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Tear Neuromediators in Subjects with and without Dry Eye According to Ocular Sensitivity

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To investigate differences of tear neuromediators between subjects with and without dry eye (DE) depending on the ocular sensitivity. Thirty-one subjects with DE and 29 subjects without DE were recruited in this study. The eyes were stimulated by exposure to an irritating product applied to the periocular region. Both DE and non-DE subjects were divided into the high sensitivity and low sensitivity groups based on the degree of ocular sensitivity to ocular irritation. Baseline tear film break-up time (TBUT) and corneal staining score were examined, and tear samples were collected. The concentrations of the tear neuromediators, including nerve growth factor (NGF), serotonin, calcitonin gene-related peptide (CGRP), substance P, neuropeptide Y, and vasoactive intestinal peptide were measured using the enzyme-linked immune sorbent assay. The baseline neuromediator concentrations were compared between subjects with and without DE based on ocular sensitivity. In both DE and non-DE subjects, baseline TBUT was significantly lower in the high sensitivity group than in the low sensitivity group. In the high sensitivity group, baseline tear NGF levels were higher in subjects with DE than in those without DE. In the low sensitivity group, baseline levels of tear CGRP were lower in subjects with DE than in those without DE. Tear neuromediators associated with DE had differences in their concentrations depending on ocular sensitivity. In patients with DE, tear NGF levels increased with high ocular sensitivity to ocular irritation, whereas tear CGRP levels decreased with low ocular sensitivity.

Key Words: *Dry Eye Syndromes; Cornea; Calcitonin Gene-Related Peptide; Nerve Growth Factor*

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INTRODUCTION

Dry eye (DE) is a complex disease characterized by changes in the ocular surface, tear film status, inflammatory reactions, and corneal sensitivity.[1,2] Tear biomarkers are potential indicators of the ocular surface status, severity and progression of DE.[1-3] Tear neuromediators have been studied to identify suitable biomarkers related to the causative conditions of DE, which include Sjögren's syndrome (SS), non-SS, refractive surgery, and ocular cicatrizing pemphigoid (OCP).[1,2,4]

Tear concentrations of nerve growth factor (NGF) increased in DE caused by non-SS, refractive surgery, or OCP.[1,4,5] Tear levels of neuropeptide Y (NPY) generally decreased in DE caused by SS or OCP.[1,4,6-8] The tear substance P levels increased after refractive surgery but it did not changed in DE with other causative conditions.[1,4,6-8] Compared to a healthy eye, the level of vasoactive intestinal peptide (VIP) remained unchanged in all causative conditions of DE.[1,4,6-8] Various results have been obtained with regard to the level of tear calcitonin gene-related peptide (CGRP), depending on the causative conditions.[1,4,6-8]

These tear neuromediators also play a role in neurosensation.[1,9,10] NGF and serotonin are involved in epithelial inflammation, and they stimulate corneal nerves.[1,9,10] CGRP and substance P are secreted at the nociceptors in response to corneal stimulations.[1,9,10] NPY and VIP are secreted at the ends of the sympathetic and parasympathetic

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nerves, respectively.[1,9,10] The role of these neuromediators suggests that their concentrations in the tear film could change with the ocular sensitivity as well as the causative conditions of DE.

Ocular sensitivity may decrease or increase in DE and vary with the subtypes, severity, and duration of disease; however, the underlying mechanism has not been elucidated.[1,11-14] Even in individuals without DE, the degree of ocular sensitivity varies to particular stimuli such as eyedrops, peri-ocular products, and cosmetics. Therefore, studies are required to examine the concentrations of tear neuromediators in patients with DE according to their ocular sensitivity. The aim of the present study was to investigate the clinical parameters and baseline levels of tear neuromediators in individuals with and without DE based on the degree of their sensitivity to ocular irritation.

