• Clinical Research •

Association between the levels of prostaglandin E2 in tears and severity of dry eye

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Abstract

• AIM: To investigate the relationship between the levels of prostaglandin E2 (PGE2) in tears and dry eye disease severity based on both clinical symptoms and signs.

• METHODS: Tear samples were collected from 36 non-Sjögren syndrome dry eye patients (10 males and 26 females, mean age 50.11±11.17y). All participants completed the Ocular Surface Disease Index (OSDI) questionnaire and underwent a detailed ophthalmic examination including, tear film breakup time (TBUT), ocular surface fluorescein staining, Schirmer I test, and meibomian gland assessment. The level of PGE2 in tears was measured using enzyme-linked immunosorbent assay (ELISA). The independent associations between tear PGE2 levels and other variables including demographics, OSDI scores, TBUT, Schirmer scores, ocular surface staining scores, and stage of meibomian gland dysfunction (MGD) were evaluated using linear regression analysis.

• RESULTS: The mean PGE2 level in tears of dry eye patients was 537.85±234.02 pg/mL. The tear PGE2 levels significantly positively correlated with OSDI scores (*R*=0.608, *P*<0.001), however, they did not significantly associate with TBUT (*R*=0.153, *P*=0.373), Schirmer scores (*R*=-0.098, *P*=0.570), ocular surface staining scores (*R*=0.282, *P*=0.095), and stage of MGD (*R*=-0.107, *P*=0.535). Male sex was significantly negatively correlated with tear PGE2 levels.

• CONCLUSION: The levels of PGE2 in tears are positively correlated with dry eye symptoms. However, no significant association was found between tear PGE2 levels and the results of other common dry eye diagnostic tests.

• KEYWORDS: dry eye; prostaglandin E2; severity; tear; dry eye tests

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INTRODUCTION

Dry eye is a multifactorial disease of the ocular surface, characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles[1]. Although dry eye is thought of as a symptomatic disease, asymptomatic form with visible ocular surface staining, early tear film breakup time (TBUT), and even tear hyperosmolarity still exists[2]. Also, a poor correlation between the symptoms of dry eye and objectively recorded sign is often clinically observed. The reason for the mismatch between symptoms and signs of dry eye may be that there are multiple factors contributing to dry eye pain, such as tear hyperosmolarity, loss of lubrication, inflammatory mediators, and change in ocular surface sensation[3].

Prostaglandins (PGs) are one of well-known inflammatory mediators, playing a key role in the generation of the inflammatory response. They are lipid autacoids derived from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes[4]. Stimulation of the ocular surface by tear hyperosmolarity might lead to the release of PGs in addition to other various cytokines and chemokines[5-6]. These PGs themselves also have algaesic properties and may stimulate sensory nerve ending[4].

There are 4 principal bioactive PGs generated *in vivo*, including prostaglandin E2 (PGE2), prostacyclin (PGI2), prostaglandin D2 (PGD2) and prostaglandin F2α (PGF2α)[4]. PGE2 is of particular interest because it is involved in all processes of inflammation and the action of PGE2 on peripheral sensory neurons and on central sites within the spinal cord and the brain can result in pain[4,7]. However, the role of PGE2 in the pathophysiology of dry eye has not been clearly determined. There have been only a few studies reporting the association between PGE2 and dry eye[5,8]. PGE2 has been shown to be elevated in the tears of dry eye patients compared with controls[5,8].

Nonetheless, the relationship between the level of PGE2 in tears and dry eye symptoms are controversial[5,8]. While dry eye symptoms were significantly associated with a reciprocal change in PGE2 and PGD2 levels but not PGE2 concentrations alone[8], there was not any correlation between dry eye symptoms and tear PGE2 levels in another study[5]. Additionally, clinical signs of dry eye can be inconsistent with symptomatology. Therefore, the purpose of this study was to investigate the relationship between PGE2 levels in tears and dry eye disease severity based on both clinical symptoms and signs.

SUBJECTS AND METHODS

Ethical Approval Institutional Review Board/Ethics Committee approval was obtained from the Mahidol University School of Medicine and all procedures conformed to the tenets of the Declaration of Helsinki. All participants were informed about the aim and protocol of the study and gave written informed consent prior to undergo the procedures.

