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ORIGINAL RESEARCH

Analysis of Clinical Characteristics and Neuropeptides in Patients with Dry Eye with and without Chronic Ocular Pain after FS-LASIK

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ABSTRACT

Introduction: Chronic ocular pain, particularly prevalent in patients with dry eye disease and post-femtosecond laser-assisted laser in situ keratomileusis (FS-LASIK) surgery, presents with unclear clinical characteristics and an undefined pathogenesis. In this study, we aimed to compare clinical characteristics and tear neuropeptide concentrations in patients with dry eye disease (DED) with and without chronic ocular pain following FS-LASIK, and investigate correlations between ocular pain, clinical characteristics, and tear neuropeptide levels.

Methods: Thirty-eight post–FS-LASIK patients with DED were assigned to two groups: those with chronic ocular pain and those without chronic ocular pain. Dry eye, ocular pain, and mental health-related parameters were evaluated using specific questionnaires and tests. The morphology of corneal nerves and dendritic cells (DCs) was evaluated by in vivo confocal microscopy. Function of corneal innervation was evaluated by corneal sensitivity. Concentrations of tear cytokines (interleukin [IL]-6, IL-23, IL-17A, and interferon-γ) and neuropeptides (α-melanocyte-stimulating hormone, neurotensin, β-endorphin, oxytocin, and substance P [SP]) were measured using the Luminex assay.

Results: Most patients with chronic ocular pain experienced mild to moderate pain; the most common types included stimulated pain (provoked by wind and light), burning pain, and pressure sensation. More severe dry eye ($P < 0.001$), anxiety symptoms ($P = 0.026$), lower Schirmer I test values ($P = 0.035$), lower corneal nerve density ($P = 0.043$), and more activated DCs ($P = 0.041$) were observed in patients with ocular pain. Tear concentrations of SP and oxytocin were significantly higher in

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patients with ocular pain ($P = 0.001$, $P = 0.021$, respectively). Furthermore, significant correlations were observed among ocular pain severity, SP, and anxiety levels.
Conclusions: Patients with DED after FS-LASIK who have chronic ocular pain show more severe ocular and psychological discomfort and higher tear levels of neuropeptides. Furthermore, ocular pain severity is correlated with tear SP levels.
Trial Registration: ClinicalTrials.gov identifier: NCT05600985.

Keywords: Dry eye; Ocular pain; Neuropeptides; Inflammatory cytokines; FS-LASIK

Key Summary Points

Why carry out this study?

Dry eye disease (DED) is the most common complication after femtosecond laser-assisted laser in situ keratomileusis (FS-LASIK). It becomes more worrisome when combined with chronic ocular pain.

Although chronic ocular pain after FS-LASIK has been reported, the description of its features remain scarce and the mechanism underlying is unclear.

In our study, we analyzed clinical characteristics in post–FS-LASIK patients with DED, comparing characteristics between those with and without chronic ocular pain. This research sheds light on pain-related clinical features and neuropeptides, offering new insights for eye care practitioners.

What was learned from this study?

Post–FS-LASIK patients with DED, who experience chronic ocular pain show more severe ocular and psychological discomfort and higher tear levels of neuropeptides. Furthermore, ocular pain severity is correlated with tear substance P (SP) levels.

INTRODUCTION

Pain is defined as "an unpleasant sensory and emotional experience" [1]. Chronic ocular pain is defined as pain originating from the ocular surface that persists for more than 3 months and significantly affects the daily activities of patients, and is common in patients with dry eye disease (DED), especially in patients after refractive surgery [2]. Although femtosecond laser-assisted laser in situ keratomileusis (FS-LASIK) has been the most frequent refractive surgery in recent years, there are still a number of patients who suffer from some form of ocular pain [3], especially those with psychiatric and neurological problems including fibromyalgia, anxiety, and depression [4]. Additionally, chronic ocular pain has a substantial impact on the quality of life and causes a huge financial burden [5].

Although chronic ocular pain after FS-LASIK has been reported previously [6], the description of its features remains scarce and the mechanism underlying chronic ocular pain in patients with DED following FS-LASIK is unclear. Due to the lack of understanding of ocular pain, there is currently no effective drug or treatment for ocular pain. Recently, a new questionnaire, the Neuropathic Pain Symptom Inventory modified for the Eye (NPSI-Eye), was validated to assess ocular pain features, which will help eye care practitioners better understand the characteristics of ocular pain [7]. There is past evidence to support that corneal nerve damage partially results in ocular pain in patients after refractive surgery [8, 9]. However, this is not sufficient to explain the pathogenesis of ocular pain. At present, increasing attention is being paid to the interactions between dendritic cells (DCs) and neuropeptides following FS-LASIK, which are the core of neuro-immune interaction [10]. However, whether tear neuropeptides and corneal DCs are involved in the pathogenesis of ocular pain, or whether it is the consequence of the pain process, remains unknown [11].

