Conjunctival and Corneal Pneumatic Sensitivity Is Associated with Signs and Symptoms of Ocular Dryness

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PURPOSE. To investigate the relationships of dry eye symptoms and corneal and conjunctival sensitivity to pneumatic stimulation, tear film stability, and clinical ocular surface characteristics in symptomatic and asymptomatic subjects.

METHODS. Ninety-seven subjects were enrolled and grouped by a questionnaire-based single score for symptoms of ocular dryness (none to trace, non-dry group; mild to severe, symptomatic group); 43 were symptomatic and 54 were non-dry. Corneal (K) and conjunctival (C) sensitivities were measured with a computer-controlled Belmonte pneumatic (room temperature) stimulus. Symptoms were assessed according to the Ocular Surface Disease Index (OSDI). Ocular surface staining with fluorescein (FL) and lissamine green (LG), noninvasive tear film break-up time (NIBUT), and the phenol red thread test (PRT) were assessed.

RESULTS. The symptomatic group showed lower K and C thresholds (P < 0.01), greater corneal FL and conjunctival LG staining, and shorter NIBUT than did the non–dry eye group (all others P < 0.05). The OSDI scores were higher in the symptomatic group (P < 0.001). K and C thresholds and NIBUT were inversely correlated with the OSDI and corneal and conjunctival staining (all P < 0.05). The K and C threshold and NIBUT (all P < 0.01) correlated positively. Step-wise multiple regression analysis showed that ocular surface sensitivity and NIBUT were significant predictors of the OSDI.

CONCLUSIONS. Ocular irritation assessed with the OSDI is associated with ocular surface hyperesthesia to cooling, corneal epitheliopathy, and tear film instability. Although cause and effect are unclear, the analysis showed that altered corneal and conjunctival sensory processing and tear film attributes are essential aspects of what characterizes dry eye. (Invest Ophthalmol Vis Sci. 2008;49:2971–2976) DOI:10.1167/iovs.08-1734

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Dry eye is characterized as an abnormality of the tears and ocular surface with a multifactorial etiology, resulting in tear film instability, symptoms of discomfort and visual disturbance, and inflammation and potential damage to the ocular surface. It is hypothesized that the ocular surface (including the cornea, conjunctiva, accessory lacrimal glands, and meibomian glands), the main lacrimal glands and the afferent and efferent innervation that connects them comprise the lacrimal functional unit. This integrated functional unit regulates the major composition of the tear film and responds to environmental, endocrine, and central neural influences. Dysfunction in any part of the functional unit potentiates alterations of the other components, thus compromising the ability of the ocular surface to respond to physiological and environmental challenges, with one possible outcome being the development of symptoms associated with dry eye (provided this is not the sole precipitating factor).

The presence of symptoms may be accounted for by the activation of sensory nerves at the ocular surface. Although the basis for symptoms in dry eye is not fully understood, a recent speculation has been that the dryness sensations after refractive surgery may be due to denervation-induced dysesthesia. Measuring ocular surface sensitivity is one technique used to evaluate the sensory nerve function and a few studies have measured corneal sensitivity in certain groups of dry eye patients. However, studies evaluating corneal sensitivity and clinical tests, such as ocular surface staining, tear film stability, and symptoms of ocular irritation have found conflicting results. In addition, there is no report of whether conjunctival sensitivity is associated with the symptoms or indeed with the clinical ocular and tear film tests commonly undertaken.

In the present study, we therefore measured corneal and conjunctival sensitivity with a computer-controlled, modified Belmonte esthesiometer and investigated its relationship with ocular surface tests and symptom severity in subjects with and without ocular dryness symptoms.

MATERIALS AND METHODS

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed consent was obtained from each subject.

