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Original Article

Ophthalmology Section

An Observational Study of Corneal Endothelial Alterations in Eyes with Unilateral Pseudoexfoliation

ARCHANA THOOL[1], RASHMI RAMANI[2], PRAVIN TIDAKE[3]

ABSTRACT

Introduction: Pseudoexfoliation (PXF) is an age-related generalised disorder of extracellular matrix primarily affecting eyes. It is characterised by deposition of pseudoexfoliative material in the anterior segment of eye. Also it is a bilateral condition but can have asymmetric presentation and can be clinically seen only in one eye. Alteration in endothelial cell morphology along with other alteration in anterior segment of the eye can compromise the surgical outcome.

Aim: To evaluate the corneal endothelial cell density and morphology in patients with unilateral PXF.

Materials and Methods: The present study was hospital-based cross-sectional observational study which included 55 patients with unilateral PXF, who came to the Department of Opthalmology, Acharya Vinoba Bhave Rural Hospital (AVBRH), Wardha, Maharashtra, India between June 2020 to November 2020. Specular microscopy was performed on all eyes to evaluate cell density and morphology, coefficient of variation in cell size and percentage of hexagonal cells in corneal endothelium and compared with fellow normal eye. Statistical analysis was done by using descriptive and inferential statistics using students unpaired t-test, p-value <0.05 was considered as level of significance.

Results: The present study included 55 patients, of which 26 were males and 29 were females. Mean age of the patients was 63.85±7.05 years. Majority of patients were in the range of 61 to 70 years (56.36%). The mean corneal endothelial cell density in the PXF eye was 2299.54±84.95 cells/mm^2, which was significantly lower as compared to fellow normal eye with p-value <0.001. Mean coefficient of variation of cell size in PXF eye was 35.49±4.62, p-value=0.064 and hexagonality was 51.12±4.91, p-value=0.13. Both the parameters were statistically non significant on comparison of two eyes. Central corneal thickness was thicker in pseudoexfoliative eye of 551.29±33.11 microns (µm), than fellow eye but was statistically non significant.

Conclusion: Eyes with PXF had alteration in endothelial cell morphology with decrease in cell density as compared to eyes without PXF clinically.

Keywords: Central corneal thickness, Endothelial cell morphology, Pseudoexfoliative material, Specular microscopy

INTRODUCTION

Pseudoexfoliation (PXF) is a common age related disease of extracellular matrix. Clinically, it is characterised by abnormal formation and deposition of abnormal extracellular fibrillar material on various anterior segment structures [1,2]. The disease is associated with mutation of Lysyl Oxidase Like 1 (LOXL1) gene located on chromosome 15. It codes for elastic fiber components of extracellular matrix suggestive that PXF is a form of elastosis due to overproduction of elastic microfibrillar components such as fibrillin-1 [3]. Intraocular cells producing such material includes nonpigmented cilliary epithelial cells, posterior pigmented cells of iris, lens capsule epithelium, corneal endothelium, trabecular cells and endothelial cells of blood vessels [4]. This material may contribute to significant biomechanical changes in the iridocorneal angle, iris and ciliary body, and crystalline lens and zonules [5].

The PXF affects up to 30% of people older than 60 years worldwide [6,7]. The PXF is predominantly bilateral condition. According to histopathological studies PXF is usually accumulated in both eyes but frequently encountered as asymmetric and clinically can be seen in only one eye of the patient. Differences in the severity of PXF may result in different morphology and biomechanical responses in both eyes of the same patient [2]. It mainly involves the anterior segment of the eye. Accumulation of these fibrillar material predisposes eye to intraocular manifestations like cataract, melanin dispersion, iridopathy and insufficient mydriasis, blood-aqueous dysfunction, anterior chamber hypoxia, posterior synechiae, zonular instability causing phacodonesis and lens subluxation and corneal endothelial decompensation [4,6,8,9]. Altered composition and increased flare intensity of the aqueous humour due to breakdown of the blood-aqueous barrier in PXF induces corneal endothelial changes [8]. These changes can lead to diffuse keratopathy. Decrease in endothelial cell count, decrease in hexagonality of endothelial cells and increase in coefficient of variation in cell size has been reported in various studies [10,11]. Endothelial changes have also been studied on various imaging modalities like specular microscopy and confocal microscopy [12-14]. Also, histopathological studies have shown atypical non-guttate Fuchs' endothelial dystrophic changes in eyes with PXF [8,15]. However, few studies did not find any significant difference between normal eyes and eyes with PXF [16-18].