Therefore, we performed a cross-sectional survey aiming to investigate chronic ocular pain features, ocular characteristics, corneal DCs,

tear film cytokines, and neuropeptides in patients with and without ocular pain, and explored the profile of clinical characteristics and pain-related neuropeptides in patients experiencing chronic pain associated with DED following FS-LASIK.

METHODS

This cross-sectional study included 38 patients with post–FS-LASIK DED. The patients were categorized into two groups based on the presence or absence of chronic ocular pain: (1) patients with DED after FS-LASIK who have chronic ocular pain and (2) patients with DED after FS-LASIK who do not have chronic ocular pain. This study complied with the principles of the Declaration of Helsinki and was approved by the Medical Science Research Ethics Committee of Peking University Third Hospital (M2023048). Written informed consent was obtained from all participants before their participation.

The sample size was estimated based on the difference in numerical rating scale (NRS) scores between post-refractive surgery patients with and without ocular pain, as documented in previous studies [12]. We employed the following formula to estimate the standard deviation (SD) from the 95% confidence intervals (CIs) of the mean NRS scores: $SD \approx [(CI\ upper - CI\ lower)/2 \times 1.96] \times \sqrt{n}$. With the estimated SDs, we conducted sample size calculations via PASS 15.0 software, opting for the method using two-sample *t*-tests allowing unequal variance. This analysis indicated that 12 patients per group would be required to achieve 90% power with an alpha level of 0.01. Therefore, 20 and 18 participants were recruited in two groups, respectively, to allow for missing data. All tests were performed on both eyes; only the data from the right eye were used for analysis to ensure consistency.

Participants

We included patients aged ≥ 18 years who had (1) undergone bilateral FS-LASIK 12 months earlier, (2) experienced DED [13] diagnosed according to the Tear Film & Ocular Surface Society (TFOS) DEWS II criteria (Ocular Surface Disease Index [OSDI] score ≥ 13 and tear breakup time [TBUT] < 10 s) for more than 6 months after FS-LASIK, and (3) experienced chronic ocular pain, which was indicated by a numerical rating scale (NRS) score ≥ 2 [8] and lasted at least 3 months. [14] The exclusion criteria were as follows: (1) any other ocular surgery, (2) ocular active infections, (3) glaucoma, (4) topical or systemic medication therapies within 2 weeks prior to recruitment, and (5) any other major systemic diseases, including diabetes, malignant tumors, and autoimmune diseases, such as Sjögren's syndrome.

Clinical Questionnaires

Ocular pain severity was assessed using the NRS (range 0–10), which rates pain intensity as none (0), mild (1–3), moderate (4–6), or severe (7–10) [15]. Ocular pain characteristics were described using the Neuropathic Pain Symptom Inventory modified for the eye (NPSI-Eye; range 0–100) [7]. The OSDI (0–100) evaluated DED-related symptoms, and the overall score classified patients into the following severity groups: 0–12, absence of symptoms; 13–22, mild symptoms; 23–32, moderate symptoms; and 33–100, severe symptoms [16]. The Hamilton Anxiety Rating Scale (HAMA) [17] (range 0–56) and Hamilton Depression Rating Scale (HAMD) [18] (range 0–52) were used to evaluate the severity of anxiety and depression, respectively.

Ocular Surface Evaluations

We performed TBUT, Schirmer I test (SIt), corneal fluorescein staining (CFS), and conjunctival lissamine green (LG) staining to evaluate ocular surface signs. TBUT was evaluated with a cobalt blue filter over a slit-lamp biomicroscope. The SIt was conducted using Schirmer paper strips (5 × 35 mm) without anesthesia. CFS and LG staining were evaluated using the National Eye Institute Workshop guidelines (total score: 0–15) [19] and the Oxford grading panel (total score: 0–15) [20], respectively. Corneal sensitivity is a method used to evaluate the function

of corneal nerves, and was measured using a Cochet-Bonnet esthesiometer (Luneau Ophthalmologie, Chartres, France).