One hundred non–contact-lens–wearing subjects were enrolled. Of these, three subjects were excluded: Two were found to have corneal striae of unknown cause, and one reported symptoms of dryness that were present only in the middle of the night. Each subject had no history of systemic or ocular disease and was not using any systemic or topical medication that would affect ocular health. Slit lamp examination was initially performed to rule out lid, conjunctival, or corneal abnormalities, other than the clinical signs of dry eye. A questionnaire-based single score for symptoms of ocular dryness (Simmons PA et al. IOVS 2003;44:ARVO EAbstract 2448), as described in Table 1, was used to classify subjects into the non-dry (scores of “none” to “trace”) or symptomatic (scores of “mild to severe”) group. The questionnaire was administered by a trained ophthalmic assistant at study entry.

Symptom Evaluation

Subjects completed the OSDI questionnaire, which consists of 12 items. Including questions related to visual function, ocular symptoms, and environmental triggers. The item scores were calculated according to the formula recommended by Schiffman et al.

Clinical Assessment

Noninvasive tear film break-up time (NIBUT) was measured using a topography system (Eyema model EH-290; Alcon, Inc., Fort Worth,
A single observer (PS) who was masked to the results of the symptom assessment conducted a training session using the central cornea and temporal conjunctiva of both eyes, with air flow varying from 0 to 200 mL/min, delivered to the central cornea and temporal conjunctiva at room temperature (20°C). This stimulus is mechanical with an easily detectable non-noxious cooling effect.22–27 The stimulus duration was 1 second, with a 15-second interval between the subject’s response and the next stimulus. Two seconds before each stimulus, a short, computer-generated tone reminded the subject to blink and view the fixation target. The subjects were informed that they were to blink freely and also to close their eyes for a few seconds to simulate a blink. Subjects were informed that they were to stop blinking and view the fixation target when the tone was heard. Subjects were also instructed to blink and view the fixation target when the esthesiometer was set 5 mm from the corneal and conjunctival surface and monitored with a calibrated video camera. The esthesiometer was rotated to ensure that the stimulus was delivered perpendicularly to the surface.

The stimuli, consisting of a series of air pulses at room temperature (20°C), with air flow varying from 0 to 200 mL/min, were delivered to the central cornea and temporal conjunctiva (~5 mm from the limbus). This stimulus is mechanical with an easily detectable non-noxious cooling effect.22–27 The stimulus duration was 1 second, with a 15-second interval between the subject’s response and the next stimulus. Two seconds before each stimulus, a short, computer-generated tone reminded the subject to blink and view the fixation target. The subjects were informed that they were to blink freely and also to close their eyes or look down between stimuli. Subjects could also interrupt the trials if necessary. Using the ascending method of limits,28 corneal and conjunctival thresholds were determined by the average flow rate of three first reports of the stimulus presence.

A single observer (PS) who was masked to the results of the subject’s group, conducted the clinical and ocular surface sensitivity tests. The tests were performed in the following order: NIBUT, PRT, corneal and conjunctival staining evaluation, and esthesiometer measurements.

**Data Analyses**

Statistical analyses were performed with commercial software (SAS ver. 9.1; SAS Institute Inc., Cary, NC), and P < 0.05 was considered to be statistically significant. Because of the characteristics of the data, the corneal and conjunctival thresholds and the NIBUT, were transformed by conversion to the natural log to achieve normality of the data distributions. Unpaired t-tests were used to compare differences between the symptomatic and non-dry groups. For corneal and conjunctival staining, the Mann-Whitney U test was used for comparison between groups. Pearson correlations were performed among transformed variables. Multiple linear regression with step-wise selection was applied to assess the relationship of OSDI scores with measurements of ocular sensitivity and other clinical signs. To eliminate potentially harmful multicollinearity,29 for highly correlated predictor variables, only one was included in model selection. In addition, response variable OSDI scores were transformed by taking the natural log to satisfy the model assumption that variance of the residuals should be approximately constant.

