Original article

Morphometric changes of corneal endothelial cells in pseudoexfoliation syndrome and pseudoexfoliation glaucoma

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ARTICLE INFO

Article history:
Received 14 December 2012
Accepted 11 June 2013
Available online 25 December 2013

Keywords:
Corneal endothelium
Central corneal thickness
Pseudoexfoliation glaucoma
Specular microscopy
Pseudoexfoliation syndrome

ABSTRACT

Objective: To evaluate the corneal endothelial morphometry and central corneal thickness (CCT) in pseudoexfoliative (PEX) eyes with and without glaucoma and to compare with normal eyes and eyes with primary open-angle glaucoma (POAG).

Method: A total of 166 patients were included in this study: 36 eyes with pseudoexfoliation syndrome (PXS), 30 eyes with pseudoexfoliation glaucoma (PXG), 40 eyes with POAG, and 60 normal eyes. Corneal endothelial cell density (ECD), coefficient of variation (CV) in cell size, and percentage of hexagonal cells were measured using a non-contact specular microscope, whereas CCT was measured with an ultrasonic pachymeter.

Results: ECD and percentage of hexagonal cells were lower in PEX groups and in the POAG group compared with normal eyes, while the CV in cell size was greater. There was a tendency for greater cell loss and morphological abnormalities of the corneal endothelial cells in PXG eyes compared to PXS eyes, when all pseudoexfoliative eyes were analyzed together. Changes in endothelial cells increased with age. There were no significant differences in mean CCT between the four groups.

Conclusion: Endothelial cell density is significantly decreased, and pleomorphism and polymegathism of cells are increased in PEX eyes, particularly when intraocular pressure is high.

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Cambios morfométricos de las células del endotelio corneal en el síndrome pseudoexfoliativo y glaucoma pseudoexfoliativo

PALABRAS CLAVE:
Endotelio corneal
Espesor corneal central
Glaucoma seudoexfoliativo
Microscopía especular
Síndrome seudoexfoliativo

RESUMEN

Objetivo: Analizar el patrón morfométrico de las células endoteliales de la córnea y el endotelio central (ECC) en los ojos con pseudoexfoliativo (PEX) y con glaucoma, y compararlos con ojos normales y con glaucoma primario de ángulo abierto (GPAA).

Método: Se incluyeron 166 pacientes en el estudio: 36 sujetos con síndrome seudoexfoliativo, 30 con glaucoma seudoexfoliativo (GPEX), 40 con GPAA y 60 pacientes normales. Los parámetros evaluados con el microscopio especular de no contacto fueron la densidad de células endoteliales (DCE), el coeficiente de variación (CV) del tamaño celular y el porcentaje de hexagonalidad del ECC se midió con paquimetria de contacto.

Resultados: La DCE y el porcentaje de hexagonalidad celular fueron menores en los sujetos con PEX y GPAA respecto al grupo control, mientras que el CV del tamaño celular fue mayor. Considerando los 2 grupos de ojos con PEX, se observó una tendencia hacia una mayor pérdida de células endoteliales y de modificaciones en los parámetros morfométricos en los ojos con GPEX. Las alteraciones en el patrón especular aumentaron progresivamente con la edad. No hubo diferencias significativas en el valor medio de ECC entre los 4 grupos.

Conclusión: La densidad de células endoteliales está significativamente disminuida y el pleomorfismo y polimegatismo celular incrementado en los ojos con PEX, especialmente cuando la presión intraocular es alta.

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Introduction

Pseudoexfoliation syndrome (PXS) is an age-related systemic disease characterized by the progressive production and accumulation of extracellular fibrilar material in various ocular tissues and other organs. These deposits can be observed through biomicroscopy as a white grayish material in various structures of the anterior chamber of the eye, mainly on the edge of the pupil and the lens capsule.

PXS is the most frequent identifiable cause of open-angle glaucoma throughout the world. Despite its significance, the exact composition of the pseudoexfoliation material (PEX) and the pathogenicity thereof are not yet entirely clear. It has been demonstrated that the various types of cells in anterior segment structures, including corneal endothelial cells, are capable of synthesizing said fibrilar material.