The morphological parameters of the corneal nerve were analyzed using ACCMetrics software (University of Manchester, UK) [21]. Participants were asked to fixate on a specially designed target to map a 1 mm^2 image of the corneal sub-basal nerve plexus at the central cornea [22]. Five representative images of the sub-basal nerve plexus of the central cornea were selected by two masked observers for analysis (resolution: 384 × 384 pixels; area: 400 mm × 400 mm [0.16 mm^2]) [23]. The nerve parameters included corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber total branch density (CTBD), and corneal nerve fiber length (CNFL). DCs are hyperreflective cells with or without processes emanating from the cell body. Activated DCs (aDCs) and non-activated DCs were distinguished according to the number of processes [24, 25]. DCs are categorized as "activated" if they have at least three processes emitting from the cell body [24, 26]. DCs and aDCs were manually counted by two independent observers masked to the clinical findings based on the previous literature [25]. A semiautomatic image processing software (ImageJ, National Institutes of Health, Bethesda, MD, USA) was used to quantify DC and aDC parameters. Marked cells of each type were averaged between both observers. Prior to commencing the study, we first examined inter-rater reliability using the intra-class correlation coefficient (ICC). Two masked readers evaluated 40 images with an ICC of 0.974 ($P < 0.001$) for the DC number and 0.960 ($P < 0.001$) for the aDC number.

Analysis of Tear Cytokine and Neuropeptides

Recent studies have shown that inflammatory cytokines and neuropeptides correlate with refractive surgery-related DED or ocular pain [12]. Therefore, we measured the levels of inflammatory cytokines (interleukin [IL]-17A, IL-23, IL-6, and interferon [IFN]-γ) and neuropeptides (α-melanocyte-stimulating hormone [α-MSH], neurotensin, β-endorphin, oxytocin, and substance P [SP]) in the two groups. In order to analyze for these substances, basal tear samples (5 μL) were collected non-traumatically from the external canthus of the patient's eyes with clean glass capillary micropipettes (Drummond Scientific Co., Broomall, PA, USA), which were collected in sterile collection tubes. Care was taken to avoid additional tear reflex as much as possible. The collection tubes were kept cold (4 °C) during collection and then immediately stored at −80 °C. The cytokines and neuropeptides were analyzed using the Luminex assay. According to previous research [27], tear collection was performed before any other test and within a maximum of 10 min.

Statistical Analyses

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Normal distribution was checked using the Shapiro–Wilk test. Quantitative data are summarized as means ± standard deviations (SD) or medians (interquartile ranges) according to their normality distributions, whereas qualitative data were summarized using percentages. If the data did not conform to a normal distribution, the Mann–Whitney *U* test was used for two independent samples. Spearman's correlation coefficient was used to analyze the correlation between neuropeptides and ocular parameters. Statistical significance was set at $P < 0.05$.

RESULTS

This study included 38 patients with a mean age of 32.03 ± 6.37 years (range 21–46 years). Among them, 20 patients were enrolled in the post–FS-LASIK DED with ocular pain group and 18 in the post–FS-LASIK DED without ocular pain group. Table 1 shows the demographic characteristics of the two groups of patients. There were no significant differences in mean age, sex ratio, or spherical equivalent between the two groups.

Table 1 Demographic characteristics of the study groups

Demographics	DED after FS-LASIK		*P* value
	With ocular pain	Without ocular pain	
Participants/eyes (*n*/*n*)	20/20	18/18	
Female/male (*n*/*n*)	16/4	15/3	0.674
Age (years), mean ± SD	33.08 ± 5.79	34.86 ± 3.45	0.141
Spherical equivalent (D), mean ± SD	−5.99 ± 1.54	−5.89 ± 2.42	0.073

DED dry eye disease; *FS-LASIK* femtosecond laser-assisted laser in situ keratomileusis; *n* number; *SD* standard deviation; *D* diopter

Table 2 Ocular pain features in post–FS-LASIK DED with ocular pain group

Ocular pain specific questionnaires	
NRS (score), median (IQR)	3 (2–4)
Mild pain, *n* (%)	12 (60%)
Moderate pain, *n* (%)	7 (35%)
Severe pain, *n* (%)	1 (5%)
NPSI-eye (score), median (IQR)	4.50 (0.50–16.75)
Burning, *n* (%)	9 (45%)
Squeezing, *n* (%)	8 (40%)
Pressure, *n* (%)	11 (55%)
Electric shocks, *n* (%)	1 (5%)
Stabbing, *n* (%)	3 (15%)
Provoked by wind, *n* (%)	12 (60%)
Provoked by light, *n* (%)	9 (45%)
Provoked by contact with cold, *n* (%)	4 (20%)
Pins and needles, *n* (%)	5 (25%)
Tingling, *n* (%)	6 (30%)

FS-LASIK femtosecond laser-assisted laser in situ keratomileusis; *DED* dry eye disease; *NRS* numerical rating scale; *NPSI-Eye* Neuropathic Pain Symptom Inventory modified for the eye; *n* number; *IQR* interquartile range

Severity and Features of Ocular Pain

Table 2 shows the ocular pain characteristics of the post–FS-LASIK DED with ocular pain group.

