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Original Article

Ophthalmology Section

Determination of Corneal Endothelial Cell Count and Morphology in Patients with Pseudoexfoliation: A Cross-control Study

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ABSTRACT

Introduction: Pseudoexfoliation Syndrome (PEX) is characterised by the formation or deposition of abnormal fibrillar material on intraocular structures. Various ocular complications, such as chronic open-angle glaucoma, zonular dehiscence causing lens subluxation/dislocation, poor mydriasis are associated with PEX. In PEX eyes, corneal endothelial changes have been demonstrated along with thinner Central Corneal Thickness (CCT).

Aim: To evaluate the CCT, corneal Endothelial Cell Density (ECD) and morphology in patients with pseudoexfoliation and to compare the results with age matched controls.

Materials and Methods: A case-control study was done on a total of 147 eyes of 81 patients with pseudoexfoliation with equal number of eyes in age matched controls were evaluated for corneal ECD, coefficient of variation in cell size, percentage of hexagonal cells and CCT using a non-contact specular microsope. The quantitative data was represented as their mean±Standard Deviation (SD). The paired t-test was used for analysing quantitative data.

Results: The average ECD in the PEX group was 2301.30±117.37 cells/mm^2. It was significantly lower than the average ECD in controls 2632.91±24.04 cells/mm^2 (p-value <0.001). The average CCT in the PEX group was 508±19.09 microns and in the age matched controls was 524.22±6.36 microns. The average CCT was low in the PEX group and difference was statistically significant (p≤0.001). The coefficient of variation and percentage of hexagonality were altered but did not show any statistical significance in both the groups.

Conclusion: ECD and CCT is significantly decreased in eyes with PEX. Pleomorphism and polymegathism was not found significant in this study.

Keywords: Central corneal thickness, Endothelium, Hexagonality, Pleomorphism

INTRODUCTION

The Pseudoexfoliation Syndrome (PEX) is the deposition of abnormal fibrillar material upon intraocular structures [1]. The pseudoexfoliative material is seen on the corneal endothelium, anterior lens surface, iris, trabecular meshwork, zonules and ciliary body [2,3]. It is often associated with ocular complications like pseudoexfoliative glaucoma, zonular dehiscence causing lens subluxation/dislocation and poor mydriasis. Exfoliative material is a homogenous, eosinophilic, periodic acid-Schiff positive staining substance. Electron microscopy demonstrates a loose fibrillar structure, with individual fibrils being generally 20-30 nm thick and having a characteristic 50 nm macroperiodicity. The source of the exfoliative material is thought to be over production of zonular tissue by the non-pigmented ciliary epithelium and the equatorial lens epithelium because the lens zonules have similar ultrastructural, staining, and immunological properties [4].

The reported prevalence of PEX in general population varies from country to country. The lowest prevalence was found to be 0% in Eskimos and the highest prevalence of 8.2% was reported in Bantu tribes of South Africa. A population based study in South India reported a prevalence of 3.41% [5].

This syndrome is slightly more common in females than males. The corneal endothelium is the posterior most surface of the cornea. It is made up of simple squamous or low cuboidal monolayer of hexagonal cells. The normal ECD is about 3000-5000 cells/mm^2 and is responsible for regulating fluid and solute transport between the aqueous and corneal stromal compartments and are rich in mitochondria [2].

These cells are derived from neuroectoderm. They do not have the ability to replicate. The ECD is approximately 5,00,000 at birth. It decreases with age because of constant wear and tear. When the endothelial cells die, neighboring cells expand to cover the empty space once occupied by them. Maintaining a healthy and transparent corneal stroma is the primary physiological function of the corneal endothelium. It also secretes a collagen matrix that forms Descemet's membrane [6]. The intraocular pressure regularly forces aqueous into the stroma from the anterior chamber to facilitate diffusion. Even though the influx of aqueous into the stroma is essential for maintenance of corneal health, however to prevent oedema, the level of corneal hydration must be controlled [7].

Exact aetiology of PEX is not known. Multiple inheritance patterns have been suggested. Three Single Nucleotide Polymorphisms (SNPs) in a lysyl oxidase like one gene have been identified. LOXL1 and elastin are expressed in the corneal endothelium, anterior lens surface, iris, trabecular meshwork, zonules and ciliary body [5,6]. The source of the exfoliative material is thought to be over production of zonular tissue by the nonpigmented ciliary epithelium and the equatorial lens epithelium because the lens zonules have similar ultrastructural, staining, and immunological properties [7].