MATERIALS AND METHODS

1. Subjects

The sample size was calculated using the G*Power software (version 3.1.9.4; Heinrich-Heine University, Germany) with a level of α=0.05 and a power of 90%, and effect size 0.8. Accordingly, a total sample size of 56 participants (28 people in each group) was found sufficient. Therefore, thirty-one subjects with DE and 29 subjects without DE were recruited for this study. Ethical approval was obtained from the Chonnam National University Hospital Institutional Review Board (CNUH-2018-023), and the study protocol followed the guidelines of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

DE was diagnosed based on an ocular surface disease index (OSDI) score $\geq$13 and a tear break-up time (TBUT) $\leq$10 s. The exclusion criteria for the study were (1) previous ocular disease other than DE, (2) the use of topical and systemic medications associated with DE, except lubricants, and (3) history of ocular surgery within 3 months.

2. Measurement of clinical parameters

The OSDI score, TBUT, and corneal staining score (CSS) were evaluated by the same investigator (K.C.Y.); only the right eye was assessed.

The OSDI questionnaire was used to quantify vision-related quality of life, and it covered the following subscales: (1) ocular symptoms (OSDI symptoms), (2) vision-related activities of daily living (OSDI visual function), and (3) environmental triggers (OSDI trigger). The total OSDI score and each subscale score, which ranged from 0 to 100, were analyzed.[15,16]

TBUT was assessed using a moistened fluorescein strip (Haag-Streit, Koeniz, Switzerland), and the time interval between the last complete blinking and the first appearance of a dry spot or disruption of the tear film was recorded in seconds. The examination was performed thrice, and the mean break-up time was used for the analysis. CSSs were obtained using the Oxford grading scale (0=absent; 1=minimal; 2=mild; 3=moderate; 4=marked; 5=severe).[17] These clinical parameters were measured at before and after ocular stimulation.

3. Assessment of ocular irritation

The eye was artificially stimulated by exposure to a product applied to the periocular skin region; the participants applied 200 μL of sunblock about 1.0 cm wide at a distance of approximately 1.0 cm from the rim of the eyelid, and 50 μL of 0.15% hyaluronate were instilled to allow the eye to be exposed to the product. Sunblock products used in this experiment included oils and surfactants that formed a cream when mixed with organic and inorganic sunscreens. After stimulation, the participants immediately completed the questionnaire. The symptom sensation questionnaire covered four symptoms with four identical analogue scales (0=none to 4=severe): burning, tearing, foreign body sensation, and tingling. Participants recorded their symptoms using the questionnaire within 10 min. The score was calculated as the multiplication of scales and time. Both DE and non-DE subjects were divided into the high sensitivity and low sensitivity groups using a cut-off value of 10.

4. Tear collection and detection of neuromediators

Tear samples were carefully obtained from the inferior tear meniscus of the right eye using glass capillary tubes (Corning, Inc., Corning, NY, USA) to avoid touching the ocular surfaces after examination of their clinical parameters. Two hundred microliters of tear samples were obtained and diluted with phosphate-buffered saline (1:4). The samples were placed in microtubes and stored at −70℃ until further examination.[18]

A specific enzyme-linked immunosorbent assay was performed on extracted proteins to quantify the concentrations of neuromediators in the tear samples of subjects following manufacturer instructions. Tear samples were centrifuged at 3000 g for 10 min and filtered through a 0.45-mm filter before the enzyme-linked immunosorbent assay. Protein samples (100 μL, 10 μg/mL) of NGF (Elabscience, Houston, TX, USA), serotonin (Abcam, Cambridge, UK), CGRP (Elabscience), substance P (R&D SYSTEMS, Minneapolis, MN, USA), NPY (Elabscience), and VIP (Elabscience) were absorbed onto each conjugate-coated plate. Levels of neuromediators in a given sample were measured by comparing its absorption with the standard curve. The minimum detectable concentrations of NGF, serotonin, CGRP, substance P, NPY, and VIP were 15.63 pg/mL, 0.5 ng/mL, 15.63 pg/mL, 78.1 pg/mL, 31.25 pg/mL, and 7.81 pg/mL, respectively.

5. Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences, version 18.0, for Windows (SPSS Inc., Chicago, IL, USA). The normality of the distribution was assessed using the Shapiro-Wilk test, and all variables were normally distributed. Data are presented as mean±standard deviation. The independent t-test was used to compare variables between groups. A p-value of less

than 0.05 was considered to be statistically significant.