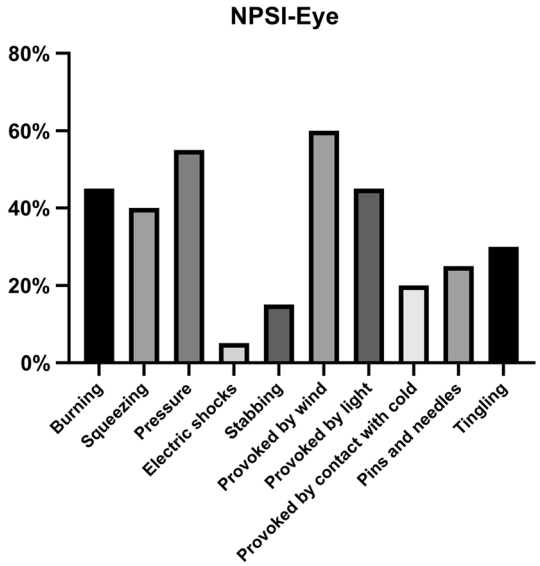

Fig. 1 The features of ocular pain in post–FS-LASIK DED with ocular pain group. *NPSI-Eye* Neuropathic Pain Symptom Inventory modified for the eye; *DED* dry eye disease; *FS-LASIK* femtosecond laser-assisted laser in situ keratomileusis

According to the NRS, 19 (95%) of patients with ocular pain experienced mild or moderate pain. Among the included patients, 12 (60%) reported mild pain (scores 1–3), 7 (35%) reported moderate pain (scores 4–6), and 1 (5%) reported severe pain (scores 7–10). The mean NRS score was 3.30. According to the NPSI-Eye, the most common types of ocular pain were stimulated pain provoked by wind (60%) and light (45%), pressure sensation (55%), and burning pain (45%) (Fig. 1).

Table 3 Clinical characteristics of patients in each study group

Characteristic	DED after FS-LASIK		*P* value
	With ocular pain	Without ocular pain	
OSDI (score), median (IQR)	25 (16.67–35.42)	14.58 (14.41–16.09)	< **0.001**
Mild (13–22)	8 (40%)	17 (94%)	
Moderate (23–32)	6 (30%)	1 (6%)	
Severe (33–100)	6 (30%)	0	
Ocular surface finding, median (IQR)			
TBUT (s)	2.5 (2–3)	3 (2–3.25)	0.484
CFS (score)	2 (0–4.5)	1.5 (0–3)	0.741
Schirmer I test (mm)	6.5 (3.25–10)	12 (6.5–18.5)	**0.035**
LG (score)	0.5 (0–2)	1 (0–3)	0.815
Corneal sensitivity (cm)	5.9 (5.6–6)	6 (5.88–6)	0.125
Corneal nerve parameters			
CNFD (n/mm^2), median (IQR)	11.46 (4.5–15)	15.62 (11.98–18.75)	**0.043**
CNBD (n/mm^2), median (IQR)	11.25 (3.12–37.5)	20.83 (10–38.75)	0.132
CNFL (mm/mm^2), median (IQR)	8.46 (5.93–12.01)	11.87 (9.12–13.02)	**0.048**
CTBD (n/mm^2), median (IQR)	26.25(8.75–56.25)	42.91 (22.5–65.93)	0.086
Dendritic cells, median (IQR)			
Dendritic cells (cells/image)	11.12 (3.12–25.60)	5.10 (2.43–6.90)	0.063
Activated dendritic cells (cells/image)	2.00 (0.44–5.95)	0.65 (0.20–1.85)	**0.041**
Mental status, median (IQR)			
HAMA (score)	6 (4–9.5)	2.5 (0–6)	**0.026**
HAMD (score)	3 (0–6)	2 (0–4.25)	0.374

DED dry eye disease; *FS-LASIK* femtosecond laser-assisted laser in situ keratomileusis; *OSDI* Ocular Surface Disease Index; *TBUT* tear breakup time; *CFS* corneal fluorescein staining; *LG* Lissamine green; *CNFD* corneal nerve fiber density; *CNBD* corneal nerve branch density; *CNFL* corneal nerve fiber length; *CTBD* corneal nerve fiber total branch density; *HAMA* Hamilton Anxiety Rating Scale; *HAMD* Hamilton Anxiety Depression Scale; *IQR* interquartile range. The Mann–Whitney *U* test was used for two independent samples