The deposition of the PEX material in the corneal endothelium leads to decreased nutrition to the stroma. There is also an increased oxidative stress to the corneal stroma which causes a decrease in the number of keratocytes and in the end leads to the damage of its extracellular structure which may result in the thinning of the cornea. The decreased number of keratocytes has been demonstrated in-vivo confocal microscopy by Zheng X et al., and Oltulu R et al., in their studies [8,9]. The progressive loss of endothelium due to chronic disorders may also affect the transparency as well as the functioning of cornea. If the ECD is less than 500/mm^2, corneal decompensation sets in causing corneal oedema and bullous keratopathy [10].

Studies conducted earlier have revealed PEX patients to have thinner corneas and increased susceptibility to develop glaucoma [11-14]. Data has reported from India on evaluation of the endothelium in patients diagnosed to have pseudoexfoliation [15]. The aim of

this study was to understand the changes in the CCT, endothelial morphology and ECD in patients with PEX and to correlate the results with age matched controls.

MATERIALS AND METHODS

A case-control study was conducted at Department of Opthalmology in Bharati Vidyapeeth Deemed to be University, Pune, Maharashtra, India, during May 2017 to September 2018 in which total of 147 eyes of 81 patients with pseudoexfoliation and equal number of age matched healthy controls were evaluated after the approval of the ethical committee (REF: BVDUMC/IEC/64. Dated: 10/10/2017). Sample size was calculated in comparison of previous study [15].

Diagnosis of the syndrome was based on the slit-lamp appearance of pseudoexfoliative material at the anterior lens capsule and/or at the pupillary margin, iris and endothelium. A written informed consent was obtained.

All patients were subjected to a complete ophthalmic evaluation which included recording of Best Corrected Visual Acuity (BCVA), anterior segment evaluation using slit lamp bio microscope and intraocular pressure using Goldmann Applanation Tonometer. Posterior segment was evaluated with Volk 90 D lens.

The corneal endothelium was evaluated using a TOPCON SP-18P specular microscope by a single observer. The endothelial morphology was quantitated by measuring parameters including cell density, coefficient of variation in the cell area, and the percentage of hexagonal cells. The CCT was also evaluated by specular microscopy.

Controls selected were age matched and had no pseudoexfoliative material at the anterior lens capsule or pupillary margin, no previous intraocular surgery, and no abnormalities of the cornea and underwent the same method of investigation.

Inclusion Criteria

- Patients having pseudoexfoliation of any age and gender.
- Patients willing to give written informed consent and follow study related procedures.

Exclusion Criteria for Controls and Case

- Patients with viral infections like Herpes zoster, Herpes simplex, Cytomegalovirus.
- Any kind of ocular trauma.
- Conditions affecting cornea like Fuch's dystrophy, Keratoconus, Aphakic bullous keratopathy, Pseudophakic bullous keratopathy.
- Operative procedure like Cataract surgery, Laser iridotomy, Penetrating keratoplatsy.
- Refractive corneal surgeries.
- Diagnosed cases of glaucoma.
- Diagnosed cases of diabetes mellitus.

STATISTICAL ANALYSIS

The quantitative data was represented as their mean±SD. The paired t-test was used for analysing quantitative data. The significance threshold of p-value of <0.05 was considered significant. All analysis was carried out by using Statistical Package for the Social Sciences (SPSS) software version 21.

RESULTS

A total of 147 eyes of 81 patients with PEX were included in this study. There were 45 females and 36 were males in both the groups. The maximum number of patients diagnosed to have PEX were in the age group 56-65 years (n=56 eyes) and 66-75 years (n=71 eyes). The average CCT in the PEX group was 508±19.09 microns and in the age matched controls was 524.22±6.36 microns. The average CCT was low in the PEX group and difference was statistically significant (p≤0.001). The age groups with a statistically significant difference in the CCT were between the groups 56-65 years (n=56 eyes) and 66-75 years (n=71 eyes) [Table/Fig-1].

Age (years) (n)	PEX (n=147)	Controls (n=147)	p-value paired t-test
<55 (06)	529.166±12.61	531.33± 16.82	0.805
56-65 (56)	515.49±16.06	530±24.56	<0.001*
66-75 (71)	506.16±20.44	522.80±27.67	<0.001*
>75 (14)	485.64±30	501.5±29	0.166
Mean CCT (microns)	508.65±19.09	524.22±6.36	<0.001*

[Table/Fig-1]: Central Corneal Thickness (CCT) in eyes with PEX and their age matched controls. (n is same for both cases and controls)
p-value was calculated using paired t-test; p-value less than equal to 0.05 was considerd significant

The mean ECD in the PEX group was 2301.30±117.37 cells/mm^2 and in the age matched controls was 2632.91±24.04 cells/mm^2. The average ECD was lower in the PEX group than the control group and the difference was statistically significant (p≤0.001) [Table/Fig-2]. Coefficient of variation was not statistically significant in any age group [Table/Fig-3]. The hexagonality of cells showed progressive decrease in all age groups in the PEX group and in control. The difference in both the groups was not statistically significant in any age group [Table/Fig-4].

