· Original article ·

Epithelial and goblet cell discordance and mixed involvement of impression cytology in patient with pseudoexfoliation syndrome

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剥脱综合征患者结膜印迹细胞学的检查特征

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摘要

目的:研究剥脱综合征对结膜细胞和泪液功能的影响。

方法:研究共纳入剥脱综合征患者 60 例 86 眼作为试验组,年龄相仿正常人 55 例 89 眼作为对照组。所有 115 例 175 眼均接受 Schirmer 泪试验及泪膜破裂时间试验,并对上方和鼻下方睑裂区球结膜进行印迹细胞学检查。印迹细胞学检查结果根据 Nelson 方法分级。对 TBUT, Schirmer 泪试验及印迹细胞学结果进行相关性分析。

结果:剥脱综合征患者的 Schirmer 试验、TBUT 均较正常组显著降低,结膜细胞也有显著改变。剥脱综合征患者的印迹细胞学分级中位数为 3 级,而正常人组为 0 级。在试验组中发现 45 例样本出现了两种以上分级共存的现象,而对照组仅发现 3 例。此外,实验组中的 45 例样本结膜上皮细胞和杯状细胞分级出现明显差异,且均表现出杯状细胞的破坏甚于上皮细胞,而对照组未发现此种现象。

结论:剥脱综合征患者的结膜细胞受到破坏,泪液分泌受到影响,极易造成眼表疾病的出现。

关键词:印迹细胞学;剥脱综合征;泪膜破裂时间;Schirmer 泪试验

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Abstract

• AIM: To evaluate the influence of pseudoexfoliation syndrome (PEX) on conjunctival cells and tear functions.

• METHODS: This study included 86 eyes of 60 patients with PEX and 89 eyes from 55 normal age-matched individuals. Tear film break-up time (TBUT), Schirmer's tests were performed and impression cytology (IC) specimens were obtained from upper and interpalpebral inferonasal bulbar conjunctiva for all 175 eyes of 115 cases. IC specimens were graded according to Nelson's method. The correlations between results of TBUT, Schirmer's test and the findings of impression cytology specimens were evaluated.

• RESULTS: Patients with PEX had lower Schirmer's tests and TBUT and also revealed significant conjunctival cell alterations when compared with normal subjects. The median cytology scores were Grade 3 and Grade 0 in PEX patients and normal subject, respectively. Mixed involvement with more than one grade type change was observed in 45 specimens in cases with PEX and only in 3 specimens in the control cases. Besides, epithelial and goblet cell discordance was found in 45 specimens in the group with PEX which was not observed in the control group.

• CONCLUSION: Destruction of conjunctival cytology combined with decreasing in tear tests demonstrates the presence of ocular surface disease in PEX.

• KEYWORDS: impression cytology; pseudoexfoliation syndrome; tear film break-up time; Schirmer's tests

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INTRODUCTION

Pseudoexfoliation syndrome (PEX) is characterized by production and progressive accumulation of gray-white fibrillogranular extracellular material in many ocular tissues such as conjunctiva, corneal endothelium, trabecular meshwork, pupillary margin of iris, anterior lens capsule, zonules and ciliary body[1-6]. Even the presence of PEX material in the conjunctiva, which is not apparent in the biomicroscopic evaluation, has been found by several ultrastructural studies[2,7,8]. The morphologic characteristics of conjunctival involvement can be evaluated by either incisional conjunctival biopsy or non-invasive impression cytology (IC). Conjunctival IC is a safe, relatively simple

painless and practical method with no side effects. It can be easily performed in an outpatient clinic[9-12].

The purpose of this study was to investigate the influence of PEX on conjunctival epithelial morphology by IC and its correlation with tear film functions.

SUBJECTS AND METHODS

The current study was designed in accordance with the Declaration of Helsinki. The patients' protocol and associated informed-consent documents were reviewed and approved by Institutional Review Board of Ataturk Education and Training Hospital. All patients were informed about the nature of the procedure and written consent was obtained.

Inclusion Criteria and Methods of Clinical Assessment: The current study included 175 eyes of 115 caucasian patient in which 86 eyes of 60 patients with PEX syndrome and 89 eyes from 55 age-matched normal individuals. Out of 60 patients 36 (60%) patient were male and 24 (40%) patients were woman in PEX group. In 55 normal subjects 35 (63.6%) were male and 20 (36.4%) were female. The sex distribution was not different in each group. (χ^2 test, $\chi^2 = 0.161$, $P = 0.689$).