Ocular Surface Parameters

Table 3 shows the clinical characteristics of the patients. The patients in the post–FS-LASIK DED with ocular pain group showed higher OSDI ($P < 0.001$) and HAMA ($P = 0.026$) scores and shorter SIt values ($P = 0.035$) than those in the post–FS-LASIK DED without ocular pain group. Concerning the OSDI questionnaire, 40% of patients reported mild symptoms, 30% reported moderate symptoms, and 30% reported severe symptoms in the post–FS-LASIK with ocular pain group; conversely, in the post–FS-LASIK DED without ocular pain group, 94% of patients reported mild symptoms, 6% reported moderate symptoms, and no patients experienced severe

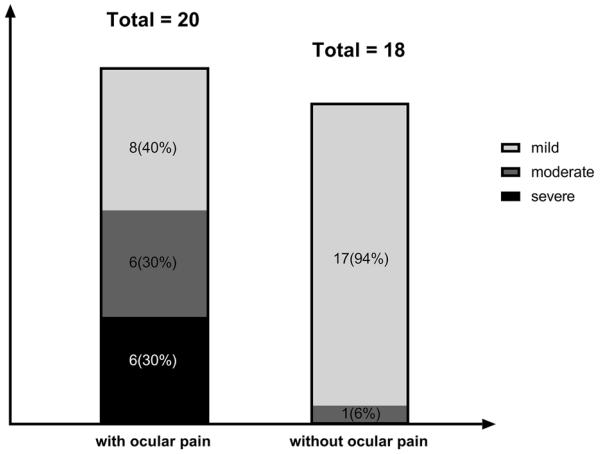

Fig. 2 The severity of dry eye symptoms in the two groups

symptoms (Fig. 2). Regarding the morphological parameters of the corneal nerve, the CNFD and CNFL were lower in the post–FS-LASIK DED with ocular pain group than in the post–FS-LASIK DED without ocular pain group ($P = 0.043$, $P = 0.048$, respectively). Corneal sensitivity was similar between the two groups ($P = 0.125$). Total DCs and activated DCs (aDCs) were reported as numbers per image (cells/image). There was no significant difference in the total number of DCs between the two groups ($P = 0.063$). However, the number of aDCs in the post–FS-LASIK DED group with ocular pain was higher than that in the post–FS-LASIK DED group without ocular pain ($P = 0.041$) (Table 3).

Cytokines and Neuropeptides Levels

Tear cytokine and neuropeptide levels are presented in Table 4. There were no significant differences in any of the inflammatory cytokines (IL-17A, IL-23, IL-6, and IFN-γ) between the two groups (all $P > 0.05$). As for neuropeptide concentrations, the post–FS-LASIK DED with ocular pain group showed higher levels of oxytocin and SP than those of the post–FS-LASIK DED without ocular pain group ($P = 0.021$, $P = 0.001$, respectively); however, there were no significant differences in α-MSH, β-endorphin, or neurotensin levels between the two groups (all $P > 0.05$) (Fig. 3).

Table 4 Concentrations of tear cytokines and neuropeptides in each study group

Concentrations in tears	DED after FS-LASIK		*P* value
	With ocular pain	Without ocular pain	
Inflammatory cytokines (pg/ml)			
IFN-γ, median (IQR)	49.28 (11.52–65.61)	24.04 (4.70–66.06)	0.273
IL-6, median (IQR)	27.99 (20.93–35.97)	24.92 (8.20–49.17)	0.465
IL-17A, median (IQR)	36.74 (18.04–44.55)	25.30 (12.84–50.63)	0.404
IL-23, median (IQR)	1678.87 (869.66–2053.43)	1257.85 (702.32–2225.22)	0.501
Neuropeptides (ng/ml)			
α-MSH, median (IQR)	1566.90 (1198.02–1992.36)	1184.84 (906.00–1821.39)	0.161
β-Endorphin, median (IQR)	1245.02 (944.10–2056.91)	1128.86 (695.98–1965.53)	0.447
Neurotensin, median (IQR)	196.02 (85.30–322.12)	106.04 (79.06–225.42)	0.248
Oxytocin, median (IQR)	585.04 (341.11–819.83)	315.46 (263.30–608.66)	**0.021**
SP, median (IQR)	417.13 (270.99–457.42)	225.98 (196.45–268.72)	**0.001**

DED dry eye disease; *FS-LASIK* femtosecond laser-assisted laser in situ keratomileusis; *INF-γ* interferon-γ; *IL* interleukin; *α-MSH* α-melanocyte-stimulating hormone; *SP* substance P; *IQR* interquartile range

