Research Article

Qualitative and quantitative changes of corneal endothelial cells and central corneal thickness in pseudoexfoliation syndrome and pseudoexfoliation glaucoma

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Abstract

Background: Pseudoexfoliation syndrome (PXS) is age-related systemic microfibrillopathy caused by the gradual deposition of extracellular grey and white material over various tissues. PXS is frequently associated with secondary open-angle glaucoma. In pseudoexfoliation eyes, corneal endothelial changes have been reported. The present study analyzed corneal endothelial morphometry and central corneal thickness in PXS and pseudoexfoliation glaucoma (PXG).

Objectives: To evaluate qualitative and quantitative changes of corneal endothelial cells and central corneal thickness in pseudoexfoliative (PEX) eyes with and without glaucoma and to compare with normal eyes and eyes with primary open-angle glaucoma (POAG).

Material and Methods: A total of 80 patients were included in this study: 20 eyes with PXS, 20 eyes with PXG, 20 eyes with POAG, and 20 normal eyes. Corneal endothelial cell density (ECD), coefficient of variation (CV) in cell size, percentage of hexagonal cells, and central corneal thickness were measured using a non-contact specular microscope.

Results: ECD (p-value < 0.0001) and percentage of hexagonal cells (p-value < 0.0001) were lower in PEX groups and in the POAG (p-value < 0.0001 and < 0.0233, respectively) group compared with normal eyes, while the CV (p-value < 0.0001) in cell size was greater. There was a tendency for greater cell loss and morphological abnormalities of the corneal endothelial cells in PXG compared to PXS, when all PEX eyes were analyzed together. Changes in endothelial cells increased with age. There were significant thin cornea (p-value < 0.0002) in PXG and POAG (p-value < 0.0001) as compare to PXS and control group.

Conclusion: Endothelial cell density is significantly decreased, and pleomorphism and polymegathism of cells are increased in PEX eyes, particularly when intraocular pressure is high. In PXG eyes, central cornea is thin as compared to PXS eyes.

KEY WORDS: Corneal endothelium, central corneal thickness, pseudoexfoliation glaucoma, pseudoexfoliation syndrome, specular microscopy

Introduction

Pseudoexfoliation syndrome (PXS) is an age-related systemic microfibrillopathy, caused by gradual deposition of extracellular grey and white material over various tissues.[1]

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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, the lens capsule, corneal endothelium, iris, trabecular meshwork, zonule, and ciliary body.[2] PXS is frequently associated with secondary open-angle glaucoma, known as pseudoexfoliation glaucoma (PXG), which is the most common identifiable form of secondary open-angle glaucoma worldwide.[2] Pseudoexfoliation (PEX) is a known risk factor for developing cataracts.[3]

The morphological and functional integrity of the endothelium is essential for the cornea to remain transparent.[4] In PEX eyes, corneal endothelial changes have been reported, including decreased cell density, higher coefficient of variation

in cell size, and lower percentage of hexagonal cells.[5–7] On the other hand, some studies reported no such significant changes in eyes with PEX.[8]

Several papers in medical literature describe changes in the characteristics of endothelial cells in eyes with PXS,[5–8] but very few papers have been published on the possible differences between patients with PXS and 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.

Material and Methods

The study included 80 patients of age > 50 including both male and female who visited the Ophthalmology Department of Sardar Patel Medical College and Associated Group of Hospitals, Bikaner. After enrolment a thorough clinical examination of both eyes including visual acuity both distant (Snellen's chart) and near (Jaeger's chart), slit lamp examination of anterior segment, gonioscopy, applanation tonometry, fundoscopy, computerized perimetry with Humphrey field analyzer, specular microscopy, and systemic examination carried out. Four groups were made, respectively, denoting 20 normal eyes serving as a control group for rest three groups (CN), 20 eyes with PXS, 20 eyes with PXG, and 20 eyes with primary open-angle glaucoma (POAG). Corneal endothelial morphometry and central corneal thickness were studied using the non-contact type TOMEY EM 3000 Specular Microscope, with automated analysis. The parameters under our study included (Figure 1): central endothelial cell density (ECD), which is the number of cells per square millimeter, coefficient of variation (C.V.), percentage of hexagonal cells and central corneal thickness (CCT). PXS based on the presence of typical pseudoexfoliation material at the pupil border on undilated examination, on anterior lens capsule on dilated examination, or on the trabecular meshwork on Gonioscopy, with or without Sampaolesi's line and pigment deposition in angle and/or corneal endothelium.

