (N = 234)</th>
<th>APAC (N = 74)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cell density (ECD, cells/mm²)</td>
<td>2518.96 ± 472.81</td>
<td>2566.02 ± 408.27</td>
<td>2523.81 ± 406.80</td>
<td>2504.01 ± 558.13</td>
<td>0.61</td>
</tr>
<tr>
<td>Adjusted ECD*</td>
<td>2562.98 ± 68.35</td>
<td>2572.01 ± 54.31</td>
<td>2531.00 ± 52.30</td>
<td>2479.64 ± 53.25</td>
<td>0.48</td>
</tr>
<tr>
<td>Coefficient of variation (CV)</td>
<td>54.38 ± 6.05</td>
<td>57.22 ± 7.70</td>
<td>57.39 ± 7.54</td>
<td>57.61 ± 7.98</td>
<td>0.07</td>
</tr>
<tr>
<td>Adjusted CV*</td>
<td>35.98 ± 1.18</td>
<td>36.92 ± 0.58</td>
<td>37.65 ± 0.55</td>
<td>37.75 ± 0.91</td>
<td>0.04†</td>
</tr>
<tr>
<td>Hexagonality (%)</td>
<td>56.08 ± 10.15</td>
<td>57.45 ± 8.10</td>
<td>57.71 ± 7.99</td>
<td>55.50 ± 8.45</td>
<td>0.23</td>
</tr>
<tr>
<td>Adjusted Hexagonality*</td>
<td>58.42 ± 55.85</td>
<td>57.67 ± 6.65</td>
<td>57.50 ± 6.64</td>
<td>55.39 ± 1.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Central corneal thickness</td>
<td>54.66 ± 30.05</td>
<td>554.61 ± 53.36</td>
<td>538.85 ± 37.45</td>
<td>544.14 ± 39.02</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

Bolded values indicate statistical significance. PACS, primary angle closure suspect; PAC, primary angle closure; PACG, primary angle closure glaucoma; APAC, acute primary angle closure.

* Adjusted for total number of glaucoma medications.
† PACS vs. PAC 0.02; PACS vs. PACG 0.01; PACS vs. APAC 0.009.
‡ PAC vs. PACG < 0.001.
TABLE 3. Age Matched Corneal Parameters Across the PAC Disease Spectrum (N = 252)

<table>
<thead>
<tr>
<th>Corneal Parameters</th>
<th>PACS (N = 42)</th>
<th>PAC (N = 84)</th>
<th>PACG (N = 84)</th>
<th>APAC (N = 42)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.30 ± 6.30</td>
<td>63.15 ± 5.96</td>
<td>63.02 ± 6.27</td>
<td>63.35 ± 6.27</td>
<td>0.99</td>
</tr>
<tr>
<td>Endothelial cell density (ECD, cells/mm²)</td>
<td>2569.3 ± 541.0</td>
<td>2629.4 ± 382.7</td>
<td>2533.5 ± 391.3</td>
<td>2407.2 ± 577.1</td>
<td>0.07†</td>
</tr>
<tr>
<td>Adjusted ECD†</td>
<td>2459.2 (2278.8, 2639.6)</td>
<td>2598.4 (2442.9, 2753.9)</td>
<td>2524.5 (2403.7, 2645.5)</td>
<td>2603.9 (2547.8, 2859.9)</td>
<td>0.60</td>
</tr>
<tr>
<td>Adjusted ECD†</td>
<td>2469.7 (2284.1, 2655.2)</td>
<td>2595.6 (2450.1, 2741.1)</td>
<td>2514.5 (2375.7, 2653.5)</td>
<td>2604.0 (2347.7, 2860.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>Coefficient of variation (CV)</td>
<td>34.41 ± 6.44</td>
<td>36.81 ± 7.16</td>
<td>36.26 ± 7.04</td>
<td>37.63 ± 8.90</td>
<td>0.23</td>
</tr>
<tr>
<td>Hexagonality (%)</td>
<td>58.40 ± 10.76</td>
<td>58.76 ± 7.33</td>
<td>58.96 ± 7.27</td>
<td>55.85 ± 9.18</td>
<td>0.23</td>
</tr>
</tbody>
</table>

PACS, primary angle closure suspect; PAC, primary angle closure; PACG, primary angle closure glaucoma; APAC, acute primary angle closure.
* Adjusted for presenting intraocular pressure (IOP), CCT.
† Adjusted for presenting IOP, total number of glaucoma medications.
‡ PAC vs. APAC: 0.05.