Identifying endothelial morphological changes in eyes with PXF is important since any rise in Intraocular Pressure (IOP) or surgical manipulations during any intraocular surgeries can lead to corneal oedema and endothelial decompensation in postoperative period [8,13]. The PXF is one of the most common causes of ocular hypertension and glaucoma [4,6,8,19]. Central corneal thickness affects the intraocular pressure measurement by applanation tonometry [20]. Corneal thickness tends to be more in eyes with PXF [16,21]. The aim of the current study was to evaluate corneal endothelial changes by specular microscopy in eyes with PXF in one eye and compare it with fellow normal eye as seen clinically.

MATERIALS AND METHODS

The study was a rural hospital-based cross-sectional observational study conducted between June 2020 to November 2020 in the Department of Ophthalmology at Acharya Vinoba Bhave Rural Hospital attached to Jawaharlal Nehru Medical College, Sawangi (Meghe) Wardha, a constituent college of Datta Meghe Institute

of Medical Sciences (DMIMS) (Deemed to be University), Nagpur, Maharashtra, India. The study was approved by the Ethics and Research committees of DMIMS (IEC no: DMIMD (DU)/IEC/May 2019/8740) and was carried out in accordance with the tenets of the Declaration of Helsinki.

For this, 55 patients with unilateral PXF were sequentially included in the study attending the ophthalmology Out Patient Department/ In Patient Department (OPD/IPD) at AVBRH after taking the inclusion and exclusion criteria into consideration. Informed consent was obtained from all subjects.

Sample size calculation: With desired error of margin, sample size was calculated using the following formula, $n=(Z_{\alpha/2})^2 \times p \times (1-p)\ d^2$, where P=Prevalence=3.8%=0.038 [22], d=Desired error of margin=5%=0.05, $Z_{\alpha/2}$=Level of significance at 5% i.e., 95% confidence interval=1.96, sample size=$1.96^2 \times 0.038 \times (1-0.038)\ 0.05^2$=55.17. Therefore, estimated sample size is 55.17 patients needed in the study.

Inclusion criteria: Patients with presence of clinical pseudoexfoliative material on the anterior lens capsule and pupillary margin in one eye and normal fellow eye as determined on slit lamp biomicroscopy were included in the study.

Exclusion criteria: Patients with previous ocular surgery, corneal pathologies, post corneal refractive surgery, glaucoma, pterygium, systemic diseases with ocular manifestations, ocular surface disease, uveitis history of chronic topical medication, ocular trauma, chronic contact lens wearers were excluded from the study.

Study Procedure

All patients underwent a complete ophthalmic examination, including best-corrected visual acuity, IOP measurement by Optilasa SL Goldmann applanation tonometry mounted on slit lamp, Aurolab slit lamp biomicroscope for anterior segment evaluation and fundus examination. On slit lamp, cornea was assessed for endothelial changes, pupil margin for pseudoexfoliative material. Pupil was dilated and the anterior lens surface was examined for pseudoexfoliative material, including pregranular radial lines and granular deposits, iris stroma for any iridopathy. Gonioscopy was performed for pseudoexfoliative material in angles. Corneal endothelial cell changes were analysed quantitatively and qualitatively by using Specular microscopy (Topcon SP-100). Both the eyes of subjects were photographed for analysis of endothelial cell density, percentage of hexagonal cells, coefficient of variation in cell size and central corneal thickness.

STATISTICAL ANALYSIS

Statistical analysis was done by using descriptive and inferential statistics using students unpaired t-test. and software used in the analysis were Statistical Package for the Social Sciences (SPSS) version 22.0 and Graph Pad Prism 6.0 version. The p-value <0.05 was considered as the level of significance.

RESULTS

A total 55 patients with unilateral PXF were included in the study. Age ranged from 41 to 80 years and mean age of the patients was 63.85±7.05 years [Table/Fig-1]. Majority of patients were in the range of 61 to 70 years (56.36%) [Table/Fig-1]. Genderwise, 26 were males and 29 were females. The mean corneal endothelial cell density can be seen from [Table/Fig-2]. The mean endothelial cell density in the PXF eyes was 2299.54±84.95 cells/mm^2. The count was significantly lower as compared to other normal eye 2599.54±84.95 cells/mm^2 (p=0.0001) [Table/Fig-2]. The mean percentage of coefficient of variation in cell size was 35.49% in PXF eyes and 33.80% in fellow normal eyes. The p-value was 0.064, which was statistically not significant [Table/Fig-3].