The mean age was 69.90±6.32y (54-82y) in PEX group and it was 69.96±6.86y (52-84y) in control group, which was statistically insignificant (t-test, $t = 0.052$, $P = 959$).

Each subject underwent a complete ophthalmic examination with special emphasis on ocular surface abnormalities and lid function. A diagnosis of PEX syndrome was made by observing characteristic gray-white PEX material either on the surface of the anterior lens capsule or pupillary border of iris, in both of their eyes, by biomicrocobic evaluation. As previously defined, tear film break-up time (TBUT) and Schirmer's tests were measured by the same observers (Altintas AGK and Eranil SH) as masked cases. Schirmer's tests were performed as both with anaesthesia and non-anaestheasia, amount of wetting portion was measured following 5min.

Patients suffering from dry eye, due to any ocular diseases such as, meibomian gland dysfunction, chronic blepharitis and systemic disorders such as Sjögren or any other autoimmune disease, patent with the history of any ocular trauma or surgery, evidence of ocular surface disorders that could interfere with tearsretion, contact lens wearers, cases with absence of Bell's phenomena and presence of lagophthalmos were not included n this study. Individuals using any topical ocular medication such as antiglaucoma drugs, receiving systemic medications, which may interface tearsretion as beta-blockers, were excluded from the study. If an individual had the history of any ocular surgery, only the single non-operated eye was evaluated. Thus, both eyes of each case were not included in the study.

Pathologic Evaluation and Grading System IC evaluation was performed according to Nelson's grading system. A total of 350 specimens were examined, 172 of them were from eyes with PEX and 178 of those specimens were obtained from normal eyes. Samples were taken from both upper bulbar (UB) and inferonasal (IN) conjunctiva of the same eye. Goblet cells are located within the epithelium and display regional variation in their distribution and density per unit area. It was found that the highest density are on the inferonasal and medial fornicial region[9,12-14]. In order to obtain more goblet cells and compare the different parts of conjunctiva, the samples, were taken from IN conjunctiva which has higher goblet cell density and also from UB conjunctiva that is covered by upper eye lid, thus that can be protected from external factors. To be able to differentiate the samples taken from different regions, cellulose acetate filter paper (Millipore pore size 0.025μm) was cut into small pieces in size of approximately 5×5mm as square and trapezoid shape. All samples were taken by two investigators (Altintas AGK and Eranil SH) with the same method from the same region of the conjunctiva, square shapes were used for UB conjunctiva and trapezoid ones were used for IN conjunctiva.

The specimens were stored under ethanol of 95% and stained with PAS (Periodic acid-Schiff). All masked specimens were evaluated simultaneously by the same observers (Altintas AGK, Eranil SH, Kandemir O) and graded between Grade 0 and Grade 3 according to grading system outlined by Nelson[12-14].

Statistical Analysis SPSS software version 10.0 (SPSS Inc, Chicago Ilinois, USA) was used to perform statistical analysis. Data were described by means of standard deviation (SD) or medians. Significant differences on averages were statistically assessed by the χ^2, t-test or Wilcoxon test. The Spearman correlation was used to assess relation among parameters. χ^2 test was applied to quantitative variables such as IC scores of UB and IN conjunctiva in two different groups (PEX group and control group). Comparisons were made by using the nonparametric Wilcoxon within dependent groups such as IC scores of UB and IN conjunctiva in the same group. t-test was performed to evaluate Schirmer's and TBUT tests. P values of less than 0.05 were considered to indicate a statistically significant difference.

RESULTS

A total of 305 specimens out of 350 samples could be classified by Nelson's grading system, according to both epithelial and goblet cell morphologic feature in same specimen.

However in 48 specimens, more than one type of grading changes were present within the same sample (Figure 1A). The transition zone was not observed in between different grade type of changes in these samples. As it is shown in Figure 1A, Grade 0 nearby Grade 3 changes were observed in same specimens without any morphological changes as a transition zone which could be as classified as Grade 1 and Grade 2 in different parts of same specimens. As far as we know this kind of mixed appearance was not reported before our observation.