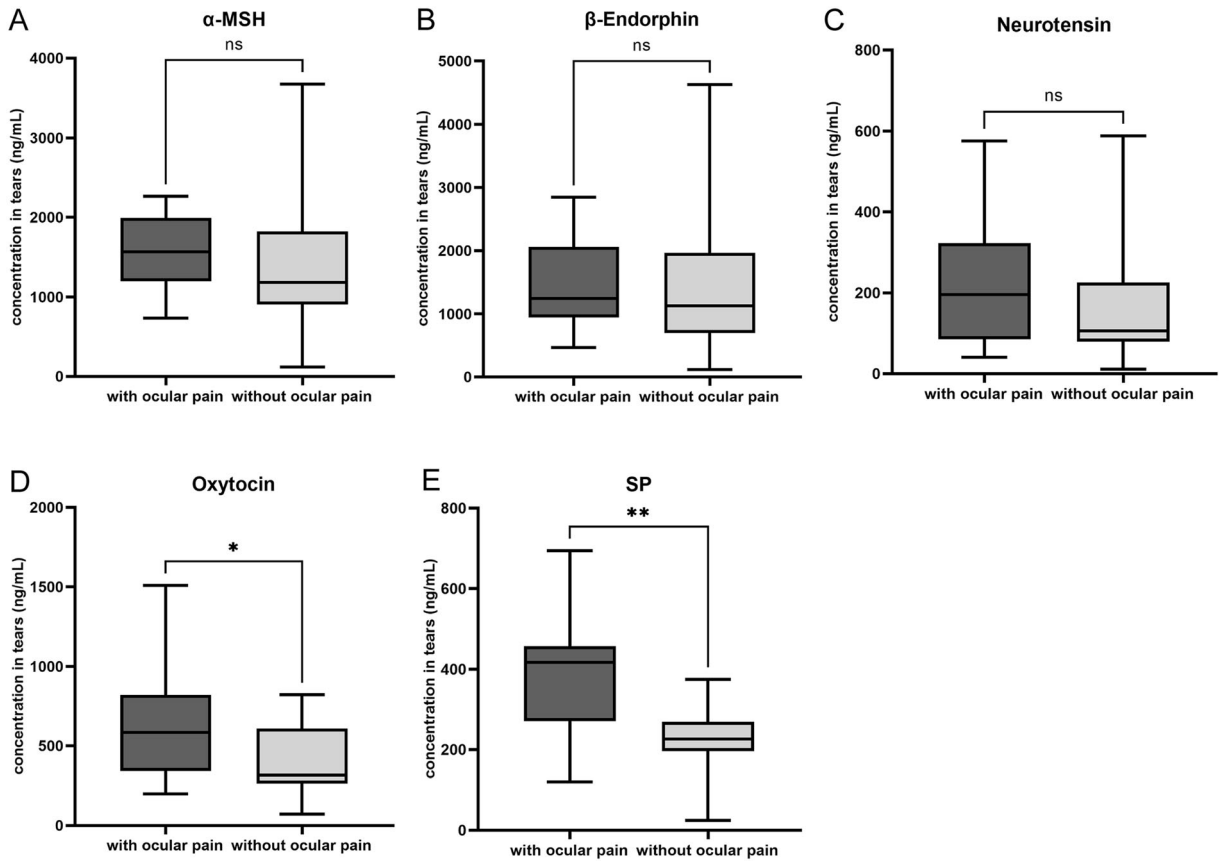

Fig. 3 The tear neuropeptide concentrations in two groups. **A** α-MSH, **B** β-endorphin, **C** neurotensin, **D** oxytocin, **E** SP. *α-MSH* α-melanocyte stimulating hormone; *SP* substance P. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, no significance

Correlations Between Ocular Pain, Neuropeptides, and Other Parameters

In patients with chronic ocular pain, there was a significant correlation between the NRS scores and SP levels ($r = 0.477$, $P = 0.033$). In addition, a significant correlation was found between the NRS and HAMA scores ($r = 0.479$, $P = 0.033$) (Fig. 4). However, no significant correlation was found between ocular pain and the other parameters.

DISCUSSION

In this study, we characterized a cohort of patients with post–FS-LASIK DED with and without chronic ocular pain. The goal of this study was to delineate the ocular pain features, ocular characteristics, corneal nerves, DCs, tear film cytokines, and neuropeptides in post–FS-LASIK patients with DED and develop tear molecular profiles that are associated with ocular pain.

The most common types of ocular pain in our study included stimulated pain (provoked by wind and light), pressure sensations, and burning pain. In previous study, the symptoms of "burning," "sensitivity to wind and light," "pins and needles," or "shooting pain" may indicate neuropathic pain instead of nociceptive pain [28]. These findings suggest that ocular pain types in post–FS-LASIK patients with DED may be neuropathic or a combination of neuropathic and nociceptive pain.