**RESULTS**

The demographic data of the two groups are reported in Table 2. The frequencies of each category classified by the symptom group are presented in Figure 1A and scatter plots of individual corneal and conjunctival thresholds versus age are shown in Figures 3A and 2B for the symptomatic and non-dry groups, respectively. Corneal and conjunctival thresholds for detection of pneumatic cool stimuli, NIBUT and PRT, corneal and conjunctival staining score, and OSDI scores for symptomatic and non-dry subjects are shown in Figures 3 to 6, respectively. The differences in all the variables except for PRT were significant between the two groups (all P < 0.05).

The correlations between corneal and conjunctival thresholds, clinical tests, and symptom severity assessed by the OSDI scores are presented in Table 3. Corneal and conjunctival threshold responses to pneumatic cool stimuli correlated inversely with staining and OSDI scores but positively with NIBUT (all P < 0.05). Inverse correlations were also found between NIBUT, staining, and OSDI scores (all P < 0.05).

The multiple regression model with OSDI as the outcome variable was statistically significant (P < 0.0001). Conjunctival threshold, NIBUT, and PRT accounted for 24% of the variation in OSDI score. With the other variables held constant, the item score was negatively related to conjunctival threshold and NIBUT and positively related to PRT. The effects of conjunctival threshold and NIBUT were significant (both P < 0.01) in the stepwise regression analysis.

**DISCUSSION**

Dysfunction of the ocular surface integrated functional unit may be manifest as dry eye with common features of symptoms of ocular discomfort, tear hyperosmolarity, interpalpebral ocular staining, reduced tear production, and/or tear instability.30 In the present study, the dry eye symptomatic group had corneal and conjunctival hypersensitivity (lower threshold), a shorter tear film break-up time, a greater degree of ocular surface staining, and a higher OSDI score than did the non-dry eye subjects, suggesting alterations in various aspects of the lacrimal functional unit. In addition, ocular surface hypersensitivity, tear film instability, epitheliopathy, and symptoms of ocular discomfort correlated significantly.

**Table 1. Questionnaire-Based Single Score for Symptoms of Ocular Dryness**

<table>
<thead>
<tr>
<th>Question</th>
<th>None (0)</th>
<th>Trace (1)</th>
<th>Mild (2)</th>
<th>Moderate (3)</th>
<th>Severe (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1: Frequency of symptom</td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Frequent</td>
<td>Always</td>
</tr>
<tr>
<td>Q2: Presence of discomfort</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q3: Interference with activity</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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**Table 2. Demographic Data of the Two Groups**

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Non-dry</th>
<th>Dry Eye Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y (range)</td>
<td>41 ± 18 (19–79)</td>
<td>49 ± 16 (19–80)</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>31/25</td>
<td>35/8</td>
</tr>
</tbody>
</table>
Neural control is one of the important aspects of the functional unit and links the components of the unit into a homeostatic loop with the primary function of protecting and maintaining the health of the ocular surface. The sensory nerves of the ocular surface together with efferent sympathetic and parasympathetic innervation control the secretory activity of the lacrimal and meibomian glands and the conjunctival goblet cells.

A few studies have evaluated the functioning of corneal sensory nerves by measuring corneal sensitivity in patients with dry eye. However, the part played by ocular surface sensitivity in relation to the natural history of different forms of dry eye is not fully understood. Tear film break-up in the interblink interval due to its instability could give rise to local drying and hyperosmolarity in the exposed surface, and to ocular surface damage and a disturbance of glycoalyx and goblet cells. It has been postulated that ocular surface damage could produce reflex stimulation of the lacrimal glands in the initial stage of dry eye, to compensate for the tear film hyperosmolarity that arises as a result of excessive evaporation or/and insufficient aqueous tear flow. Stimulation of the lacrimal functional unit in the absence of a protective tear film could result in neurogenic inflammation, further damaging the ocular surface. The present study revealed ocular surface hypersensitivity in the dry eye symptomatic group, indicating an alteration of the sensory nerve function. The altered sensory input was related to an index of tear film instability and epitheliopathy of the ocular surface, each part of a vicious circle of interacting mechanisms: The worse the quality of the tear film and the greater the degree of the epithelial staining, the higher the sensitivity (perhaps due to sensitization of sensory fibers).