Study Design This was a cross-sectional pilot study analyzing the level of PGE2 in tears of patients with dry eye. The study was conducted at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand between December 2015 and December 2017.

Participants A total of 36 patients from dry eye clinic who have been diagnosed with dry eye disease were recruited into this study. The inclusion criteria were as follows: patients aged 18 years or over, documented history of dry eye disease for at least 3mo, having at least one or more of the dry eye symptoms often or all the time (dryness, foreign body sensation, burning, ocular discomfort, heaviness of the eyelids, photophobia or ocular fatigue), and fluorescein TBUT≤10s. Patients were excluded if: 1) They had a history of other coexisting ocular surface diseases except for meibomian gland dysfunction (MGD), ocular surgery or trauma within the past 3mo; 2) They were being treated with any eye drops or systemic medications in the group of ω-3 supplement, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, immunomodulators, and antibiotics within the past 3mo; 3) They had been using any systemic medications which might affect tear film and meibomian gland function, including isotretinoin, antiandrogens, antidepressants, diuretics, anti-hypertension drugs, antihistamines, contraceptives, postmenopausal hormone therapy, and chemotherapy within the past 3mo; 4) They had diabetes mellitus; 5) They had a history of intensive and prolonged use of digital devices; 6) They had a history of contact lens wear within the past 3mo; 7) They had an abnormality of the nasolacrimal drainage apparatus or permanent or temporary occlusion of lacrimal puncta in any eye; and 8) They were pregnant or lactating women.

Interventions At baseline, the Ocular Surface Disease Index (OSDI) questionnaire (Allergan Inc., Irvine, CA, USA) was administrated by a single trained interviewer to assess symptom severity scores. This questionnaire comprises 12 questions and evaluates the frequency of symptoms over the preceding week. Each question is graded from 0 (“none of the time”) to 4 (“all of the time”). The total score, ranging from 0 to 100, is calculated based on the following formula: OSDI=(sum of scores for all questions answered ×100)/(total number of questions answered ×4). Higher scores represent greater ocular surface disease. The patients’ symptoms were categorized as normal (0-12), mild dry eye (13-22), moderate dry eye (23-32), or severe dry eye (33-100)[9].

A detailed examination of parameters of dry eye was performed sequentially at the same visit by another examiner as follows: slit-lamp examination, TBUT measurement, corneal and conjunctival fluorescein staining, Schirmer I test without anesthesia, and meibomian gland assessment[10].

TBUT was evaluated by introducing a fluorescein strip moistened with 1 drop of non-preserved normal saline into the inferior conjunctival fornix with minimal stimulation. The quantity of saline was also controlled by carefully shaking the fluorescein strip to remove excess fluid. The patient was asked to blink several times and then hold the eye open. The cornea was scanned with a slit-lamp using cobalt blue illumination. Time from the last complete blink to the first appearance of a random dry spot on the cornea was recorded in seconds. The test was repeated 3 times in each eye, and the mean time for 3 consecutive measurements was obtained. The test was considered positive if the average TBUT was less than 10s.

For corneal and conjunctival fluorescein staining, presence of any fluorescein staining of the ocular surface was recorded and graded using the Oxford scheme 6-point scale (from 0 through 5).

The Schirmer I test without anesthesia was then performed. A standard 5×35-mm^2 strip of dry filter paper was placed in each lower fornix at the junction of the lateral and middle thirds, taking care to avoid touching the cornea, and left in place for 5min. After 5min, the strips were removed, and the amount of wetting in millimeters was recorded. The test results were

considered positive if the length of wetting obtained was less than 10 mm in 5min.

Lid margin abnormalities were graded from 0 to 4 according to the number of these abnormalities present in each eye: plugged meibomian gland orifices, vascular engorgement, posterior displacement of the orifices, and irregularity of the lid margin. To assess an obstruction of meibomian gland orifices, firm digital pressure was applied to the lower tarsus. Meibum quality was evaluated in each of 8 glands of the central third of the lower lid on a scale of 0 to 3 for each gland: 0, clear; 1, cloudy; 2, cloudy with debris (granular); and 3, thick, like toothpaste (total score range, 0 to 24). Expressibility was assessed on a scale of 0 to 3 in 5 glands in the lower or upper lid, according to the number of gland expressible: 0, all glands; 1, 3 to 4 glands; 2, 1 to 2 glands; and 3, no glands. Based on Tear Film and Ocular Surface Society (TFOS) workshop on MGD, stage of MGD was determined by evaluating clinical symptoms, corneal and conjunctival fluorescein staining, lid margin abnormalities, meibum quality, and meibum expressibility[11].

