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ASSESSMENT OF CORNEAL SUBBASAL NERVE PLEXUS USING CONFOCAL *IN VIVO* MICROSCOPY IN PATIENTS WITH PSEUDOEXFOLIATION SYNDROME AFTER PHACOEMULSIFICATION

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✧ Phacoemulsification (PE) is the leading method of cataract surgery. ***Purpose.*** To assess the impact of PE on corneal subbasal nerve plexus in patients with pseudoexfoliation syndrome (PEX) using confocal *in vivo* microscopy. ***Methods.*** 42 patients (42 eyes) were enrolled in the study. The main group consisted of 24 patients (24 eyes) with PEX syndrome, and 18 patients (18 eyes) without it composed the control group. Confocal *in vivo* microscopy was performed before and after PHACO. ***Results.*** In patients with PEX after PE, an increase in number of nerve branches and pellet-like structures in them were noticed ($p < 0,05$).

✧ ***Keywords:*** phacoemulsification; confocal microscopy; pseudoexfoliation syndrome.

ОЦЕНКА СОСТОЯНИЯ СУББАЗАЛЬНОГО НЕРВНОГО СПЛЕТЕНИЯ РОГОВИЦЫ ПО ДАННЫМ КОНФОКАЛЬНОЙ *IN VIVO* МИКРОСКОПИИ У ПАЦИЕНТОВ С ПСЕВДОЭКСФОЛИАТИВНЫМ СИНДРОМОМ ПОСЛЕ ФАКОЭМУЛЬСИФИКАЦИИ

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✧ Факоэмульсификация (ФЭ) является ведущим способом хирургического лечения катаракты. ***Цель*** — оценить влияние ФЭ на состояние суббазального нервного сплетения роговицы у пациентов с псевдоэксфолиативным синдромом (ПЭС) по данным конфокальной *in vivo* микроскопии. ***Материалы и методы.*** Было обследовано 42 пациента (42 глаза). Основную группу составили 24 пациента (24 глаза) с ПЭС, группу контроля — 18 пациентов (18 глаз) без ПЭС. Всем пациентам до и после ФЭ выполняли конфокальную *in vivo* микроскопию. ***Результаты.*** У пациентов с ПЭС после ФЭ наблюдалось увеличение количества ветвей нервных волокон и увеличение количества гранулоподобных структур в них ($p < 0,05$).

✧ ***Ключевые слова:*** факоэмульсификация; конфокальная микроскопия; псевдоэксфолиативный синдром.

INTRODUCTION

Changes in the eye surface are often observed in the elderly and in those with pseudoexfoliation syndrome (PEX) [3, 5, 11]. PEX is usually associated with a shorter time of tear film break-up, severe conjunctivochalasis, reduced corneal sensitivity,

and increased subjective complaints [3]. The etiology and pathogenesis of these conditions in PEX remain poorly understood. Changes in the corneal sub-basal nerve plexus may aggravate the disorders of the eye surface. We previously demonstrated that individuals with PEX have a significantly increased number of nerve branches and granular structures in the corneal sub-basal nerve plexus [2, 4]. Here, we assessed the effect of phacoemulsification (PHACO) on the cornea in PEX-affected eyes [6].

We evaluated the impact of PHACO on corneal sub-basal nerve plexus in patients with and without PEX.

MATERIAL AND METHODS

The study included 42 patients (42 eyes) admitted to the 5th Department of Ophthalmology of City Hospital No. 2. All participants were divided into two groups: study (24 patients (24 eyes) with PEX) and control (18 patients (18 eyes) without PEX) groups. PEX was diagnosed based on the detection of pseudoexfoliation material in the anterior lens capsule, on the pupillary border of the iris, or in the corner of the anterior chamber angle. Patients in both groups were matched for sex and age (Table 1).

The exclusion criteria were as follows: corneal dystrophy, glaucoma, contact lens wear, use of IOP-lowering drops or artificial tears, diabetes mellitus, and history of eye surgery. In addition to the standard ophthalmological examination, all patients underwent corneal confocal microscopy prior to PHACO and in 1 month after it.

The procedure was performed under epibulbar anesthesia using the Heidelberg Retina Tomograph 3 (HRT3; Heidelberg Engineering GmbH, Heidelberg, Germany) fitted with a confocal laser-scanning microscope (Rostock Cornea Module (RCM)). The image area was 400 × 400 μm; acquired images had a resolution of 384 × 384 pixels.

Microscopic examination was performed in the central cornea by the researcher blinded to patient clinical data.