Mixed involvement was observed in total of 45 samples, 24 (27.9%) samples taken from superior and 21 (24.4%) samples from IN conjunctiva in subjects with PEX as opposed

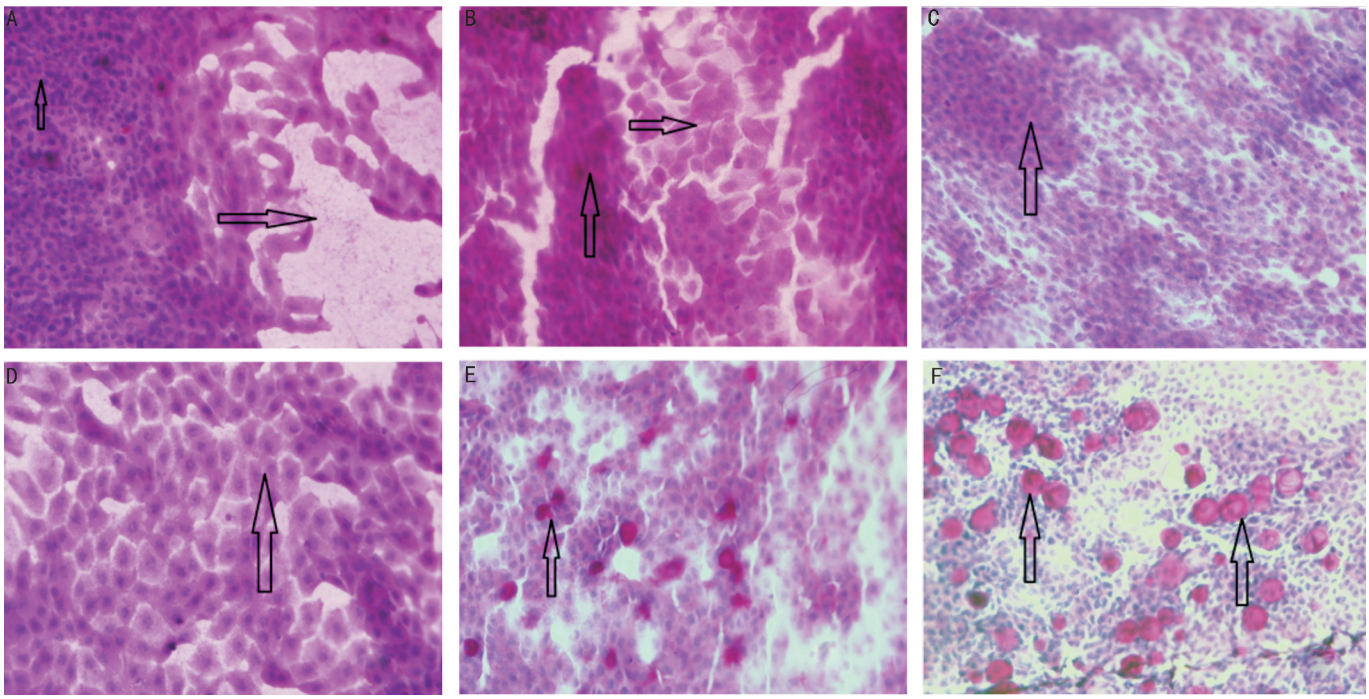

Figure 1 Impression cytology (IC) of different stages (periodic acid-Schiff stain, ×200 magnification) A: Mosaism of specimen obtained from inferonasal interpalpebral conjunctiva [Grade 0 (upside arrow) and 3 (horizontal arrow) changes in the same area]; B: Mosaism of the area from upper bulbar conjunctiva [Grade 1 (upside arrow) and 3 (horizontal arrow) changes in the same area]; C: Epithelial-goblet cell miscorrelation on the inferonasal interpalpebral area (Epitel cells are Grade 1 but there is no goblet cells compatible with this level); D: Epithelial-goblet cell miscorrelation on the inferonasal interpalpebral conjunctiva (Epitel cells are Grade 2 but there is no goblet cells compatible with this level); E: IC of Grade 2 on the superior bulbar conjunctiva; F: Grade 0 on the superior bulbar conjunctiva.

to only 3(3.37%) specimens obtained from normal subjects, one of which was from UB and 2 of them were from IN interpalpebral conjunctiva. In specimens with mixed involvement the higher grading scores were accepted in classification for statistical analyses.