Tear sampling was done on the next day to ensure that the previous diagnostic tests would not disrupt tear film quality and quantity and result in false-positive PGE2 elevation. Patients were instructed to lie down in a supine position and tilt their heads to the same side of the eye in which tear was being collected. Unstimulated tear samples were gathered from the inferior tear meniscus, using a glass microcapillary tube (10 μL Microcaps®, Drummond Scientific, Broomall, PA, USA). The microcapillary tube was placed gently at the lateral canthus, taking care not to irritate the conjunctiva, cornea, or lid margin, to avoid additional tear reflex as much as possible. Patients were allowed to blink naturally. Up to 10 μL of tear samples was collected from each eye at the same time of the day. The tear samples were immediately transported in an insulated cooler at 4℃ to a -80℃ freezer, where they remained frozen until analyzed. The samples were obtained from both eyes of each individual separately, and were not pooled[12-13]. In order to ensure that our tear collection technique was suitable, tears had been collected from 5 healthy volunteers and found that this technique had not caused any significant reflex tearing, potentially leading to iatrogenic high PGE2 expression.

Tear concentrations of PGE2 were measured by another examiner using a monoclonal enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. This PGE2 ELISA kit is a competitive assay that can be used for quantification of PGE2 and has a range from 7.8-1000 pg/mL and a sensitivity of approximately 15 pg/mL. Each kit contains PGE2 standard, PGE2 monoclonal antibody, AChE tracer, Ellman's reagent, and a goat anti-mouse IgG coated 96-well plate. The diluted tear samples (50 μL) was placed in a 96-well plate in triplicates and 200 μL Ellman's reagent was added to each well for 90min and the absorbance at 414 nm was recorded with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). All measurements and interviews were conducted in a double-masked fashion by separate observers. Adverse reactions and complications were recorded.

Statistical Analysis Statistical analyses were performed with the statistical software package STATA version 14 (Stata Corp, College Station, TX, USA). The data from the worse eye which was defined as having lower TBUT value of each patient were used for analysis. If the TBUT values in both eyes were equal, the scores of ocular surface fluorescein staining, MGD, and Schirmer I test were used to define the worse eye. For continuous data, normally distributed variables were presented as mean and standard deviation (SD); non-normally distributed variables were presented as median and range. Frequency and percentage were used for categorical data. Linear regression analysis were applied to assess the independent association between tear PGE2 levels and other variables including demographics, dry eye symptom severity scores (OSDI scores), TBUT, Schirmer I test, corneal staining score, and MGD stage. Variables with $P<0.10$ in univariate analysis were selected for multivariate linear regression analysis. $P<0.05$ was considered to be statistically significant.

RESULTS

A total of 36 symptomatic dry eye patients were recruited and completed the study. There were 26 women (72.2%) and 10 men (27.8%). The mean age was 50.11±11.17y (51.70y for females and 45.18y for males; range, 27-72y). The average OSDI score, TBUT, ocular surface staining Oxford scale, Schirmer I score, and MGD stage based on the TFOS workshop on MGD[11] are shown in Table 1.

Based on scores generated by the OSDI questionnaire, 19 of the 36 patients (48.7%) reported mild dry eye symptoms, 12 (30.8%) reported moderate dry eye symptoms, and 8 (20.5%) reported severe dry eye symptoms. Thirty-four patients (94.4%) had symptoms plus at least 1 positive dry eye tests. Only 2 patients had only dry eye symptoms without positive dry eye tests. MGD of any severity was recorded in 23 patients (63.9%). Because all patients included into this study must have had at least one or more of the dry eye symptoms often or all the time, there was no asymptomatic or nonobvious MGD identified in our study. None of our patients had rheumatic disorders.

The mean tear PGE2 level was 537.85±234.02 pg/mL. The PGE2 levels in tears of dry eye patients significantly correlated with OSDI scores ($R=0.608$, $P<0.001$), as shown in Figure 1.