The condition of the sub-basal nerve plexus was assessed using the CCMetrics Image Analysis Software v. 1.1. We estimated the number of nerve fibers and their branches, nerve fiber density, and coefficient of tortuosity calculated according to the method developed by Kinard et al. [10]. The density of nerve fibers and their branches was estimated per 1 mm^2 as follows: length of nerve fibers × coefficient (0.00075) / size of the scanning area. The tortuosity coefficient was determined automatically. In addition to the abovementioned parameters, we evaluated the changes in nerve structure by measuring granules using a score system (score from 0 to 3).

All patients underwent uncomplicated PHACO using the phaco chop technique performed with the Infinity vision system (Alcon, Inc., USA). Akreos AO intraocular lenses (Bausch and Lomb, USA) were implanted. During the postoperative period, all participants received standard anti-inflammatory treatment with dexamethasone for 4 weeks (with gradual dosage reduction) and levofloxacin for 2 weeks.

Statistical analysis was performed using SPSS Statistics v.20.0. Data were checked for normality using the Kolmogorov–Smirnov test. The *t*-test was used to compare quantitative variables between the two independent groups. Differences were considered significant for $p < 0.05$.

RESULTS

The number of nerve fibers per mm^2 prior to PHACO did not significantly differ between the groups (20.3 ± 6.8 vs. 24.3 ± 7.6 in the study and the control groups, respectively; $p = 0.43$); after PHACO, these numbers were 18.7 ± 4.5 and 21.9 ± 6.6, respectively ($p = 0.35$). Both groups showed an insignificant decrease in the number of nerve fibers by 7.7% and 10.0%, respectively, after PHACO (Fig. 1).

The number of nerve branches per mm^2 was 47.9 ± 13.5 and 16.0 ± 4.94 ($p = 0.045$) before PHACO and 80.5 ± 12.5 and 21.1 ± 6.9 ($p = 0.04$) after PHACO in the study and the control groups, respectively. Both groups demonstrated a significant increase in the number of nerve branches by 68.1% and 32.0%, respectively ($p = 0.0001$; Fig. 2).

Table 1

Patients distribution by sex and age

Таблица 1

Распределение групп по полу и возрасту

Parameter		Study group, $n = 24$	Control group, $n = 18$	Significant difference, p
Age		74.6 ± 3.8	75.3 ± 4.1	0.51
Sex	Male	6 (25%)	6 (33.3%)	0.23
	Female	18 (75%)	12 (66.6%)	
Note: n, number of patients				

The density of nerve fibers (mm/mm^2) was 6.49 ± 1.3 and 6.13 ± 1.1 ($P = 0.81$) before PHACO and 6.07 ± 1.2 and 5.67 ± 0.8 after PHACO in the experimental and control groups, respectively. There was an insignificant decrease in the density of nerve fibers in both the groups ($p = 0.89$; Fig. 3).

The tortuosity coefficient was 0.067 ± 0.002 and 0.052 ± 0.002 ($p = 0.48$) before PHACO and 0.082 ± 0.008 and 0.064 after PHACO in the study and the control groups, respectively. Patients in both groups demonstrated an insignificant increase in nerve fiber tortuosity by 22.4% and 23.1%, respectively ($p = 0.16$; Fig. 4).

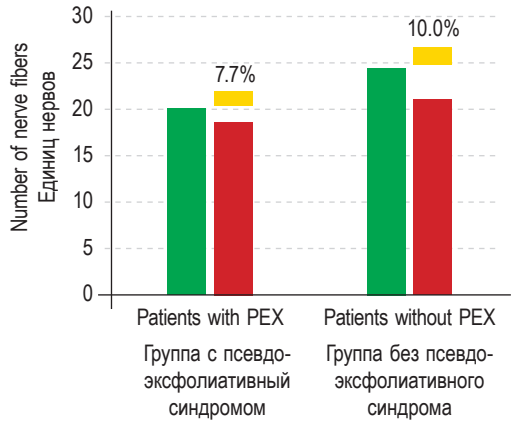
Therefore, patients with PEX had a significant increase in the number of nerve branches per mm^2 after PHACO ($p = 0.0001$; Fig. 5). The remaining parameters did not significantly differ between the groups.

Granular changes in nerve cells were observed in both groups; however, the experimental group showed significantly more pronounced changes before and after PHACO. Mean score before PHACO was 2.1 and 1.1 in the study and the control groups, respectively, compared to 2.4 and 1.3, respectively, after PHACO ($p = 0.03$ and $p = 0.04$, respectively; Fig. 6).