In our study, the SIt value was lower in the post–FS-LASIK DED with ocular pain group than in the group without ocular pain. Decreased tear secretion can lead to ocular inflammation, which further sensitizes the polymodal and

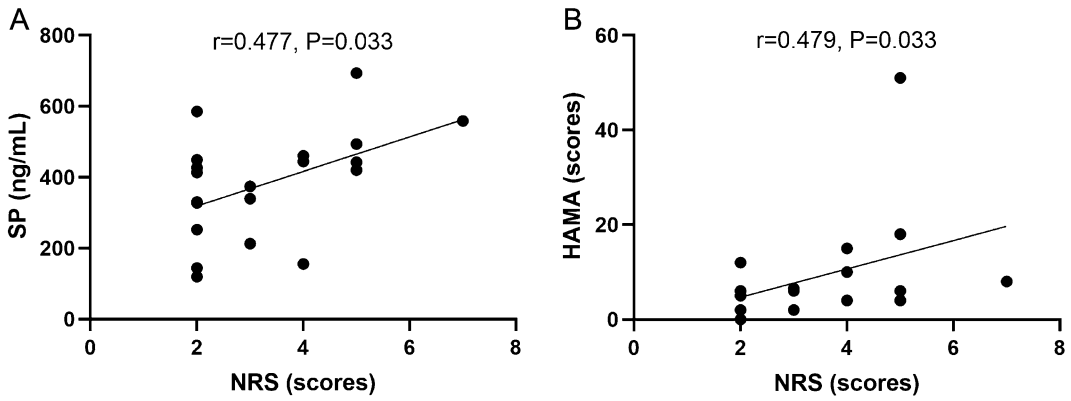

Fig. 4 Correlations between NRS scores and tear SP levels and HAMA scores in post–FS-LASIK DED with ocular pain group. Correlation of NRS scores with tear levels of SP (**A**). Correlation between NRS scores and HAMA scores (**B**). The *r* and *P* values were determined using Spearman's correlation coefficient. *NRS* numerical rating scale; *HAMA* Hamilton Anxiety Rating Scale; *SP* substance P; *DED* dry eye disease; *FS-LASIK* femtosecond laser-assisted laser in situ keratomileusis

mechanonociceptor nerve endings, eventually inducing ocular pain [29]. Structural and functional dysfunctions in the ocular sensory pathways ultimately lead to neuropathic ocular pain. However, there were no significant differences in TBUT, CFS, or LG staining between the two groups, which indicates that tear film instability may not be the main cause of the ocular pain.

Our findings revealed that although the ocular surface signs were similar in both groups, patients in the post–FS-LASIK DED with ocular pain group reported more severe dry eye symptoms and anxiety compared to those without ocular pain. Moreover, there was a positive correlation between the NRS and HAMA scores. Our findings suggest that chronic ocular pain may aggravate DED and anxiety symptoms in post–FS-LASIK patients with DED. These results are consistent with those of a previous study, which demonstrated that patients with more severe ocular pain exhibit higher OSDI scores and less healthy mental health indices [30, 31]. On the basis of these findings, a thorough psychiatric and social history and referral for mental health evaluation may be beneficial for patients with chronic ocular pain.

Regarding the morphology of the corneal sub-basal nerves, the ocular pain group had significantly lower CNFD and CNFL values than the patients without ocular pain. Zhang et al. [32] compared the corneal sub-basal nerve between patients with ocular pain and healthy participants and found that both CNFD and CNFL were decreased in patients with ocular pain. This finding suggests that poor corneal nerve recovery may be related to the pathogenesis of ocular pain. The cornea is densely innervated by sensory neurons [33], which are responsible for pain perception when the ocular surface is exposed to harmful stimulation or inflammation [34]. The injury of the ocular surface nerve upregulates the voltage-gated sodium channel of the neuron, thus reducing the threshold of signal transmission, including pain [35].Interestingly, both groups showed similar results in terms of corneal sensitivity. This may be because corneal perception mainly represents the density of the subepithelial nerve endings and does not completely reflect the density and length of the corneal sub-basal nerve.

DCs are antigen-presenting cells that constitute the majority of immune cells in the cornea and are considered a key role in neuroimmune crosstalk [36, 37]. Dendrites of DCs are one of the morphological characteristics of "activation." When the immune response is activated, DCs become larger and have longer dendrites [38, 39]. In the present study, the total number of corneal DCs was similar between the

two groups. However, aDC density was significantly higher in patients with ocular pain than in those without. These novel findings indicate new mechanisms by which DCs may be involved in regulating ocular pain. More aDCs showed an enhanced response to the antigens. Consequently, immune-targeted therapies may be effective strategies for treating ocular pain.