The presence of symptoms of discomfort suggests nociception at the ocular surface evoked by the activation of sensory nerves. It has been reported that the activities of corneal sensory fibers could be modified by injury and inflammation of the ocular surface. Sensitization of corneal polymodal nociceptors, a decrease in threshold to one or more stimulus modalities, and/ or increased responsiveness and spontaneous activity to suprathreshold stimulation, can be induced by certain inflammatory mediators. Interleukin (IL)-1, a proinflammatory cytokine found in the tears and conjunctiva (using impression cytology) in patients with keratoconjunctivitis sicca has been reported to induce hyperesthesia. The effect of sensitization increases the probability that a given stimulus will activate the target receptor (i.e., sensitized nociceptors.

![Figure 1](image1.png)

**Figure 1.** The prevalence of the single score for symptom of ocular dryness and the grouping criteria. Score: none to trace, non-dry group; mild to severe, symptomatic group.

![Figure 2](image2.png)

**Figure 2.** Scatterplots of corneal and conjunctival threshold versus age for symptomatic (A) and non-dry group (B).

![Figure 3](image3.png)

**Figure 3.** Box plots of corneal and conjunctival thresholds in response to pneumatic cool stimuli in the symptomatic and non-dry groups. Data are the mean ± 95% CI. Both LN corneal and LN conjunctival thresholds (natural log transformed) were lower in the symptomatic group. *P = 0.003 and **P < 0.001.
can be triggered by stimulation that would be insufficient to activate intact nerve endings under normal conditions).

On the other hand, the peptides and neurotransmitters, such as substance P and calcitonin-gene–related peptide released by activated nociceptors from peripheral nerve terminals, facilitate production of the “inflammatory soup.”9,37 Thus, it is also possible that the sensory changes themselves are the initiating factor and the resultant alterations in the tear film and surface follow because of neurogenic inflammation.10

In the present study, we found that ocular surface sensitivity and the index of tear film stability were significant predictors for the severity of the symptoms. It may be possible that increased sensory input from sensitized receptors, resulting from inflammatory mediators and related to the aforementioned interacting mechanisms between tear film instability and disruption of the ocular surface, account for the symptoms in these subjects.

In addition to the activation of nociceptors at the ocular surface, the abnormal dryness sensation in dry eye may relate to altered sensation (dysesthesia). During sensory processing, the voltage-gated ion channels expressed by nociceptors contribute to the propagation of the signals detected by primary afferents.38 The changes in expression of voltage-gated sodium channels play a key role in the pathogenesis of neuropathic pain39,40 such as dysesthesias,41 and in the pain and hypersensitivity associated with tissue inflammation.40 A recent study showed sensitized cold sensory receptors (non-nociceptors) in surgically lesioned guinea pig corneas and the abnormal activities of sensitized cold receptors were attenuated by application of lidocaine, suggesting that the cause of the enhanced nerve ending activity of a cold receptor is the increased expression of sodium channels (Belmonte C et al. IOVS 2007;48: ARVO E-Abstract 3470). Evidence also suggests that, to a certain extent, the phenomena found in both inflammatory and neuropathic pain share common physiologic mechanisms, including contributions from sodium channels.40 Altered expression of ion channels leads to an enhanced excitability of the membrane in primary sensory neurons and gives rise to abnormal neuronal activities and altered responsiveness to stimuli.

In the present study, the stimuli used to measure sensitivity consisted of thermal cooling23,27 and mechanical26 components that usually produce a non-noxious “cooling sensation” at threshold.22 The enhanced sensitivity to this non-noxious stimulation in the symptomatic group suggests that altered sensation, possibly due to the increased expression of ion channels, may have the therapeutic potential to be a complementary treatment for dry eye.