DISCUSSION

The ocular surface is an integrated anatomical and functional structure that includes conjunctival epithelium, limbus, and cornea [1], and the interaction between these components is impossible without normal ocular adnexa, i. e., eyelids, eyelashes, and various glands [1, 3].

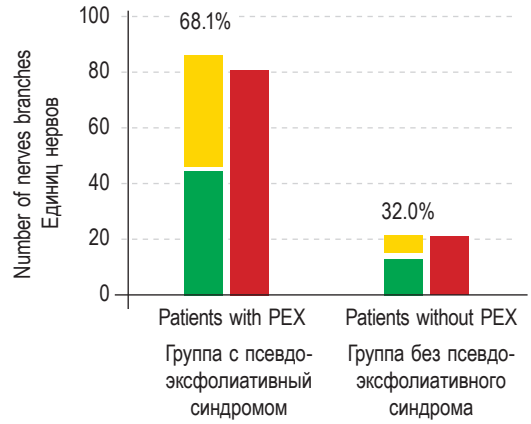
Dry eye syndrome (DES) is a complex disorder of the eye surface manifesting with discomfort, visual impairment, pain, and reduced quality of life [9]. Multiple studies have demonstrated ocular surface lesions in patients with PEX [3, 7, 9]. These patients usually have normal basal tear production, but tear film stability is often impaired, possibly due to meibomian gland dysfunction and an atonic lower eyelid. These conditions have the following symptoms: decreased number of functioning meibomian glands, increased viscosity of meibomian secretions, shorter time of tear film break-up, reduced orbicularis muscle tone, and decreased function of the lower eyelid retractors [3]. Moreover, many investigators



	Patients with PEX	Patients without PEX
Before PHACO / До факоэмульсификации	20.3	24.3
After PHACO / После факоэмульсификации	18.7	21.9
Absolute difference / Абсолютная разница	1.6	2.4

Fig. 1. Number of nerve fibers in 1 mm^2 before and after PHACO

Рис. 1. Количество нервных волокон на 1 мм^2 до и после факоэмульсификации



	Patients with PEX	Patients without PEX
Before PHACO / До факоэмульсификации	47.9	16
After PHACO / После факоэмульсификации	80.5	21.1
Absolute difference / Абсолютная разница	32.6	5.1

Fig. 2. Number of branches of nerve fibers in 1 mm^2 before and after PHACO

Рис. 2. Количество ветвей нервных волокон на 1 мм^2 до и после факоэмульсификации