The morphological and functional integrity of the endothelium is essential for the cornea to remain transparent. Normal corneal endothelium is like a mosaic of hexagonal cells with well-defined limits. As of birth, endothelial cells lose the ability to divide, although they are able to extend and functionally compensate cell losses which can occur as a consequence of aging, traumatisms or corneal diseases. This process induces morphological changes in the cells which express as a progressive increase in size (polymegatism) and a modification of the polygonal profile (pleomorphism), thus reducing the percentage of hexagonal cells.

The condition of the endothelial layer is determined by measuring corneal thickness, an indirect reflection of its condition, and quantitative and qualitative analysis of its cells by means of specular microscopy which provides valuable information about the function and feasibility of the cornea by allowing in vivo observation of endothelial cells with large increases.

Several papers in medical literature describe changes in the characteristics of endothelial cells in eyes with PEX, but very few papers have been published on the possible differences between patients with pseudoexfoliative syndrome and pseudoexfoliative glaucoma (PXG). This paper assesses qualitative and quantitative changes in the corneal endothelial cells and the modifications in central corneal thickness of patients with PXS and PXG with the objective of establishing the existence of differences between these 2 groups as well as comparing them with normal subjects and glaucomatous patients.

Subjects, material and methods

The study included 166 patients who visited the Ophthalmology Service of Salamanca University Hospital. They underwent a complete ophthalmological exploration comprising slit lamp biomicroscopy, intraocular pressure (IOP) measured with Goldmann appplanation tonometry, gonioscopy, central corneal pachymetry, capillary funduscopic assessment, computerized perimetry with Humphrey field analyzer and retinal nerve fiber layer thickness assessment by means of optic coherence tomography (OCT). The patients were consecutively selected and assigned to one of the 4 groups of the study, i.e., pseudoexfoliative syndrome, pseudoexfoliative glaucoma, open-angle primary glaucoma (OAPG) and healthy patients. Approval was obtained from the Ethical Committee of the hospital and informed consent from participating patients.

The pseudoexfoliation diagnostic was based on the biomicroscopic observation of the PEX material in the pupil edge or lens. The glaucoma diagnostic was established by means
of IOP > 22 mmHg, glaucomatous morphology of papilla (thin neuroretinal ring with increased excavation), alteration of the nervous fiber layer in OCT and/or glaucomatous damage in the visual field. Patients with glaucoma were divided into 3 groups: subjects with topical medication for at least one year prior to their inclusion in the study, patients treated less than one year and patients with no treatment.

The study excluded subjects with history of ocular traumatism or surgery, previous laser treatments, and ocular diseases (corneal disease, intraocular inflammation) and contact lens users. As the prevalence of the pseudoexfoliation syndrome increases with age, the study also excluded patients under 60 years of age.

Only one eye of each patient was included in the analysis and accordingly the number of studied eyes was the same as the number of participants.

The corneal endothelium was evaluated by means of non-contact specular microscopy (Topcon SP-3000P, Topcon, Corp., Tokyo, Japan). Images of the central cornea with an overall number of cells above 100 were taken, and the value was the mean of 3 measurements. The images were automatically analyzed by the IMAGEnet 2000 system. Parameters included in the calculation were endothelial cell density (ECD) (cells/mm²), cell size coefficient of variation (CV) (an objective polymegatism parameter), and the hexagonality percentage (pleomorphism index).

Central corneal thickness (CCT) was measured with the contact pachymeter (DGH 5100e Technology, Inc., Exton, PA, USA) under topical anesthesia. Five measurements were taken and the mean was registered.

Data were analyzed with the SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). A descriptive analysis was performed and the adjustment to normality of the data were verified. The T for student test was used to compare the results between 2 groups and variance analysis (ANOVA) with Bonferroni correction was used to identify differences between the age groups classified patients between 60 and 69 years, between 70 and 79 years and over 80 years. Pearson’s correlation coefficient ($r$) was used for the analysis between pairs of quantitative variables. The value of $p < 0.05$ was considered for statistical significance.