There was also a significant association between the OSDI category (mild, moderate, severe) and the concentrations of

Table 1 Clinical information of 36 dry eye patients

Dry eye characteristic	Mean±SD (median, range)
Dry eye symptoms (OSDI score)	23.51±8.94 (12.5-41.7)
Fluorescein TBUT (s)	6.66±1.61 (4-10)
Ocular surface staining Oxford scale	0.56±1.27 (0, 0-5)
Schirmer I score without anesthesia (mm)	8.11±3.56 (8, 2-16)
Stage of MGD based on the TFOS workshop on MGD[10]	0.94±1.09 (1, 0-4)
Tear PGE2 level (pg/mL)	537.85±234.02 (533.78, 149.68-972.56)

OSDI: Ocular surface disease index; TBUT: Tear film breakup time; MGD: Meibomian gland dysfunction; TFOS: Tear Film and Ocular Surface Society; PGE2: Prostaglandin E2.

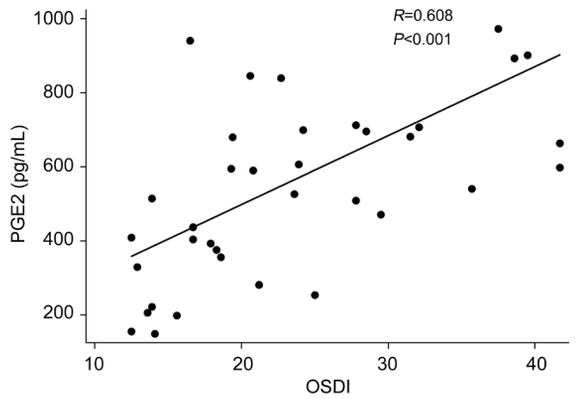

Figure 1 Correlation between tear PGE2 levels and the OSDI score.

PGE2 in tears (R=0.561, P<0.001). However, tear PGE2 levels did not significantly correlate with TBUT (R=0.153, P=0.373), Schirmer I scores (R=-0.098, P=0.570), ocular surface fluorescein staining (R=0.282, P=0.095), and MGD stage (R=-0.107, P=0.535), as shown in Figure 2. No any adverse reactions and complications were observed during the study period.

Univariate analysis demonstrated a significant positive relationship between OSDI scores and levels of PGE2 in tears (Table 2). From the multivariate analysis, OSDI scores remained significantly associated with tear PGE2 levels and male sex was significantly negatively correlated with tear PGE2 levels (Table 3).

Table 2 Univariate analysis of the association between patient variables and levels of PGE2 in tears

Variables	PGE2			
	Data coefficients	95%CI	t	P
Age	-5.71	-12.74-1.31	-1.65	0.108
Male sex	-155.87	-327.01-15.27	-1.85	0.073
OSDI scores	15.91	8.66-23.15	4.46	0.000
OSDI category				
Moderate symptoms	174.02	15.47-332.57	2.23	0.032
Severe symptoms	328.13	148.70-507.56	3.72	0.001
TBUT	22.33	-27.89-72.55	0.90	0.373
Ocular surface fluorescein staining	51.82	-9.55-113.20	1.72	0.095
Schirmer I scores	-6.44	-29.24-16.36	-0.57	0.570
MGD stage	-22.87	-97.00-51.26	-0.63	0.535

PGE2: Prostaglandin E2; OSDI: Ocular surface disease index; TBUT: Tear film breakup time; MGD: Meibomian gland dysfunction.

Table 3 Multivariate analysis of the association between patient variables and levels of PGE2 in tears

Variables	PGE2			
	Data coefficients	95%CI	t	P
OSDI scores	14.20	7.17-21.23	4.11	0.000
Male sex	-145.34	-281.81 to -8.87	-2.17	0.038
Ocular surface fluorescein staining	37.90	-11.58-87.38	1.56	0.129

PGE2: Prostaglandin E2; OSDI: Ocular surface disease index.