A total of 45 specimens that were obtained from 35 eyes could not be classified according to Nelson's grading system; 25 of which were taken from UB conjunctiva and 20 were obtained from IN interpalpebral conjunctiva. A significant discrepancy between epithelial and goblet cells morphologies was observed on those specimens. According to normal epithelial morphology, the specimen should be classified as Grade 0, on the other hand, according to markedly decrease in number of goblet cells, decrease of cell dimensions and structural changes, same specimen could be classified higher grades such as Grade 2 or Grade 3. The normal epithelial morphology and the absence of goblet cells were also noticed together on some of the specimens (Figure 1C, 1D). However, inverse discrepancy such as epithelial metaplasia, and abundant normally structured goblet cells were not observed in any specimen. In all samples having discordance in epithelial and goblet cell involvement, the goblet cell destruction was more significant than epithelial involvement.

Epithelial and goblet cell involvement discordance was found only in the specimens those were belonging to eyes with PEX. It was noticed in both UB and nasal IN conjunctiva of 8 eyes. Additionally, this discordance was observed only in nasal conjunctiva in 12 eyes and found in only superior side in 17 eyes. These specimens were classified according to degree of epithelial metaplasia for statistical analyses. As far as our knowledge this presented discordance had not been reported before current study in eyes with PEX.

In only 2 specimens, mixed appearance combined with epithelial and goblet cell involvement discordance were observed together, both of which were taken from subjects with PEX.

Distribution of the UB and IN conjunctival specimen finding is given in the Table 1. In the evaluation, which was made by considering mixed involvement and epithelial goblet cell involvement discordance (EGID), the median IC scores from UB conjunctival specimens on PEX patients was Grade 2 (Figure 1E) and that was statistically significantly higher than normal subjects' median scores of grade 0 (Figure 1F; $\chi^2 = 91.342$, $P = 0.000$). Subjects with PEX had significantly higher median grading scores (Grade 3) than normal subjects' (Grade 0) in IN interpalpebral area too ($\chi^2 = 89.174$, $P = 0.000$).

Comparison of the IC findings between the UB and the IN conjunctiva in both groups showed that interpalpebral conjunctiva had significantly higher grading scores than UB conjunctiva where was normally covered by upper eyelid (Wilcoxon test $P = 0.010$ for eyes with PEX, $P = 0.003$ for normal control eyes). The grading scores between UB and IN region in same subjects showed positive correlation coefficients in Spearman correlation test ($r = 0.340$, $P = 0.001$). This result testified that, even the grading scores were different in

Table 1 Comparison of IC scores of superior and inferonasal bulber conjunctiva areas from patients with PEX and normal subjects *n*(%)

Region	Groups	Grade 0	Grade 1	Grade 2	Grade 3
Superior bulbar conjunctiva	PEX	3(3.5)	23(26.7)	34(39.5)	26(30.2)
	Normal	50(56.2)	34(38.2	5(5.6)	0(0.0)
Inferonasal interpalpebral conjunctiva	PEX	5(5.8)	11(12.8)	24(27.9)	46(53.5)
	Normal	36(40.4)	40(44.9)	13(14.6)	0(0.0)

Table 2 Comparison of tear function tests of PEX patients with and without epithelial-goblet cell involvement discordance on inferonasal bulber conjunctiva and normal subjects $\bar{x}\pm s$

Schirmer tests	With Epithelial-goblet cell involvement discordance on inferior quadrant(n=20)	Without Epithelial-goblet cell involvement discordance on inferior quadrant(n=49)	Control (n=89)	^1P	^2P
Schirmer test without anesthesia	8.8±2.52	9.48±3.76	17.03±5.17	0.455	0.000
Schirmer test with anesthesia	6.95±2.32	7.55±3.34	13.29±3.93	0.467	0.000
TBUT	4.6±2.21	5.1±2.01	9.84±3.25	0.364	0.000

[1]Statistical comparison of Schirmer tests between eyes with epithelial and goblet cell involvement discordance and without discordance group;[2]Statistical comparison of Schirmer tests between eyes with epithelial and goblet cell involvement with discordance and control group.