Results

The study included the data of 166 patients, who were divided into 4 groups: 36 subjects with PXS, 30 with PXG, 40 with OAPG and 60 healthy controls. The demographic characteristics of the participants are summarized in Table 1. No significant differences were found between sex and age among the patients of the different groups.

The characteristics of the analyzed eyes are summarized in Table 2. The mean CCT value was significantly lower in the groups with PEX and with OAPG vis-à-vis the control group. In addition, the endothelium of eyes with PXS, PXG and OAPG exhibited a lower percentage of hexagonal cells and higher cell size CV in comparison with the endothelium of healthy eyes. Considering the 2 groups of eyes with PEX, no significant differences were found in the mean values of analyzed endothelial parameters, even though a tendency was observed toward loss of cells and higher morphological anomalies in eyes with PXG.

The assessment of CCT did not produce statistically significant differences between mean values in the PXG, PXG and OAPG groups and the control group.

The mean IOP was significantly higher in the PXG and OAPG compared to the control group.

The analysis of endothelial parameters of patients with ocular hypertension (PXG and OAPG) on the basis of the use of topical medications did not produce significant differences (Table 3).

Table 4 illustrates the mean values of studied corneal parameters by age groups. The CCT results indicate that said parameters diminish together with the increase in patient age. Statistically significant differences were found between the younger and older groups. In addition, a tendency was observed toward increased pleomorphism and polymegatism with age but without significant differences between groups.

The mean CCT value also diminished with age in each group of patients (normal, PXS, PXG and OAPG). This parameter was lower in eyes with PXG for each age group (Table 5).

Taking into account the data of all participants, a positive correlation was observed between CCT and the hexagonality percentage ($r = 0.21$, $p < 0.001$), and a negative correlation between the hexagonality percentage and cell size CV ($r = -0.30$, $p < 0.001$). However, no correlation was found between CCT and cell size CV, or between IOP and CCT with endothelial parameters.

Discussion

The conditions which give rise to corneal endothelial lesions not only diminish its density but also determine alterations in the morphological pattern thereof. Accordingly, cell size and shape variations are more specific endothelial damage indicators than only cell density.

There are a number of studies that describe the reduction of endothelial cells with age because these cells appear to have little or no possibility of dividing after birth. The loss of these cells involves an increase in size and a reduction of hexagonality. The results of this study are consistent with the evidence reported in medical literature as an increase in endothelial morphology and density alterations together with the increase in patient age was found.

However, there are factors that can contribute to reducing the number of endothelial cells faster than normal age-related reductions. One of these factors seems to be the presence of PEX material. By means of electronic microscopy, it has been demonstrated that in eyes with PEX, the corneal endothelium actively participates in the production of said pathological material. The sedimentation on cells can lead to their degeneration and, with time, produce alterations in their number and morphology. In addition, hypoxic changes have been described in the anterior chamber as well as variations in the composition and dynamics of the aqueous humor caused by the rupture of the blood barrier in eyes with PEX, which could affect the function of the endothelium. These findings contribute to explain the results of this study in which it has been observed that eyes with PEX lose cells in the corneal endothelium together with significant variations in the cell size and shape.
shape when compared to eyes without PEX in patients within the same age group.

The first study to analyze said endothelial changes in eyes with PEX was carried out by Miyake et al. who observed a marked endothelial density reduction as well as significant modifications in cell pleomorphism and polymegatism. Other studies referred similar results, including Naumann and Schlötzer-Schrehardt who related the

| Table 1 – Demographic characteristics of patients in each group of the study. |
|---------------------------------|---|---|---|---|
|                               | Normal | PSX | PXG | OAPG |
| No.                            | 60     | 36  | 30  | 40   |
| Age (years)                    |        |     |     |      |
| Mean ± SD                      | 77.2 ± 7.1 | 76.5 ± 6.9 | 77.2 ± 7.3 | 75.9 ± 7.2 |
| Range                          | 60–87  | 62–88 | 60–89 | 61–87 |
| Age distribution, n (%)        |        |     |     |      |
| 60–69                          | 20 (33.3) | 12 (33.3) | 9 (30) | 13 (32.5) |
| 70–79                          | 21 (35)  | 12 (33.3) | 10 (33.3) | 14 (35) |
| ≥80                            | 19 (31.6) | 12 (33.3) | 11 (36.6) | 13 (32.5) |
| Sex, n (%)                     |        |     |     |      |
| Man                            | 28 (46.6) | 18 (47.3) | 14 (46.6) | 18 (45) |
| Women                          | 32 (53.3) | 19 (52.7) | 15 (53.3) | 22 (55) |