Age groups (years)	No. of patients	Percentage
41-50	2	3.64
51-60	14	25.45
61-70	31	56.36
71-80	8	14.55
Total	55	100
Mean±SD	63.85±7.05 (41-80 years)	

[Table/Fig-1]: Age distribution of the study sample.

Eye	N	Mean (cells/mm^2)	Std. Deviation	Std. Error mean	t-value, p-value
Pseudoexfoliative eye	55	2299.54	84.95	11.45	18.71, p<0.001**
Normal fellow eye	55	2599.54	84.95	11.45	

[Table/Fig-2]: Corneal endothelial cell density.
**p-value <0.001 is considered highly significant

Eye	N	Mean	Std. Deviation	Std. Error mean	t-value, p-value
Pseudoexfoliative eye	55	35.49	4.62	0.62	1.87, p=0.064
Normal fellow eye	55	33.80	4.85	0.65	

[Table/Fig-3]: Comparison of Coefficient of variation between two eyes.

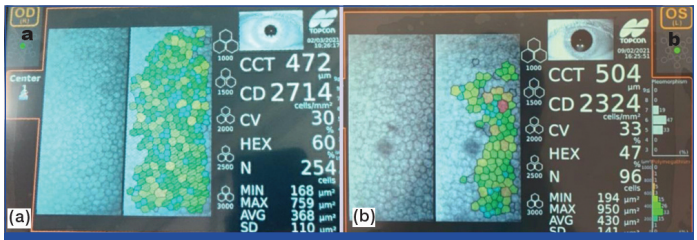
The mean percentage of hexagonal cell was 51.12% in PXF eyes and 52.54% in fellow normal eyes. The p-value was 0.13. the difference was statistically not significant [Table/Fig-4]. The mean central corneal thickness was 551.29 microns in PXF eyes and 521.07 microns in fellow normal eyes. The p-value was 0.77. The difference was statistically non significant [Table/Fig-5]. Photograph from specular microscopy for normal eye and eye with Pseudoexfoliation (PXF) [Table/Fig-6].

Eye	N	Mean	Std. Deviation	Std. Error mean	t-value, p-value
Pseudoexfoliative eye	55	51.12	4.91	0.66	1.49, p=0.13
Normal fellow eye	55	52.54	5.06	0.68	

[Table/Fig-4]: Comparison of hexagonality % between two eyes.

Eye	N	Mean (in microns)	Std. Deviation	Std. Error mean	t-value, p-value
Pseudoexfoliative eye	55	551.29	33.11	4.46	0.28, p=0.77
Normal fellow eye	55	521.07	33.38	4.50	

[Table/Fig-5]: Comparison of central corneal thickness (in microns) between two eyes.



[Table/Fig-6]: a) Normal eye b) Pseudoexfoliation eye (Photograph from specular microscopy). (Magnification: (x254).

DISCUSSION

Pseudoexfoliative disease is characterised by deposition of extracellular fibrillar material on the surface of various ocular structures. Clinically, pseudoexfoiliative material is seen as white dandruff-like material on pupillary margin, anterior surface of lens, angle of anterior chamber with increase in trabecular meshwork pigmentation. It is also associated with iris atrophy, iris transillumination defects, inadequate pupil dilation, lens instability and corneal endothelial abnormalities [4]. It is a bilateral disease but presentation is asymmetric, clinically seen as unilateral PXF [2,23-25]. The present study was conducted to evaluate the endothelial cell morphology and central corneal thickness by specular microscopy in eyes with clinical unilateral PXF and compared

with apparently normal fellow eye. Corneal endothelial morphological changes both quantitative and qualitative has been studied by various imaging modalities in pseudoexfoliative eyes, which shows damaged endothelium [1,11-13]. In a study done by Schlotzer-Schrehardt UM et al., electron microscopy was used which showed large clumps of pseudoexfoliative material were found to be adherent to the corneal endothelium, and also pseudoexfoliative material are incorporated into the posterior Descemet membrane [15]. These may lead to early corneal endothelial decompensation and diffuse keratopathy [8]. Reports on thickness of cornea are variable. Some studies mention it to be thicker [16,21] where as some studies reported it to be thinner [12,26].