RESULTS

This study included 31 subjects with DE and 29 subjects without DE. All subjects were female. The mean age was 23.3±2.93 (20-35) years. Baseline levels of NGF were higher in subjects with DE than in those without DE (p=0.01). In contrast, baseline CGRP was lower in subjects with DE than in those without DE (p=0.02). Subjects with DE were divided into two groups: the high sensitivity group, comprising 19 subjects and the low sensitivity group comprising 12 subjects. Subjects without DE were also divided into two groups: the high sensitivity group comprising 12 subjects and the low sensitivity group comprising 17 subjects (Table 1).

Fig. 1 shows the comparison of TBUT and CSS between the high sensitivity and low sensitivity groups in subjects with and without DE. Among the subjects with DE, TBUT was significantly lower in the high sensitivity group (5.08±1.73 s) than in the low sensitivity group (6.86±2.47 s; p=0.03). In addition, TBUT in subjects without DE was significantly lower in the high sensitivity group (9.43±2.77 s) than in the low sensitivity group (12.54±2.32 s; p<0.01). Among subjects with DE, no significant difference was observed between the CSSs of the high sensitivity and low sensitivity groups (0.78±1.51 and 1.08±0.90, respectively; p=0.54). There was no significant difference between the CSSs of the high sensitivity and low sensitivity groups in subjects without DE (0.08±0.28 and 0.35±0.49, respectively; p=0.10). Fig. 2 shows the comparison of NGF and CGRP between the high sensitivity and low sensitivity groups in subjects with and without DE. There was no significant difference between each group.

Table 2 shows the comparison of baseline levels of tear neuromediators between subjects with and without DE in the high sensitivity group. The baseline NGF level was higher in subjects with DE than in those without DE (910.7± 704.3 and 431.7±265.5 pg/mL, respectively; p=0.03). No significant differences were observed in the levels of other

TABLE 1. Baseline characteristics and tear neuromediators of subjects with and without dry eye (DE)

	DE (n=31)	Non-DE (n=29)	p-value
Age, year	23.32±2.97	23.31±2.84	0.99
Clinical parameters			
Ocular surface disease index	38.83±17.46	11.50±6.93	<0.01
Tear film break-up time, sec	5.77±2.19	11.26±3.06	<0.01
Corneal staining score	0.90±1.30	0.24±0.44	0.01
Tear neuromediators			
NGF, pg/mL	858.2±716.6	475.5±275.7	0.01
Serotonin, ng/mL	47.14±18.71	48.32±18.07	0.81
CGRP, pg/mL	802.3±330.1	1021.1±379.9	0.02
Substance P, pg/mL	8611.0±3066.1	8417.4±2982.2	0.81
NPY, pg/mL	20970.8±15165.1	22404.1±14253.2	0.71
VIP, pg/mL	948.4±516.9	1047.3±541.3	0.47
Corneal sensitivity, n			0.12
High	19	12	
Low	12	17	

Data are expressed as mean±standard deviation, unless otherwise indicated. NGF: nerve growth factor, CGRP: calcitonin gene-related peptide, NPY: neuropeptide Y, VIP: vasoactive intestinal peptide.

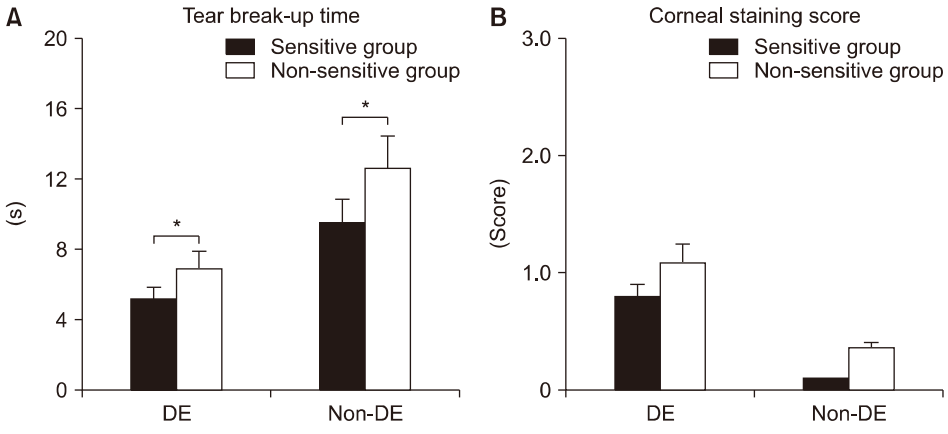

FIG. 1. Comparison of ocular surface parameters between the high sensitivity and low sensitivity groups of subjects with and without dry eye (DE): (A) tear break-up time and (B) corneal staining score (*p<0.05).