SD, standard deviation; OAPG, open angle primary glaucoma; PXG, pseudoexfoliative glaucoma; No, sample size; PXS, pseudoexfoliative syndrome.

| Table 2 – Corneal endothelial characteristics, corneal thickness and intraocular pressure of the study groups. |
|---------------------------------------------------------------|---|---|---|---|
|                               | Normal | Mean ± SD | PXS | Mean ± SD | PXG | Mean ± SD | OAPG | Mean ± SD |
|                               |       |           |    |        |    |        |    |        |
| Endothelial cell density (cells/mm²)                          | 2565.14 ± 270.17 | 2346.50 ± 256.88 | 0.000 | 2246.10 ± 251.63 | 2294.77 ± 235.82 | 0.000 |
| Cell size variation coefficient                                | 33.06 ± 4.2 | 35.41 ± 7.03 | 0.04 | 36.14 ± 6.89 | 35.93 ± 6.77 | 0.01 |
| Hexagonality (%)                                               | 57.55 ± 6.8 | 53.22 ± 6.4 | 0.003 | 51.03 ± 6.3 | 51.15 ± 6.15 | 0.000 |
| Central corneal thickness (µm)                                 | 540.96 ± 28.5 | 533.51 ± 31.2 | 0.23 | 531.23 ± 29.6 | 550.30 ± 29.1 | 0.11 |
| Intraocular pressure (mmHg)                                   | 15.3 ± 2.4 | 15.8 ± 2.7 | 0.35 | 17.6 ± 3.7 | 17.2 ± 3.4 | 0.001 |

SD, standard deviation; OAPG, open angle primary glaucoma; PXG, pseudoexfoliative glaucoma; No, sample size; PXS, pseudoexfoliative syndrome.

<table>
<thead>
<tr>
<th>Table 3 – Corneal endothelial morphometric analysis of glaucoma patients without treatment and patients receiving over one year of treatment.</th>
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<tr>
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<td></td>
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<tr>
<td>Endothelial cell density (cells/mm²)</td>
</tr>
<tr>
<td>Cell size variation coefficient</td>
</tr>
<tr>
<td>Hexagonality (%)</td>
</tr>
</tbody>
</table>

Data shown as mean ± standard deviation.
OAPG, open angle primary glaucoma; PXG, pseudoexfoliative glaucoma.

| Table 4 – Corneal endothelial morphometric analysis per age group. |
|-------------------------------------------------------------------|---|---|---|---|
|                                                           | 60–69 (n = 54) | 70–79 (n = 57) | ≥80 (n = 55) | p   |
| Endothelial cell density (cells/mm²)                            | 2394.92 ± 286.17 | 2506.95 ± 259.72 | 2394.07 ± 257.25 | 2285.79 ± 301.21 | 0.000** |
| Cell size variation coefficient                                 | 34.82 ± 5.90 | 34.05 ± 6.42 | 34.62 ± 5.74 | 35.78 ± 5.49 | 0.29 |
| Hexagonality (%)                                                 | 53.89 ± 7.02 | 54.29 ± 7.14 | 53.75 ± 7.43 | 53.63 ± 6.58 | 0.87 |

Data shown as mean ± standard deviation.
* Statistically significant differences between 60–69 year and ≥80 year groups.
endothelial alterations observed in eyes with PEX to true keratopathy with its own entity capable of causing corneal decompensation with moderate IOP increases or after cataract surgery. However, other studies did not find significant modifications in endothelial morphometric parameters in eyes with PEX.8,15

High IOP is another factor that could contribute to increase in endothelial cell loss. In this study the authors observed that OAPG cases had lower CCT and higher alterations in the size and form of these cells than normal eyes. This finding is similar to that of previous studies.16–18 Gagnon et al.19 described that patients with glaucoma had a lower number of endothelial cells than subjects without glaucoma at the same age. The mechanism they propose is direct damage of the cornea due to high IOP, congenital corneal endothelium alterations, topical medication toxicity or a combination thereof.