Biomarkers in tears can potentially be used as indicators of ocular surface innervation status. Although the number of studies examining neuropeptides and their role in DED is increasing [40, 41], only a few studies have investigated the relationship between neuropeptides and chronic ocular pain. In our study, higher tear SP and oxytocin neuropeptide levels were observed in patients with DED after FS-LASIK who experienced ocular pain, and a positive correlation was observed between NRS scores and tear SP levels. However, the levels of inflammatory cytokines in the tears were similar between the two groups. This suggests that nervous system function may account for ocular pain.

SP plays a key role in the migration of immune cells and the expression of chemokines [42]. Corneal nerve stimulation induces the local release of SP [43]. Factors released from neurons are recognized as mediators of persisting pain [44]. Similar to neuroinflammation, pain is not merely a "messenger" of peripheral tissue damage, but it can also trigger pro-inflammatory activity in the brain [45]. Pain impairs trigeminal neuronal regulatory activity in the brain, which triggers the continuous release of SP and possibly other neuromediators into the ocular surface, leading to an excessive inflammatory response [46]. Lasagni et al. revealed that stimulation of corneal nerves promoted ocular inflammation and initiated pain through the release of SP, and provided evidence that SP modulation can be exploited therapeutically [47]. Furthermore, reduced corneal pain has been observed in SP-knockout mice [48]. Our findings indicate that SP levels vary among the studies examined. This variation may be interpreted within the framework of methodological heterogeneity, including differences in assay techniques such as enzyme-linked immunosorbent assay (ELISA) and Luminex assay. Furthermore, the discrepancy in SP levels could be due to the variable severity of ocular surface conditions present within the study cohorts, as well as the innate biological variation in SP levels across distinct patient populations.

Oxytocin influences the immune and nervous systems and serves as an anti-inflammatory agent. [49] The second possible role of oxytocin is to accelerate nerve regeneration, probably by increasing the level of nerve growth factor. [50, 51] However, few studies have investigated the role of oxytocin in ocular diseases. Interestingly, we found that oxytocin levels were higher in patients in the ocular pain group; however, previous studies did not find any correlation between oxytocin expression and other ocular parameters. The elevated tear oxytocin concentration in patients with ocular pain could be due to corneal nerve damage and ocular inflammation caused by ocular surgery and DED, which could also be supported by the elevated aDCs and reduced corneal nerves in our patients.

This study had some limitations. First, we specifically concentrated on patients who underwent FS-LASIK, and consequently, we did not incorporate a healthy control group. This means that the findings are primarily applicable to the FS-LASIK patient population and may not be generalized beyond this group. Second, we did not differentiate between nociceptive and neuropathic pain in the patients. We will develop more detailed questionnaires and diagnostic protocols to further differentiate types of ocular pain, such as the Modified Single-Item Dry Eye Questionnaire (mSIDEQ), anesthetic challenge test, Belmonte's gas esthesiometry, and analysis of the presence of microneuromas. This approach, especially the investigation of microneuromas as a potential objective biomarker of corneal neuropathic pain [52], is expected to facilitate a more nuanced differentiation of ocular pain types and enhance our understanding of the pathogenic mechanisms underlying this condition. Third, since this was a cross-sectional observational study, we could not conclusively determine the causative effect for ocular pain. Further longitudinal studies are needed to evaluate the relationship between ocular pain

severity and the dynamic changes in other ocular parameters in post–FS-LASIK patients with DED.

CONCLUSION

In summary, our study found that patients with DED after FS-LASIK who experienced ocular pain exhibited more severe dry eye and anxiety symptoms than those without ocular pain. This group also exhibited higher neuropeptide levels, lower corneal nerve densities, increased aDCs, and lower tear secretions. Understanding the characteristics and mechanisms of ocular pain can assist eye care practitioners in identifying diagnostic and management needs, targeting treatments, and improving outcomes.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Lu Zhao, Yifan Zhou, Hongyu Duan, Yu Zhang, Baikai Ma, Tingting Yang, Jiawei Chen, Yueguo Chen, and Hong Qi declare that they have no conflicts of interest to disclose.

Ethical Approval. This study complied with the principles of the Declaration of Helsinki and was approved by the Medical Science Research Ethics Committee of Peking University Third Hospital (M2023048). Written informed consent was obtained from all participants before their participation. The study was registered at ClinicalTrials.gov before study initiation under the registry number NCT05600985.

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