Table 3 Comparison of tear function tests of PEX patients with and without epithelial-goblet cell involvement discordance (EGID) on superior bulber conjunctiva and normal subjects

Schirmer tests	With EGID on superior quadrant (n=25)	Without EGID on superior quadrant (n=49)	Control (n=89)	^1t	^1P	^2t	^2P
Schirmer test without anesthesia	9.68±2.98	8.83±3.22	17.03±5.17	0.752	0.455	6.908	0.000
Schirmer test with anesthesia	7.56±2.53	7.44±2.63	13.29±3.93	0.732	0.467	6.926	0.000
TBUT	4.64±2.60	5.18±1.76	9.84±3.25	0.912	0.364	6.84	0.000

[1]Statistical analysis between eyes with epithelial-goblet cell involvement discordans and without discordans group; [2]Statistical analysis between eyes with epithelial-goblet cell involvement discordans and control group.

different parts of conjunctiva, the histopathologic changes were correlated in the same eyes.

In the tear functional tests, which were made as masked to patients' condition, the average value of Schirmer's test with anesthesia was 7.49±3.01mm/5min (2-15mm/5min) in the PEX group and was found as 13.29±3.93mm/5min (7-25mm/5min) in normal subjects, the difference was statistically significant (t-test, P=0.000). The average value of Schirmer's test without anesthesia in subject with PEX (9.40±3.39, 4-20mm/5min) was significantly lower than normal subjects' average value (17.03±5.18, 8-35mm/5min; t-test, P=0.000).

The average values of TBUT were 4.91±2.59s (1-16) in subjects with PEX and 9.84±3.26s (4-20) in normal subjects (Figure 2). The average TBUT value was lower in patient with PEX than in normal subjects (t-test, P=0.000).

Both Schirmer's tests and TBUT were significantly lower in PEX patients with epithelial and goblet cell involvement discordance than normal subjects (Tables 2, 3).

Correlations between the Schirmer's test scores with IC scores were statistically insignificant in both PEX group and normal subjects in either side (P>0.05). The correlation between TBUT and IC scores of UB conjunctiva was insignificant in both groups either. On the other hand, TBUT displayed on inverse correlation with IC of interpalpebral IN conjunctiva in both PEX group and normal subjects. (r=-0.262, P=0.015). According to these results, metaplasia in IN conjunctiva, in which dense goblet cells are present, effects tear functions much more than UB conjunctival changes.

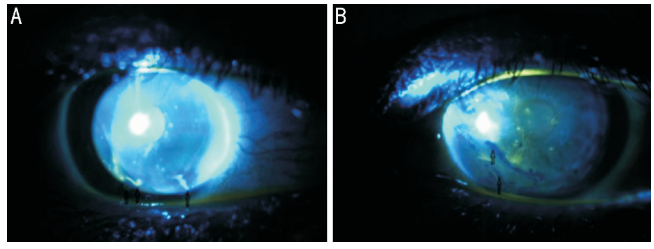

Figure 2 Dry eye in eyes with PEX A: Mucoidsretion with fluorescein stain in eyes with PEX; B: Dry spots in TBUT in eyes with PEX.

The average TBUT test was lower (4.64±2.60s) in patients with epithelial and goblet cell involvement discordance on UB conjunctiva than patients without discordance (the average value of 5.18±1.76s). However, the difference was not statistically significant (P=0.240, t=1.184). Even the average TBUT test was lower (4.60±2.21s) in patients with

epithelial and goblet cell involvement discordance on IN conjunctiva than patients without discordance (5.10±2.01s), but the difference was also statistically insignificant ($P=0.364$, $t=0.921$). The presence or absence of epithelial and goblet cell involvement discordance and both Schirmer's tests were not significantly correlated in PEX patients. According to these findings, the presence of PEX causes reduction of tear film functions but epithelial and goblet cell involvement discordance are not significantly correlated with tear film tests in these patients.

DISCUSSION

The presence of PEX material in the conjunctiva of the eyes with PEX syndrome has been reported by several investigators by ultrastructural studies. Conjunctiva IC is a safe, relatively simple, non-invasive method for evaluation of the morphology of the superficial cell layers. Different grading methods for classification of conjunctival metaplasia are available such as Tseng's, Saini's and Nelson's grading system. Among the all these grading systems, Nelson's system is the most accepted, well known and widely used[10,14-17]. For this reason, Nelson's grading system was preferred in this study.