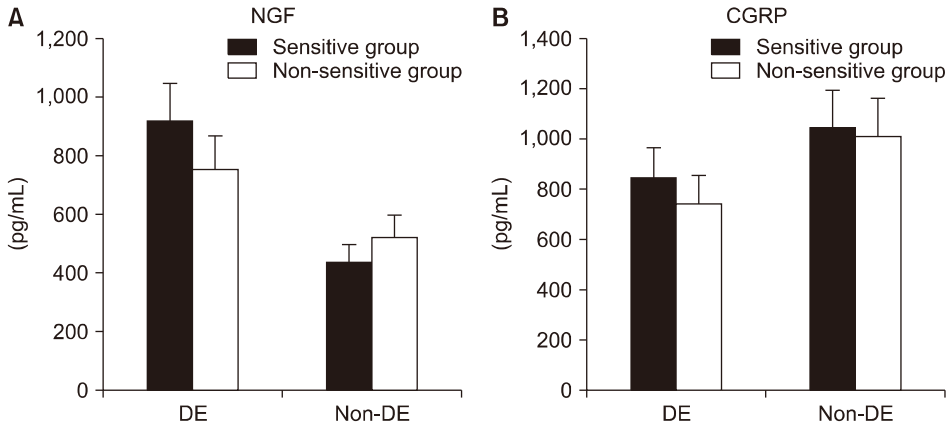

FIG. 2. Comparison of neuromediators between the high sensitivity and low sensitivity groups of subjects with and without dry eye (DE): (A) nerve growth factor (NGF) and (B) calcitonin gene-related peptide (CGRP).

TABLE 2. Comparison of baseline levels of tear neuromediators between subjects with and without dry eye (DE) in the high sensitivity group

	DE	Non-DE	p-value*
NGF, pg/mL	910.7±704.3	431.7±265.5	0.03
Serotonin, ng/mL	51.7±15.8	46.5±10.0	0.42
CGRP, pg/mL	840.3±293.7	1039.8±382.0	0.13
Substance P, pg/mL	7952.7±2103.8	7921.0±2489.8	0.97
NPY, pg/mL	22504.4±16308.5	22967.9±14497.8	0.94
VIP, pg/mL	1011.4±549.6	1200.3±641.2	0.41

Data are expressed as mean±standard deviation, unless otherwise indicated. NGF: nerve growth factor, CGRP: calcitonin gene-related peptide, NPY: neuropeptide Y, VIP: vasoactive intestinal peptide. *Compared subjects with and without dry eye using the independent *t*-test.

TABLE 3. Comparison of baseline levels of tear neuromediators between subjects with and without dry eye (DE) in the low sensitivity group

	DE	Non-DE	p-value*
NGF, pg/mL	753.1±729.4	519.3±278.7	0.24
Serotonin, ng/mL	39.9±21.8	49.5±22.0	0.37
CGRP, pg/mL	743.5±337.3	1009.1±339.8	0.04
Substance P, pg/mL	9748.0±5588.4	8738.6±3294.6	0.55
NPY, pg/mL	18542.7±13472.4	22006.1±14512.4	0.48
VIP, pg/mL	845.3±464.6	935.2±444.7	0.62

Data are expressed as mean±standard deviation, unless otherwise indicated. NGF: nerve growth factor, CGRP: calcitonin gene-related peptide, NPY: neuropeptide Y, VIP: vasoactive intestinal peptide. *Compared subjects with and without dry eye using the independent *t*-test.

neuromediators between groups (all p>0.05).

Table 3 shows the comparison of baseline levels of tear neuromediators of subjects with and without DE in the low sensitivity group. The baseline CGRP level was lower in subjects with DE (743.5±337.3 pg/mL) than in those without DE (1009.1±339.8 pg/mL; p=0.04). Similarly, there were no significant differences between the tear levels of the other neuromediators in the groups (all p>0.05).