The reports in the literature on corneal sensitivity to pneumatic mechanical stimuli in patients with dry eye are contradictory.12-14 Hyperesthesia has been found in patients with dry eye and in those with post-LASIK dry eye, and this correlates positively with corneal epithelial barrier function.13 Others reported hypoesthesia in patients with dry eye and suggest that the decreased corneal sensitivity in dry eye is due to the damage of sensory innervation.12,15-17 We also found corneal and conjunctival hypersensitivity in dry eye symptomatic subjects, supporting the findings reported by de Paiva and Pflugfelder.14 The reconciliation of these apparently discrepant results remains to be elucidated. If, as discussed earlier, corneal and conjunctival sensitivity represent the functioning of the
sensory nerves of the ocular surface, hypersensitivity (sensitization of the sensory nerves) and hyposensitivity (damage of the sensory nerves) may not be contradictory, but rather, may be indicators of different states of compensation during the continuum of the condition (including a failure to compensate) in the integrated functional unit.

In our study, tear volume measured using PRT was not different between the dry eye symptomatic and non–dry eye groups. In addition, most of the subjects in the dry eye symptomatic group fell into the category of mild to moderate. Also, some symptomatic subjects in the sample had none of the signs of dry eye assessed in this experiment. It is plausible that increased sensory input from the ocular surface may have produced an augmented tear secretion in these patients, to compensate for tear hyperosmolarity resulting from a compromised functional unit (regardless of the etiology). This augmented secretion could be a factor contributing to the weak correlation between corneal and conjunctival sensitivity and staining. This correlation may be expected to be higher in a sample that includes more subjects with severe disease who are unable to adapt to the disrupted ocular surface functional unit.

Many studies have shown that the associations between symptoms and clinical dry eye tests are sometimes statistically significant, but typically not strongly so.11,12,13,14,16,18,42–45 In the present study, we developed a model that included sensitivity, NIBUT, and PRT, to predict the severity of ocular discomfort measured by the OSDI. The model accounted for about one fourth of the variance and the relatively low variance may reflect the diverse etiologies and interacting mechanisms related to dry eye development, giving rise to a disease with a continuum of the condition (including a failure to compensate) in the integrated functional unit.

In conclusion, the symptoms of ocular irritation consistent with dry eye disease assessed using the OSDI appeared to be associated with ocular surface (particularly conjunctival) hyperesthesia to cooling stimulation, corneal epitheliopathy, and tear film instability. The interacting mechanisms of disruption of the barrier function, tear film instability, and altered sensory processing associated with neurogenic inflammation within the lacrimal functional unit may lead to or be influenced by the dry eye symptoms. In addition, changes in expression and function of ion channels isoforms in the peripheral and probably the central nervous systems may play a role in the sensation of abnormal dryness in dry eye.

References


<table>
<thead>
<tr>
<th>K Threshold</th>
<th>C Threshold</th>
<th>NIBUT</th>
<th>PRT</th>
<th>OSDI Score</th>
<th>K FL Staining</th>
<th>C LG Staining</th>
</tr>
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<tbody>
<tr>
<td>K threshold</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>C threshold</td>
<td>0.78</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>NIBUT</td>
<td>0.41</td>
<td>0.40</td>
<td>—</td>
<td>—</td>
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<tr>
<td>PRT</td>
<td>NS</td>
<td>NS</td>
<td>0.32</td>
<td>—</td>
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<td>OSDI score</td>
<td>0.22</td>
<td>0.20</td>
<td>—</td>
<td>0.39</td>
<td>NS</td>
<td>—</td>
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<tr>
<td>K FL staining</td>
<td>—0.41</td>
<td>—0.29</td>
<td>—</td>
<td>0.47</td>
<td>NS</td>
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<tr>
<td>C LG staining</td>
<td>—0.30</td>
<td>—0.27</td>
<td>—</td>
<td>0.45</td>
<td>0.28</td>
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All P < 0.05.