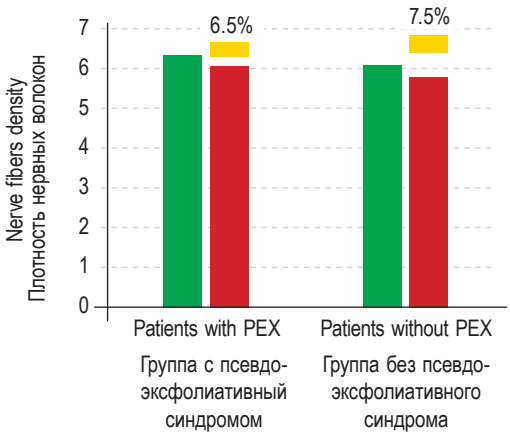


Fig. 3. Nerve fibers density before and after PHACO

Рис. 3. Плотность нервных волокон до и после факоэмульсификации, мм/мм^2

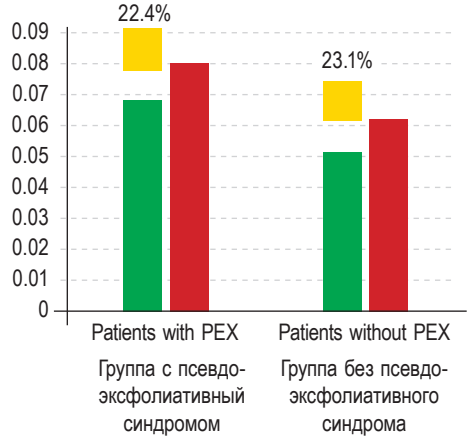


Fig. 4. Tortuosity coefficient of nerve fibers before and after PHACO

Рис. 4. Коэффициент извитости нервных волокон до и после факоэмульсификации

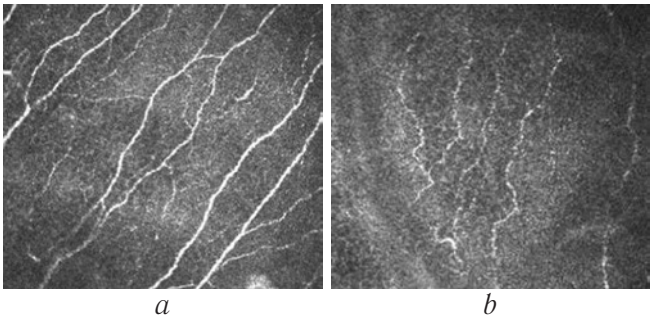


Fig. 5. Tortuosity of subbasal nerve plexus: *a* — in control group; *b* — in PEX group

Рис. 5. Извитость суббазальных нервных волокон: *a* — у пациентов без псевдоэксфолиативного синдрома; *b* — у пациентов с псевдоэксфолиативным синдромом

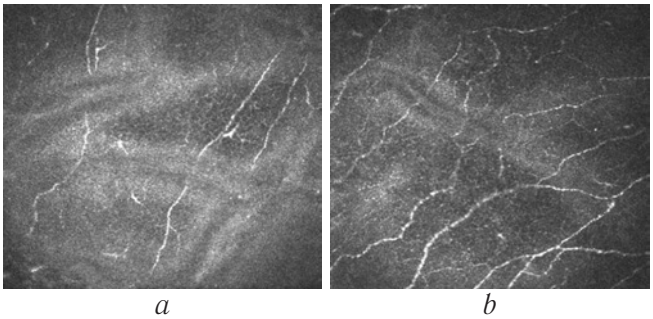


Fig. 6. Subbasal nerve plexus: *a* — in control group; *b* — in PEX group

Рис. 6. Изменение нервных волокон по типу гранул: *a* — у пациентов без псевдоэксфолиативного синдрома; *b* — у пациентов с псевдоэксфолиативным синдромом

have reported reduced corneal sensitivity in patients with PEX [11, 17, 20].

Innervation of the cornea and bulbar conjunctiva is primarily provided by the ophthalmic branch of the trigeminal nerve and sympathetic and parasympathetic nerve fibers [14, 15]. In addition to sensory functions, corneal nerves provide protective and trophic functions and also regulate corneal epithelial integrity, proliferation, and wound healing [18]. Moreover, stimulation of the nerve fibers and subsequent damage are believed to be a pathophysiologic mechanism involved in DES development [9]. Furthermore, activation of trigeminal nociceptors may cause clinical manifestations of DES [9].

In vivo confocal microscopy can be used for qualitative and quantitative analysis of the corneal subbasal nerve plexus. However, studies assessing the condition of the corneal sub-basal nerve plexus in patients with ocular surface disorders showed controversial results [13], which can be attributed to the

complexity of DES, its varying severity, and use of different methods for assessing the corneal sub-basal nerve plexus [7, 13].

In vivo confocal microscopy has allowed for the identification of various morphological characteristics of the cornea in PEX, including increased density of dendritic cells and hyper-reflective intercellular inclusions, increased epithelial desquamation, thickening of Bowman's membrane, and reduced density of basal and wing cell layers and stromal keratocytes [2, 17, 20]. These changes are hypothetically associated with chronic inflammatory and dystrophic processes typical of DES. Our findings suggested that patients with PEX have an increased number of nerve branches and pronounced granular changes in nerve cells after PHACO, which indicates the aggravation of morphological manifestations of corneal neuropathy during the postoperative period. The most probable causes are either damage to nerve fibers or an increase in their metabolic activity in response to chronic inflammation affecting the tissue of the ocular surface [7].

Similar to any other corneal surgery that requires long-term postoperative anti-inflammatory therapy, PHACO impairs tear film stability, which may result in DES development or aggravation of its symptoms [9, 13, 20].

CONCLUSION

We demonstrated PEX-associated morphological changes in the corneal sub-basal nerve plexus as well as the effect of PHACO. Patients with PEX have increased number of nerve branches per mm^2 and pronounced granular changes in nerve cells. After PHACO, these changes become even more significant and reflect PEX-related damage to the ocular surface.

The authors declare no conflicts of interest related to the current manuscript.

Authors' contribution:

Research concept and study design: V.V. Potyomkin and E.V. Ageeva

Data collection and processing: V.V. Potyomkin, T.S. Varganova, I.V. Terekhova, and E.V. Ageeva

Data analysis and drafting the manuscript: V.V. Potyomkin and E.V. Ageeva

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