Age (years) (n)	PEX (n=147)	Control (n=147)	p-value
<55 (06)	2531.16±238.968	2670.33± 95.26	0.2295
56-65 (56)	2310.163±230.157	2641.16±16	<0.001*
66-75 (71)	2303.633±276.128	2640±253.064	<0.001*
>75 (14)	2156.214±260.69	2531.41±374.566	0.0053
Mean ECD (cells/mm^2)	2301.30±117.37	2632.91±24.04	<0.001*

[Table/Fig-2]: Corneal Endothelial Cell Count (ECD) in patients with pseudoexfoliation.
paired t-test

Age (years) (n)	PEX (n=147)	Controls (n=147)	p-value
<55 (06)	35.01±2.94	34.33±4.32	0.761
56-65 (56)	35.42±5.25	35.34±4.11	0.9293
66-75 (71)	36.46±4.97	35.79±6.17	0.1595
>75 (14)	36.12±3.92	35.91±2.17	0.1235
Mean coefficient of variation (μm^2)	35.04±3.53	35.35±8.48	0.6839

[Table/Fig-3]: Co-efficient of variation in cell size (μm^2) in PEX group and controls.
paired t-test

Age (years) (n)	PEX (n=147)	Control (n=147)	p-value
<55 (06)	60±6.21	60.83±5.38	0.8097
56-65 (56)	56.58±8.71	58.05±7.62	0.1283
66-75 (71)	54.63±9.87	57.21±8.36	0.2234
>75 (14)	53.92±11	55.83±4.04	0.2293
Mean	53.60±1.41	57.56±16.97	0.1664

[Table/Fig-4]: Co-relation between percentage of hexagonal cells in eyes with PEX and their age matched controls.
paired t-test

DISCUSSION

The corneal endothelium is a single layer of hexagonal cells of uniform size. The number of cells decrease by 0.5-0.6% (100–200 cells, approximately) per year due to wear and tear. These cells are unable to regenerate. The cell loss is compensated by other endothelial cells migrating and increasing in size to fill the gaps. This in turn causes reduction of the tight junctions between the endothelial cells which leads to corneal oedema due to leak in the corneal endothelium [5,12].

PEX is an age-related disease which can be clinically diagnosed by the formation or deposition of abnormal fibrillar material upon intraocular structures. Cornea is involved in about 70% of patients

with PEX. The observed disorders include increased fragility and progressive loss of the corneal endothelium which lead to pleomorphism and polymegathism [2,3].

A total of 147 eyes of 81 patients diagnosed to have PEX. Of the 81 patients included in the study, bilateral PEX was present in 79 patients (97.53%). Unilateral PEX was present only in two patients. The maximum number of patients included in our study were between the age groups 56-65 years (38.09%) and 66-75 years (48.29%).

Central Corneal Thickness (CCT)

In our study, the average CCT in the PEX group was 508.65±19.09 microns and that of the control group was 524.22±6.36 microns. This difference was statistically significant (p<0.001). Average CCT in controls in our study was slightly lower as compared to other studies [10,15].

A study done by Vijaya L et al., in South Indian population the average CCT for the population was 511.4±33.5 microns, which was significantly thinner than the average CCT found in other countries [10]. The average CCT in a Turkish study in the non PEX patients was 543±23 and in Japanese population was 549±28 microns [13,14].

In this study pateints were grouped into increasing age groups, where we detected progressive thinning as the age increased in PEX group as well as in control group. In patients with PEX, the average CCT in age group <55 years was 529.166±12.61 microns. In the age groups of between age groups 55-65 years and 66-75 years had CCT of 515.49±16.06 and 506±20.44 microns, respectively. There was further thinning seen in the age group above 75 years where CCT was 485±30 microns.

In this study, a statistically significant difference in the CCT in the age groups of 56-65 years (p≤0.001) and 66-76 years (p<0.001) in comparison with their age matched controls was found. In a recent Indian study done by Priyadarshini et al., the values were comparable with this study. The mean CCT in their study was 515±0.07 microns in the control group and 501+0.07 microns in PEX group (p-value=0.001) [15].

Inoue K et al., also found the central corneas to be significantly thinner in the PXS eyes 529±31 microns than in the non-PXS eyes 547±28 (p=0.03) [14]. Fujisawa A et al., also reported significantly thinner corneas in PEX (524.33±32.79) as compared to the control group (543.23±30.33) [16].