DISCUSSION

Dry eye can have a variety of symptoms such as irritation, blurred vision, and ocular pain including dryness, burning, and foreign body sensation, as well as photophobia. Within dry eye-related symptoms, some patients report recurring episodes of transient pain whereas others complain of chronic pain. Besides hyperosmolar tears itself, PGE2, the subtype of PGs, is recognized to be the principal pro-inflammatory mediator and contributes to pain hypersensitivity by modulating multiple sites in the nociceptive pathways, including peripheral and central sensitization[7]. Furthermore, metabolites of PGE2 may activate nociceptive neurons independent of PG receptors[14]. Clinically, the PGE2 level in the tears of dry eye patients was higher than in that in the control group[8]. These clinical results were also supported by increased COX-2 and PGE synthase expression levels seen in tear-producing tissue of dry eye mice[8]. In addition, topical COX-2 inhibitor reduced inflammatory cells on the ocular surface, suggesting the role of COX-2/PGE2 axis in the pathogenesis of dry eye[6]. Topical NSAIDs has also been observed to improve corneal fluorescein staining in a dry eye mouse model[15]. However, the association between the concentrations of PGE2 in tears and dry eye parameters is still controversial.

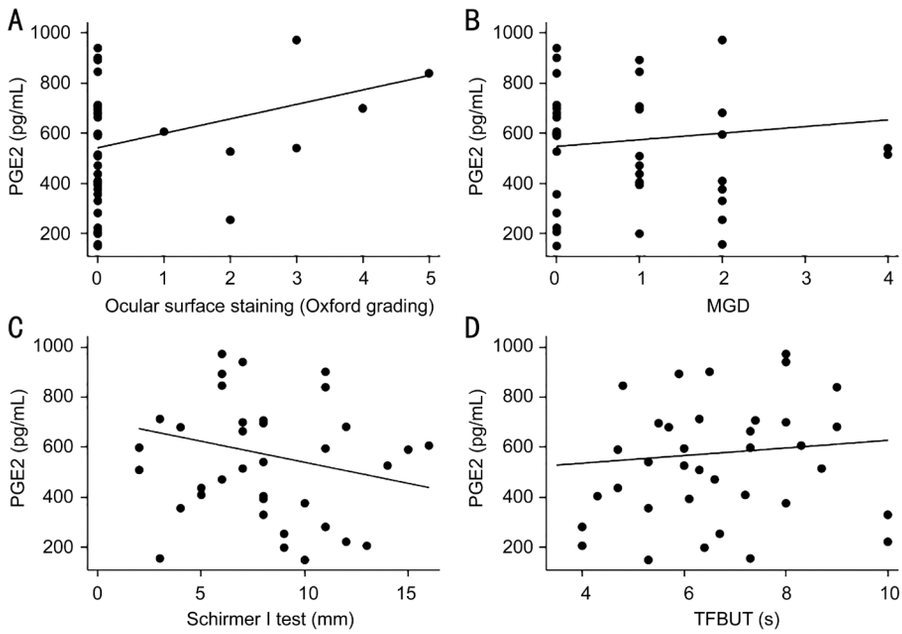

Figure 2 Correlation between tear PGE2 levels and other dry eye clinical signs including ocular surface fluorescein staining score (Oxford scheme) (A), MGD stage (B), Schirmer I test without anesthesia (C), and fluorescein TBUT (D).

Our study demonstrated that the increased tear PGE2 levels in non-Sjögren dry eye patients positively correlated with only their OSDI scores but not other clinical dry eye parameters including TBUT, Schirmer score, ocular surface staining score, and MGD stage. A previous study by Shim *et al*[8] showed that although tear PGE2 levels were elevated significantly in dry eye patients, the PGE2 concentrations alone did not associate with dry eye symptom scores. However, a reciprocal change in PGE2 and PGD2 levels correlated well with patients' symptom scores. In another study by Walter *et al*[5], there was no correlation between dry eye symptoms and tear PGE2 levels. Additionally, tear PGE2 levels were not correlated with corneal sensation, TBUT, Schirmer score, meibomian gland atrophy, and meibum quality. Nonetheless, higher levels of PGE2 were associated with lower tear osmolarity, more meibomian gland plugging, and more corneal staining.