Diagnosis of PXG was based on the presence of typical exfoliation material on the anterior lens capsule in one or both eyes with typical glaucomatous cupping and visual field defect, IOP > 22 mmHg with an open angle in gonioscopic examination.

Diagnosis of POAG was based on the presence of two out of following three parameters – typical glaucomatous cupping and visual field defect and IOP >22 mmHg with open angle in gonioscopic examination.

The study excluded patients with previous history of mechanical or chemical trauma, known corneal degeneration and dystrophies, inflammation, history of contact lens wear, previous laser treatment, under 50 years of age, previous surgery and angle closure glaucoma.

The data was analyzed by Student's unpaired *t*-test by SPSS 6 version software. The value of $p < 0.05$ was considered for statistical significance.

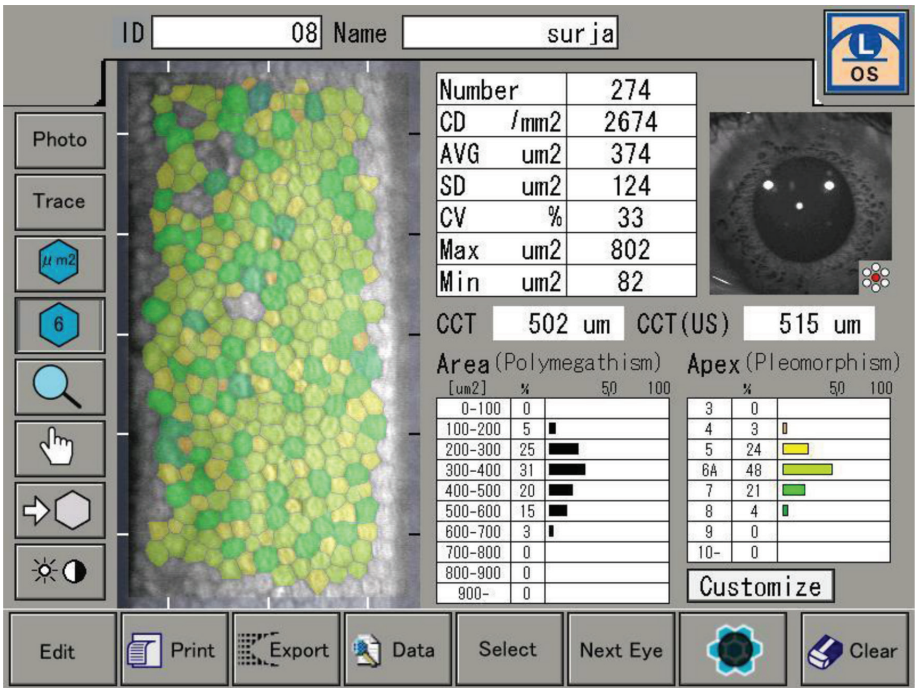

Figure 1: Specular photomicrograph from non-contact type TOMEY EM 3000 Specular Microscope

Ethical Issues

The study was conducted after taking permission and approval from Institute Review Board and Ethical Committee.

Results

The study included the data of 80 patients, who were divided into 4 groups: 20 subjects with PXS, 20 with PXG, 20 with POAG and 20 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 patients with PXS had statistically significant decreased mean value of endothelial cell density (2124 ± 116 cells/mm^2) with in lower percentage of hexagonal cells (46.70 ± 6.45%) and higher cell size coefficient of variation (39.05 ± 3.0%) (p-value < 0.05) as compared to age matched individuals in control group. Patients with PXS had no statistically significant difference in central corneal thickness (524 ± 16.19 μm) as compared to matched individuals in control group (530 ± 26.20 μm).

The patients with PXG decreased mean value of endothelial cell density (2062 ± 121.1 cells/mm^2), central corneal thickness (504.4 ± 11.13 μm) and percentage of hexagonal cells (48.25 ± 6.30%) and increased Coefficient of variation of cell size (42.10 ± 4.94%) (p-value < 0.05) as compared to age matched individuals in control group.