In the age group of <55 years (p=0.805), although the cornea was thinner, there was no statistical significance in comparison to the controls. This may be due to less duration of corneal exposure to the PEX material and less number of patients in this age group. In a study done by Wang M et al., there was no significant change in CCT in the PEX and control group [17].

Deposition of PEX material in the corneal endothelium leads to decreased nutrition to the stroma. There is also an increased oxidative stress to the corneal stroma which causes a decrease in the number of keratocytes and ultimately leads to the damage of its extracellular structure which results in the thinning of the cornea. The decreased number of keratocytes has been demonstrated by invivo confocal microscopy by Zheng X et al., and Oltulu R et al., in their respective studies [8,9].

PEX is the most common cause of secondary open angle glaucoma. The evaluation of intra ocular pressure is an important parameter for diagnosing glaucoma. The thinner corneas in PEX give a false low reading in the early stages which may lead to a delay in diagnosing pseudoexoliatory glaucoma. Therefore, evaluation of CCT should be done in every patient of PEX syndrome.

Endothelial Cell Density (ECD)

In this study, the average ECD in patients with PEX was 2301.30±117.37 cells/mm^2 which was significantly lower (p≤0.01) than in the control group 2632.91±24.04 cells/mm^2 with reduction of 12.57%. The mean ECD (cells/mm^2) in the age group 56-65 years was 2310.163±230.157 and in that of 66-75 was 2303.633±276.128. In both these age groups the ECD was significantly lower(p≤0.001) than their age matched controls [Table/Fig-5]. A comparision of previous studies was done with the present study [14,15,18].

	Present study N=147	Inoue K et al., [14] N=21	Quiroga L et al., [18] N=61	Priyadharsini et al., [15] N=40
PEX	2301.30±117	2336±383	2315	2124±116
Non PEX	2632.91±24.04	2632±327	2482	2511±171
p-value	<0.001*	<0.001*	<0.002*	0.003*

[Table/Fig-5]: Comparison of ECD (cells/mm^2) between the present study and the previous studies [14,15,18].

Although the ECD was less than age matched controls in the age group of <55 years, it was not statistically significant (p=0.2295) probably due to lesser duration of the disease process.

PEX influences the cell density of corneal endothelium. PEX material, settles on the endothelium that penetrates into the Descemet's membrane that eventually breaks the connections between individual six-sided cells, resulting in accelerated apoptosis of these cells. Other factors which may affect the ECD include hypoxia of the anterior chamber, changes in the fibroblasts and elevated concentration of TGF factor [9].

PEX usually affect elderly patients, who are likely to undergo intraocular surgery for cataract and glaucoma. These patients are at a higher risk of endothelial cell loss during surgery because of associated conditions like non-dilating pupil, zonular laxity. Hence, it is advisable to do a specular microscopic evaluation of all patients prior to any intraocular surgery.

Pleomorphism and Polymegathism

In this present study the average coefficient of variation in cell size was 35.04±3.53% in the PEX group and 35.35±8.48% in the controls. This difference was not statistically significant (p <0.6839). coefficient of variation up to 30% is considered normal [9].

The average percentage of hexagonal cells in PEX group was 53.60±1.41% and in the controls was 57.56±16.97 %. The difference between the PEX group and controls was not statistically significant in any age group (p <0.1664). However, there was an overall decrease of the percentage of hexagonal cells in both groups with increasing age. A study done by Inuoue K et al., and Sultana N et al., found similar results to the present study in both pleomorphism and polymegathism [14,19].

A study conducted by Sarowa S et al., found a significant decrease in the co-efficient of variation in PEX group 39.05±3% and in controls 32.23±2.66% (p <0.05) [20]. A similar study done by Wali UK et al., in Omani patients found a significant difference in the co-efficient of variation 37.09±12.43 which was very low in comparison to the controls 33.12±11.44. (p <0.005) [21]. This disparity in the findings from our study may be explained by the fact that both these studies had included patients with PEX.

Accelerated apoptosis of the endothelial cells leads to healing by the expansion of the surrounding cells leading to polymegathism. The decrease in the coefficient of variation in the cell area and the percentage of hexagonal cells could be explained by the fact that mild intraocular disturbances or endothelial damages developed slowly over a long period can later result in a decrease in the corneal endothelial cells and their enlargement without transformation of their hexagonal shape [22].

Limitation(s)

This was a time bound study with a smaller sample size. The refractive errors of the patients were not taken into consideration.

CONCLUSION(S)

A significant decrease in the ECD and CCT in PEX group in comparison to the controls. There was no significant difference in the coefficient of variation of the cell area and percentage of hexagonal cells between the PEX group and the controls in our study.

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