DISCUSSION

DE is a multifactorial disease of the ocular surface associated with tear film instability, tear hyperosmolarity, ocular surface inflammation and damage, and neuro-sensory abnormalities.[1] The ocular surface is densely innervated with nerves, and this maintains its integrity by detecting potentially harmful stimuli and evoking reflexive responses to them.[1,19] The coordination between the sensory nerves and the autonomic nervous system is important for homeostasis of the ocular surface.[1,20,21] Therefore, tear neuromediators concentrations could reflect the condition of the ocular surface and could be potential indicators of the DE status and corneal sensitivity.[1-3] In this study, we investigated ocular surface parameters and the concentrations of tear neuromediators in subjects with and without DE based on their sensitivity to ocular irritation.

NGF in tears was upregulated following damage to the ocular surface or its nerves.[1,22] One study showed that tear NGF level was elevated after laser-assisted in-situ keratomileusis (LASIK), and it remained elevated until 6 months after surgery.[5] Tear NGF concentrations may also be elevated in non-SS DE, contact lens-related DE, and OCP.[1,4,7] In contrast, CGRP was reduced in tears of non-SS DE and OCP.[1,4] After refractive surgery, there was no change in CGRP level in the LASIK group; however, it increased in the photorefractive keratectomy group.[1,6,8] In our study, tear NGF levels were lower while levels of CGRP were higher in subjects with DE than in those without DE, consistent with non-SS findings in previous studies.

Baseline TBUT was significantly lower in the high sensitivity group than in the low sensitivity group in subjects with and without DE. Therefore, individuals with higher sensitivity to ocular irritation could be presumed to have DE with short TBUT. Kaido et al.[23] reported that corneal sensitivity, induced mechanically using the Cochet-Bonnet esthesiometer, was higher in subjects with short TBUT than in the DE controls. Although the methodology was different from what was used in the present study, the associa-

tion between TBUT and ocular sensitivity was similar in both studies.[23]

In terms of corneal neuro-sensory reaction, both NGF and CGRP were positively correlated with the nociceptive stimulation. NGF was involved in the stimulation of corneal nerves, and CGRP was secreted at the nociceptors with response to corneal stimulation.[1,9,10] However, tear neuromediators had different concentrations depending on the causative condition of DE.[4,6-8] In non-SS DE, the concentration of NGF tended to be high and that of CGRP tended to be low.[4,6-8] In this study, in the high sensitivity group, baseline NGF was higher in subjects with DE than in those without DE. In the low sensitivity group, baseline CGRP was lower in subjects with DE than in those without DE.

One study showed that tear CGRP levels were inversely correlated to DE severity such as corneal staining and the Schirmer test results.[4] The other studies showed that exogenously delivered CGRP could facilitate corneal epithelium repair in vivo and in vitro.[1,24] These suggested that CGRP was involved in DE pathogenesis. CGRP had a complex role of modulating the nervous system and inflammatory reactions through the inhibition of (1) the antigen presentation by Langerhans cells and (2) the impairment of contact hypersensitivity by tumor necrosis factor secreted from mast cells.[4,25] Therefore, a low tear concentration of CGRP could reflect low ocular sensitivity in patients with DE.

This study has several limitations. The first limitation is the inclusion of a specific population (e.g., women aged 20-35 years). DE and neuropathic pain are more prevalent in women than in men. However, future studies that include subjects of both sexes, various ages, and social backgrounds are required. Second, the mechanism of underlying the stimulation to the corneal nociceptors used in this study is unclear. Additionally, it may be helpful to use the stimulation source such as a jet esthesiometer, which could affect all three types of nociceptors.

Despite these limitations, the current study is the first to investigate the level of tear neuromediators in DE based on the ocular sensitivity. Our results suggest that ocular sensitivity as well as causative conditions of DE should be considered in the interpretation of concentrations of tear neuromediators. In conclusion, tear neuromediators associated with DE may have different concentrations depending on ocular sensitivity. In patients with DE, tear NGF levels increased with high ocular sensitivity to ocular irritation, whereas tear CGRP levels decreased with low ocular sensitivity.

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CONFLICT OF INTEREST STATEMENT

None declared.

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