The effect of topical antiglaucoma drugs on corneal endothelial cells is the subject of controversy. Ranno et al.20 confirmed that patients with this medication exhibit diminished cell densities. However, Baratz et al.21 did not observe differences between subjects with and without treatment. The patients of this study with chronic topical medication exhibited cell densities similar to those who were not under treatment, although it must be taken into account that the number of patients in this subgroup was small. Even though it is likely that high IOP damages endothelial cells in these eyes, the amount of time the endothelium must be exposed to high pressure to begin to reduce cell density is unknown.

No differences were found in CCT, cell size CV or hexagonality percentage between the group of patients with PXS and PXG, although both exhibited significant changes in these parameters vis-à-vis the control group. However, in eyes with PXG endothelial counts tended to be lower and qualitative changes in cell size and shape tended to be higher when compared with eyes with PXS and OAPG. Therefore, the functional capacity of endothelial cells that translate morphometric indicators would be more altered in eyes with PEX and glaucoma than eyes with PEX only. This indicates that the pseudoexfoliation process per se could be the cause of endothelial specular pattern changes, while IOP increase is an added factor that increases said changes.

In the medical literature, there is no consensus about CCT in eyes with PEX. Several studies have pointed out that the cornea is thinner in these cases,7,22 although other studies have not confirmed this.23,24 These alterations are probably secondary to changes in endothelial cells because, as commented above, corneal thickness is an indirect indicator of endothelial function. In this study, pachymetry did not exhibit significant variations between the 4 groups and no correlation was found between CCT and the analyzed endothelial parameters.

Even though eyes with PEX do not exhibit manifest endothelium dysfunction at exploration, a study of cell density and morphology when considering a cataract operation becomes particularly relevant because this surgery is another factor that induces aggression against endothelial cells.24 Tissue characteristics in eyes with PEX predispose patients to complications during said operation1 and this could induce greater risk of losing endothelial cells, which is worsened if the corneal endothelium is already affected.

In conclusion, this study confirms the existence of qualitative and quantitative modifications in endothelial cells of eyes with PEX, particularly when IOP is high and patients are older and are not conditioned by antiglaucoma treatments or in relation to pachymetry values. Identifying corneal endothelium alterations prior to cataract surgery in these patients must lead us to consider the adequate measures to minimize intra-surgery endothelial cell loss and avoid post-surgery corneal failure.

**Table 5 – Endothelial cell density per age in each group of patients.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Normal Mean ± SD</th>
<th>PXS Mean ± SD</th>
<th>PXG Mean ± SD</th>
<th>OAPG Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>60–69</td>
<td>2658.30 ± 270.17</td>
<td>2497.64 ± 163.01</td>
<td>2350.12 ± 285.90</td>
<td>2455.87 ± 174.79</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>70–79</td>
<td>2568.23 ± 200.16</td>
<td>2308.86 ± 262.35</td>
<td>2366.20 ± 231.45</td>
<td>2263.59 ± 238.75</td>
<td>0.000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥ 80</td>
<td>2463.64 ± 335.84</td>
<td>2272.95 ± 259.69</td>
<td>2056.35 ± 182.69</td>
<td>2167.24 ± 203.87</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SD, standard deviation; OAPG, open angle primary glaucoma; PXG, pseudoexfoliative glaucoma; No, sample size; PXS: pseudoexfoliative syndrome.

<sup>a</sup> Statistically significant differences between PXG and normal group.
<sup>b</sup> Statistically significant differences between PXS, PXG, OAPG and normal group.
<sup>c</sup> Statistically significant differences between PXG, OAPG and normal group.

**Conflict of interests**

No conflict of interests has been declared by the authors.

**REFERENCES**