In the evaluation of all cases the higher cytology scores of Nelson's Grading system were observed in specimens obtained from interpalpebral inferonasal conjunctiva than upper bulber conjunctiva in both PEX patients and normal age-matched controls. That observation may be related to protective effect of the upper eye lid, which covers the upper bulber conjunctiva and destructive effect of environmental factors such as UV radiation on inferonasal interpalpebral conjunctiva.

The more advanced morphologic destruction with higher cytology scores were observed in specimens obtained from patient with PEX comparing to normal subjects. In the cases with PEX, the median IC scores obtained from IN conjunctiva and UB conjunctival specimens were Grade 3 and Grade 2 respectively, while Grade 0 was in both areas in the control group. This histological observation confirms that the patients with PEX may have ocular surface changes.

In current study, some of the conjunctival specimens demonstrated more than one grade without any intermedial zone in between two far end of cytologic spectrum that belongs to different gradings level in the same specimen. We used the term of mixed pattern which was not reported before us as for as we know. Mixed involvement was observed in 45 specimens in the group with PEX and only in 3 specimens in the normal group. This observation confirms that the patients with PEX have ocular surface disease. Erdogan *et al*[10] who studied IC in patient with PEX, had not reported any observation like mixed pattern involvement in their study. They did not either notice any difference in cytology scores between temporal and nasal side of conjunctiva. They only reported that IC scores were significantly higher in patient with PEX than normal subjects' as we found in our series too.

PEX material can be found bilaterally, even the cases clinically appeared to be unilateral[2,4,6,18]. In our study the most of patients both eyes were evaluated. Therefore we could have chance to compare the both eyes so, finding of the mixed pattern conjunctival changes in one eye in 3 normal subjects may indicate that these eyes are more prone to developing PEX manifestations in the following period. According to our observation conjunctival surface changes including mixed pattern involvement may be considered as a preceding finding of clinically undiagnosed PEX syndrome.

Epithelial cell and goblet cell involvement discordance was only observed in our cases with PEX. According to epithelial cell morphology, the specimen should be graded as normal, but according to metaplasia of goblet cells, the same sample area could be classified in higher grades. However, inverse miscorrelation was not observed in any specimen. In our opinion, the goblet cell destruction may exist prior to epithelial metaplasia and this finding is an objective data proving ocular surface disease in the cases with PEX. As far as we know an interesting unexpected discrepancy between epithelial morphology and goblet cell metaplasia has not been reported in eyes with PEX before us.

In this study, grading was made according to epithelial cell changes in statistical analysis of the specimens having epithelial goblet cell discrepancy. For the evaluation of early phased ocular surface disease, modifications on Nelson's grading system or establishment of a new one may provide better classification to separately assess epithelial cell metaplasia and goblet cell changes.

The major function of goblet cell is mucin production that is thought to play an important role in tear-film stability tested by TBUT[7,9,17-21]. Conjunctiva epithelial cell alteration disturbs not only tear film stability but also increase of evaporation and decrease in Schirmer's tests. Kozobolis *et al*[7] reported that both Schirmer's test and TBUT scores were significantly lower in the PEX patient than in control group. In the current study, cases with PEX displayed significantly lower scores in both Schirmer's and TBUT tests than normal subjects. We found, statistically significant correlation with increase of conjunctival metaplasia and decrease in TBUT too. Although mean values of TBUT tests were lower in PEX group with epithelial - goblet cell involvement discordance than the PEX patient having no such discordance; the differences were not statistically significant.

To our knowledge, one important finding in the current study is that mixed appearence on the conjunctival surface changes in patients with PEX. Another important new finding that as far as we know, this study is the first study that presents discordance between conjunctival epithelial and goblet cell involvement which indicates goblet cell morphology and density appears to be significantly altered prior epithelial cell changes in subject with PEX. All of this findings prove the presence of ocular surface disease in PEX histologically and decrease in both TBUT and Schirmer's test values showed that tear functions were also affected in this syndrome.

As a conclusion, conjunctival involvement may be more complicated in subject with PEX than classical knowledge as

previously reported. Even pseudoexfoliative subjects may be prone to develop dry eye, PEX may influence the conjunctiva in a different way than other patients with conjunctival surface disorders. As far as we know, our research is the first detailed study which demonstrated that subject with PEX has a very significant probability of having coexistent ocular surface disease with all related implications including keratitis sicca. The further studies are necessary to investigate exact nature of ocular surface manifestations of PEX.

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