The mean value of endothelial cell density (cells/mm^2) and hexagonality (%) were decreased in PXG patients as compared to PXS group, but it was statistically insignificant with (p = 0.1051 NS) and (p = 0.447 NS), respectively. However patients with PXG had statistically significant increased coefficient of variation of cell size (p = 0.0235*) and decreased central corneal thickness (p < 0.0001***) as compared to patients with PXS.

The mean value of endothelial cell density (cells/mm^2) and hexagonality in PXG patients was decreased as compared to primary open-angle glaucoma group which was statistically significant with p-value (p = 0.0010**) and (p < 0.00001***),

Table 1: Demographic characteristics of patients in each groups in the study

Characteristics	CN (Group A) (*N* = 20)	PXS (Group B) (*N* = 20)	PXG (Group C) (*N* = 20)	POAG (Group D) (*N* = 20)
Age (years)				
55–65	15 (75%)	6 (30%)	4 (20%)	16 (80%)
66–75	4 (20%)	8 (40%)	12 (60%)	2 (10%)
>75	1 (5%)	6 (30%)	4 (20%)	2 (10%)
Mean ± SD	64.80 ± 5.435	71.95 ± 11.31	70.65 ± 6.167	64.60 ± 6.353
Range	55–81	57–95	62–83	56–80
Gender				
Male	12 (60%)	11 (55%)	10 (50%)	13 (65%)
Female	4 (40%)	9 (45%)	10 (50%)	7 (35%)

Table 2: Corneal endothelial characteristics and central corneal thickness of the study groups

	CN (Group A), Mean ± S.D	PXS (Group B)		PXG (Group C)		POAG (Group D)	
		Mean ± S.D.	*p*-Value	Mean ± S.D.	*p*-Value	Mean ± S.D.	*p*-Value
Endothelial cell density / (cells/mm^2)	2511 ± 171.3	2124 ± 116.0	< 0.0001***	2062 ± 121.1	< 0.0001***	2257 ± 120.1	< 0.0001***
Cell size variation coefficient (%)	32.23 ± 2.686	39.05 ± 3.0	< 0.0001***	42.10 ± 4.94	< 0.0001***	36.0 ± 3.129	0.0002***
Hexagonality (%)	59.30 ± 2.227	46.70 ± 6.45	< 0.0001***	48.25 ± 6.30%	< 0.0001***	56.50 ± 4.807	0.0233**
Central Corneal thickness (µm)	530.8 ± 26.20	524.8 ± 16.19	0.3892NS	504.8 ± 11.13	0.0002***	500.4 ± 18.61	0.0001***

Table 3: Comparison of corneal endothelial characteristics and central corneal thickness between PXS and PXG

	PXS (Group B), Mean ± S.D.	PXG (Group C)	
		Mean ± S.D.	*P*-Value
Endothelial cell density (cells/mm^2)	2124 ± 116.0	2062 ± 121.1	0.1051
Cell size variation coefficient	39.05 ± 3.0	42.10 ± 4.94	0.0235*
Hexagonality (%)	46.70 ± 6.45	48.25 ± 6.30	0.447
Central corneal thickness (µm)	524.8 ± 16.19	504.8 ± 11.13	< 0.0001***

respectively. Coefficient of variation of cell size and central corneal thickness (μm) was statistically significant more in PXG patients as compared to primary open-angle glaucoma group ($p = 0.0032$**) and ($p < 0.0001$***), respectively.

Endothelial cell density per age in each group is shown in Table 5. The inference drawn from Table 5 is that the endothelial cell loss increases more progressively with advancing age in PXS and PXG patients as compared control group.

Discussion

Since 1917 when the Finnish ophthalmologist Lindberg first described the PXS, the main intraocular production sites of pseudoexfoliation material have been identified as the epithelial cells of the lens capsule, the iris, the non-pigmented ciliary epithelium, and the trabecular meshwork, as well as the corneal endothelial cells.[1] 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 endothelium 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 have little or no possibility of dividing after birth. The loss of these cells involves an increase in size and a reduction of hexagonality.[8] 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.

The age group of patients included for the study ranged from 55 to 95 years of age. Maximum number of patients of PXS and PXG were in between 66 and 75 years of age (Table 1). This reflects that disease is more prevalent in the older age group. The study also showed that endothelial cell loss increases more progressively with advancing age in PXS and PXG patients as compared to control group. de Juan-Marcos et al's[10] study also showed the similar results.