TABLE 4. Correlation of ECD and CV With Other Variables

<table>
<thead>
<tr>
<th>Corneal Parameters</th>
<th>Presenting IOP</th>
<th>Total Glaucoma Medications</th>
<th>Duration of Disease</th>
<th>ACD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cell density (ECD)</td>
<td>−0.091</td>
<td>0.003</td>
<td>−0.045</td>
<td>−0.018</td>
</tr>
<tr>
<td>Pearson Correlation Sig. (2-tailed)</td>
<td>0.07</td>
<td>0.93</td>
<td>0.31</td>
<td>0.68</td>
</tr>
<tr>
<td>Coefficient of variation (CV) Pearson Correlation Sig. (2-tailed)</td>
<td>0.101*</td>
<td>−0.034</td>
<td>−0.048</td>
<td>0.001</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>4.49 ± 0.92</td>
<td>3.80 ± 0.35</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Glaucoma medications</td>
<td>0.80 ± 1.09</td>
<td>0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Corneal curvature</td>
<td>7.64</td>
<td>7.67</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Endothelial cell density (ECD, cells/mm²)</td>
<td>2546.77</td>
<td>2432.20</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation (CV)</td>
<td>37.80</td>
<td>37.87</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Hexagonality (%)</td>
<td>57.47</td>
<td>55.90</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

IOP, intraocular pressure; ACD, anterior chamber depth.
* Correlation is significant at the 0.05 level (2-tailed).

Significant endothelial loss and corneal decompensation have been reported in eyes that underwent LPI. Some of the proposed mechanisms for endothelial damage from LPI include an impairment of the blood-aqueous barrier; alterations in aqueous dynamics and shock waves generated by the laser, and, direct endothelial damage from the laser.26–28 Our study comprised exclusively of subjects with angle closure who had undergone LPI, including those with PACS, the earliest stage of the disease spectrum. This allowed a more homogeneous comparison as all subjects had undergone LPI and would have similar impact on corneal endothelium.

Our study also revealed no significant differences in the ECD in eyes with previous APAC when compared to their fellow PACS eyes. It is likely that changes in structure and function of corneal endothelial cells following an acute increase in IOP is transient and revert to pre-elevated IOP level once the acute crisis resolves. A study by Chen et al.29 found no significant differences in ECD of acute PACG eyes when compared with fellow PACG or chronic PACG eyes, while there was significantly lower ECD when compared with normal. Interestingly, a recent study on a rat model showed that an acute increase in IOP resulted in structural and functional damage of corneal endothelial cells, which gradually reversed when the acute rise in IOP resolved.18 However, some studies11–15 have shown significant decrease in ECD in eyes with a history of APAC. The variable interval between performing specular microscopy and an acute attack or duration of the acute attack across different studies, may contribute to the conflicting associations of APAC and ECD. Unfortunately, we do not have data on the duration of the acute
attack, but we speculate that early and rapid resolution of an acute attack reduces the risk of corneal endothelial damage in the long term.

Our findings of the lack of correlation between ECD and disease duration and ocular biometric parameters corroborates the findings of Chen et al.\textsuperscript{29} in their study of acute PACG eyes. We also noted no correlation between ECD and glaucoma medications. There are conflicting reports on the association of corneal ECD with topical glaucoma medications. Whilst Gagnon et al.\textsuperscript{6} found that subjects receiving three or more glaucoma medications had lower cell counts when compared to those receiving either one or two medications, Korey et al.\textsuperscript{17} noted no significant differences between treated and untreated ocular hypertension with respect to ECD. A study by Lass et al.\textsuperscript{50} showed latanoprost and fixed combination are equivalent to timolol regarding its usage due to the perceived impaired endothelial contraindication for donor corneas, there is concern regarding long-term corneal effects after 1 year of treatment.

Although a positive history of glaucoma is not an absolute contraindication for donor corneas, there is concern regarding its usage due to the perceived impaired endothelial status. Our study shows that subjects with angle closure disease do have acceptable endothelial reserve to serve as donor corneas corroborating with the findings of Korey et al.\textsuperscript{17} though their study was mainly on ocular hypertension and POAG subjects.

The strength of our study was the relatively large sample size with subjects across the entire angle closure disease spectrum using standardized disease classification and definition. Moreover, our study comprised of subjects who have all undergone LPI, thus allowing a more uniform comparison. One of the study limitations was the cross-sectional nature of the study; hence it is difficult to establish temporal or causal relationships. Secondly, the duration of IOP spike in eyes documented with acute attack was not available. In addition, it is likely that some subjects who may have presented with severe APAC attack may have undergone early surgical intervention and were therefore not included in the study, thus limiting the information of eyes with severe acute attack on ECD. Additionally, it is also possible that the current sample size for APAC subjects may not be sufficient to reveal statistical difference between the groups. Thirdly, although our subjects underwent sequential LPI under standardized settings, however, data on the energy used for individual patients were not available. Lastly, ECD is an indirect measure of corneal thickness response to intraocular pressure. Acta Ophthalmol. 1980;58:250–256.