Differences in outcomes between our study and the previous studies may be due to the disparities in baseline characteristics of patient populations, dry eye questionnaires, and tests used. Shim *et al*[8] included non-Sjögren dry eye patients similar to our study but their patients appeared to be slightly older (mean age=57y) and had more severe dry eye based on their eligible criteria of having all abnormal symptoms and signs. Visual analog scale (VAS; 0-10 cm) was used to obtain symptom severity scores from their patients instead of OSDI. The relationship between dry eye test results and tear PGE2 levels was also not investigated in their study. Meanwhile, unlike our study, the series of Walter *et al*[5] was comprised of late middle-aged and elderly, mostly male subjects (mean age=62y). Approximately 60% and 20% of patients were taking NSAIDs and ω-3 supplements respectively, which might affect the PGE2 levels. Furthermore, 81% of their patients had moderate to severe dry eye according to the OSDI scores. On the other hand, our patients (79.5%) are mainly classified as mild to moderate dry eye based on either the OSDI and other dry eye diagnostic scores.

Interestingly, our study revealed that male sex was negatively correlated with levels of PGE2 in tears of dry eye patients. Although many studies have shown the roles of PGs on various functions of sex steroids, there have been few reports on the effects of sex on PGE2 production in the literature[16-20]. In previous animal studies, lack of female sex hormones significantly increased the levels of PGE2 in neural tissues[16], whereas PGE2 concentrations in renal tissue and neutrophils was lower in male rats than in female rats[17-18]. In rabbits, neither female sex hormones nor pregnancy alone caused a significant increase in PGE synthesis in gallbladder[19]. However, in a human laboratory study, PGE2 production by human peripheral monocytes was reduced by testosterone, while progesterone and estradiol at a specific concentration enhanced its production[20]. Therefore, the effects of sex on physiological and pathological production of PGE2 in several kinds of tissue is still unclear. Nevertheless, our data might support the importance of sex in local PGE2 biosynthesis. The sex-related difference in tear PGE2 levels in dry eye patients might be attributed to the impacts of sex hormones, sex chromosome complement, sex-specific trait, sex-specific autosomal factors, and other sex-related factors.

Our findings suggest that PGE2 may involve in pathogenesis of dry eye symptoms. Although the role of PGE2 in dry

eye symptoms are not clearly known, there is evidence that hyperosmotic state stimulated the release of pro-inflammatory cytokines including PGE2 from the damaged corneal epithelial cells[21]. Therefore, even though dry eye symptoms may arise after several kinds of internal and external insults, common subsequent hyperosmolarity and/or inflammation could activate nociceptors through the PGE2 pathway. Also, new research has revealed that dry eye is a complicated disorder with potentially complex neuropathologic mechanisms. It is likely that a subset of dry eye patients has neuropathic pain and central sensitization[22]. Specialized corneal nociceptors tuned to tear film evaporation have been shown to affect the generation of chronic sensations of dry eye[23]. Thus, PGE2 which is a potent nociceptor stimulus, probably play an important role in the mechanism of dry eye. However, as both structural and functional alterations of subbasal corneal nerves have been documented in dry eye patients and were related to the severity of dry eye[24], the modifications of corneal nerves may account for some discrepancy between the tear PGE2 levels and dry eye symptoms and signs particularly in certain cases. Greater corneal epitheliopathy in patients with severe dry eye might expose subapical nerve endings and change nerve response by lowering the stimulation threshold[25]. Consequently, low levels of inflammatory mediators including PGE2 could abnormally sensitize corneal nerves, inducing spontaneous pain or hyperesthesia. Thus, the increased excitability of corneal nerves may interfere with the correlation.

Limitations of this study are no control group, a predominantly female population, small sample size, insufficient representation of the moderate to severe groups of dry eye, and no measurement of corneal sensation or other tear cytokines and chemokines. Future case-control studies enrolling more participants with representation in the greater disease severity range are needed to investigate the effects of PGs on dry eye symptoms. Measuring blood PGE2 levels synchronized with tear PGE2 levels will be more useful. The clinical role of PGE2 inhibition in improving dry eye symptoms should also be assessed. Additionally, other substances that may cause ocular irritation such as bradykinin or substance P should be evaluated for their relationship to dry eye. This might lead to the development of new and more effective treatments for this prevalent disease.

In summary, the concentrations of PGE2 in tears are positively correlated with dry eye symptoms. However, no significant association was found between tear PGE2 levels and the results of other widespread diagnostic tests. Considering dry eye as a unique type of ocular neuropathic pain, PGE2 may be one of the sources for dry eye symptoms and a good candidate for targeted therapies in the future.

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