The study showed that decreased cell density of the corneal endothelium occurred in eyes of patients from the PXS group. Our study also showed that endothelial cell density in PXG group was more decreased, however, no significant statistical variance has been shown between these groups ($p = 0.1051$). In the control group the cell density of the endothelium was the highest (2511±171.3 cells/mm^2) and was significantly different from the values obtained for the other study groups ($p < 0.0001$). Our study also showed that endothelial cell density in PXG group was statistically significant ($p < 0.0001$) lower as compared to patients in primary open-angle glaucoma group.

PXS significantly influences cell density of corneal endothelium of people with this disease. The cause of lower endothelial cell density of patients with PXS is the pseudoexfoliation material, appearing at the earliest stages of pseudoexfoliation, which settles on the endothelium penetrating it in the direction of the Descemet's membrane and breaking the connections between individual six-sided cells, which results in local accelerated apoptosis of these cells. Other factors recognized by researchers, excluding the accumulation of PEX material causing the reduction of the number of cells within the layer of the corneal endothelium, include hypoxia of the anterior chamber, changes in the fibroblasts of the endothelium, and elevated concentration of TGF-α1.[9,10] The simultaneous occurrence of glaucoma further intensifies and accelerates the deterioration of endothelial cells.

Other studies similar results, including Wang et al,[11] Seitz et al,[12] Quiroga et al,[6] Inoue et al,[13] Knorr in 1991,[14] Miyake et al,[5] and de Juan-Marcos et al.[10]

PEX eyes with or without glaucoma have more polymegathism as compare to other group. Similar changes have been reported by Miyake et al[5] and de Juan-Marcos et al.[10] PEX eyes with glaucoma have more polymegathism than PEX eyes without glaucoma.

Table 4: Comparison of corneal endothelial characteristics and central corneal thickness between PXG and POAG

	PXG (Group C), Mean ± S.D.	POAG (Group D)	
		Mean ± S.D.	*p*-value
Endothelial cell density (cells/mm^2)	2062±121.1	2257±120.1	<0.0001***
Cell size variation coefficient	42.10±4.94	36.0±3.129	<0.0001***
Hexagonality (%)	48.25±6.30	56.50±4.807	0.3752 NS
Central corneal thickness (μm)	504.8±11.13	500.4±18.61	<0.0001***

Table 5: Endothelial cell density per age in each group of patients

Age in years	CN (Group A)	PXS (Group B)	PXG (Group C)	POAG(Group D)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
55–65	2572±148.0	2219±84.49	2214±82.25	2297±79.14
66–75	2342±81.37	2132±95.58	2054±81.02	2133±80.61
> 75	2265±0.0	2018±83.22	1930±88.55	2059±197.3

PXS and PXG both exhibited significant changes in these parameters compared to 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 POAG. Therefore, the functional capacity of endothelial cells that translate morphometric indicators would be more altered in eyes with PXG than eyes with PXS only.

The results showed that PEX eyes with or without glaucoma have more pleomorphism as compared to other groups. But no statistically significant difference found between PXS and PXG ($p = 0.447$).Similar results were obtained in studies done by Naumann and Schlotzer-Schrehardt et al,[15] de Juan-Marcos et al,[10] and Miyanke et al.[5]

The results obtained prove that the thinnest corneas occur in eyes of patients with primary open-angle glaucoma (500.4 ± 18.6 μm). Additionally, it has been shown that patients with PXS but without glaucoma had the thick cornea; however, in comparison with the control group this variance was not statistically significant ($p = 0.3892$). However subjects with pseudoexfoliative glaucoma group had statistically significant thinner cornea compared to PXS group ($p < 0.0001$) and control group ($p < 0.0002$). Shah et al[16] proved that patients with PXG had thinner corneas than people with PXS without glaucoma (530.7 versus 553.9 μm) recording a variance in statistical significance reaching a level of $p < 0.001$. Yagci et al[17] and Sobottka et al. also noticed that people with PXG had thinner corneas than the patients of the control group. de Juan-Marcos et al[10] observed that there were no significant differences. Similar results found by Aghaian and colleagues[18] and Bechmann and colleagues.[19]

Conclusion

The present study confirms the existence of significant qualitative and quantitative modifications in endothelial cells of eyes with PEX, particularly when IOP is high which may increase the risk of corneal decompensation after intraocular surgeries. In patients with PXG, CCT is thinner than in the PXS and control group.

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