The present study included total 55 patients with unilateral PXF. Age ranged from 41 to 80 years and mean age of the patients was 63.85±7.05 years. Majority of patients were in the range of 61 to 70 years (56.36%), which was comparable to other studies [2,24]. In the present study, the mean endothelial cell density in the PXF eyes was 2299.54±84.95 cells/mm^2. The count was significantly lower as compared to fellow normal eye (2599.54±84.95 cells/mm^2 ($p<0.001$). The findings were comparable with other studies [1,10-12]. In a previous study done by Inoue K et al., the corneal endothelial cell density was significantly lower in PXF eyes 2336±383 cells/mm^2 than in the non-PXF eyes 2632±327 cells/mm^2, p=0.003 [12]. Similarly Wang M et al., concluded that mean corneal endothelial cell density in the PXF eyes was 2298± 239 cells/mm^2, significantly lower than in the cataract eyes 2652±18 cells/mm^2, with p-value of 0.026 [1]. In the current study, Coefficient of variation in cell size between two eyes variation in cell size was more in PXF eyes 35.49±4.62% than fellow eye 33.80±4.85% with p-value of 0.064. Similarly, percentage of hexagonal cells was less in PXF eye (51.12±4.91%) than fellow eye (52.5±5.06%) with p-value of 0.13. However, both these parameters were statistically nonsignificant and was comparable to other Studies [1,12,16].

In a previous study, by Wang M et al., it was seen that coefficient of variation in cell size was 34.7±7.1% in PXF eyes and 34.6±1.4% in fellow normal eye. Percentage of hexagonal cells were 54.5±2.8% and 59.4±9.9% in fellow normal eye. Both the parameters were statistically non significant [1]. In Inoue K et al., study, there was no significant difference between the coefficient of variation in the cell area in the PXF Group (0.324±0.033) compared to the control group (0.336±0.041). There was no significant difference between the percentage of hexagonal cells in the PXF group (58.4±8.1%) compared to the control group (58.9±6.6%) [12].

However, Miyake K et al., observed significant decrease in endothelial cell density along with reduced cell hexagonality and increased coefficient of variation in cell size in the PXF eyes as compared to fellow normal eye [10]. Mean endothelial cell density was 2669±502 cell/mm^2 in PXF eye and 2847±540 cell/mm^2 in normal fellow eye. Mean coeffecient of variation was 0.339±0.073 in PXF eyes and 0.343±0.097 in fellow normal eye. Mean percentage of hexagonal cells were 57.1±7.1 in PXF eyes and 55.3±9.3 in fellow normal eye. The p-value for all parameters in this study was <0.05. On comparison of central corneal thickness between two eyes, the thickness was 551±33.11 µm in PXF eye and 521±33.38 µm in fellow normal eye. But the difference was not stastically significant, which was comparable to other studies [16,21]. However, in Krysik K et al., study used three different imaging modalities i.e., Pentacam-Scheimpflug device, time-domain Optical Coherence Tomography (OCT) Visante, and swept-source OCT Casia to assess the corneal thickness whereas, in the present study corneal thickness was measured only on specular microscope [21]. In a previous study by Paivi P et al., the PXF eyes had thicker central cornea than normal eyes. Values were 528±30 µm in PXF eyes and 523±32 µm in normal eye. The p-value <0.01, which was statistically significant [16].

Thus, eyes with PXF have altered endothelial morphology which can be assessed quantitatively and qualitaively on imaging modalities like specular microscope which cannot be detected on slit lamp examination. These changes should be considered before any intraocular surgeries, since it can lead to endothelial decompensation following intraocular surgeries [8]. Also central corneal thickness in PXF eyes should be considered because it affects measurement of intraocular pressure.

Limitation(s)

Limitations of the current study were small sample size and being a hospital based study, population did not represent Indian population as whole.

CONCLUSION(S)

In the present study, pseudoexfoliation eyes had decreased endothelial cell density, altered morphology of cells and thicker cornea as compared to eyes without evidence of pseudoexfoliation clinically as seen on specular microscopy. Further advanced investigations like anterior segment optical coherence study or confocal microscopy should be considered to identify structural changes in eyes with unilateral cases.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Ophthalmology, Jawharlal Nehru Medical College, Wardha, Maharashtra, India.
2. Junior Resident, Department of Ophthalmology, Jawharlal Nehru Medical College, Wardha, Maharashtra, India.
3. Professor, Department of Ophthalmology, Jawharlal Nehru Medical College, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Archana Thool,
Assistant Professor, Department of Ophthalmology, Jawharlal Nehru Medical College, Wardha, Maharashtra, India.
E-mail: rashmi.ramani9@gmail.com

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