Tear Osmolarity as a Biomarker for Dry Eye Disease Severity

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Purpose. To study the association between tear osmolarity and dry eye severity grade, based on a modified Dry Eye Workshop (DEWS) scale, and between osmolarity and the signs and symptoms that determine dry eye disease severity.

Methods. Nineteen patients with dry eye disease were asked to complete an evaluation of dry eye signs and symptoms composed of the Ocular Surface Disease Index (OSDI) questionnaire, corneal staining with fluorescein, conjunctival staining with lissamine green, tear-film breakup time (TFBUT), Schirmer’s test with anesthesia, and tear sample collection. Tear samples were collected in 5-μL microcapillaries. Tear osmolarity was measured in the right eye with a tear osmometer.

Results. Tear osmolarity correlated significantly with dry eye severity grade (modified DEWS). Schirmer’s test and tear osmolarity correlated significantly at \( r = -0.52 \), with Schirmer’s test result, with adjustment for age, contributing significantly to the independent estimate of tear osmolarity.

Conclusions. Tear osmolarity correlates with dry eye severity and therefore could provide a biomarker for disease severity. (Invest Ophthalmol Vis Sci. 2010;51:4557–4561) DOI:10.1167/iovs.09-4596

Dry eye disease, or keratoconjunctivitis sicca, is a common eye problem that involves irritation and blurry vision caused by damage to the ocular surface by insufficient tear production or excessive tear evaporation and that can affect quality of life. It is prevalent in the aging population, with a higher incidence in women. Although it is a common diagnosis, physicians rely on several symptoms and diagnostic tests to confirm its presence. The Dry Eye Workshop (DEWS) report and the 2006 International Task Force Delphi Expert Consensus outlined seven signs and symptoms for use in determining dry eye disease severity. The five relevant to this study are the Ocular Surface Disease Index, corneal staining, conjunctival staining, tear film breakup time (TFBUT), and Schirmer’s test. In addition to this, tear hyperosmolarity was recognized as part of the definition of dry eye in 1995 by the International DEWS.

Tear hyperosmolarity is considered to be a phenomenon that leads to dry eye disease, sometimes attributable to everyday lifestyle choices, such as contact lens wear. Findings in studies have long shown a difference in tear osmolarity between eyes with keratoconjunctivitis sicca and normal eyes. Measurement of tear osmolarity is therefore a diagnostic test for clinical use that has potential to be used as an efficient, quantifiable biomarker for dry eye disease, in addition to the five commonly used signs and symptoms.

Successful measurement of tear osmolarity was thought to necessitate collection of a large amount of tears. With technological advances in its measurement, tear osmolarity is now more feasible to use in assessing dry eye. A clinical osmometer with the advantage of requiring a significantly smaller tear sample (0.5 μL, model 3D2; Advanced Instruments, Norwood, MA) was found to be acceptable for clinical use after tests for repeatability and accuracy, in comparison with a U.S. Food and Drug Administration (FDA)-approved clinical osmometer by the same manufacturer (model 3D2; Advanced Instruments) that requires 250 μL. The new instrument was used for the purpose of measuring tear osmolarity in the present study.

As there is room for more study of the relationship of tear hyperosmolarity and dry eye disease severity, we focused on the association of tear osmolarity and dry eye severity grade (DEWS), as well as the associations with the signs and symptoms commonly used to determine DEWS severity grade. Since the diagnostic criteria of dry eye disease had not been quantified, a modified DEWS scale (Table 1) was used for the purposes of this study.

Materials and Methods

Patient Information

The study comprised 19 volunteer patients (52.1 ± 16.4 years old), all of whom had established dry eye diagnosed by experienced clinicians who used standard techniques during regular eye examination. Dry eye was diagnosed by the physician by the patient’s report of ocular discomfort, TFBUT, corneal staining with fluorescein, and conjunctival staining with lissamine green. These tests were used to qualify patients for inclusion in the study and for grading dry eye disease severity.

Meibomian gland dysfunction was not used to include or exclude patients. The dry eye patients were a mixed group with aqueous-deficient or evaporative dry eye disease. Research in this study adhered to the tenets of the Declaration of Helsinki.

After providing informed consent according to Mount Sinai School of Medicine’s Program for the Protection of Human Subjects (PPHS), the patients completed an evaluation of the signs and symptoms of dry eye disease that included the Ocular Surface Disease Index (OSDI) questionnaire, corneal staining with fluorescein, conjunctival staining with lissamine green, TFBUT, and Schirmer’s test with anesthesia. Corneal staining with fluorescein was evaluated using the National Eye Institute (NEI) method, a standardized scale (0–3) for each of the five regions of the cornea: central, inferior, nasal, superior, and temporal.
Conjunctival staining with lissamine green was evaluated by using the Oxford Schema (0–4) for three regions of the conjunctiva: central, nasal, and temporal. TFBUT was measured as the time from a patient’s blink to the appearance of the first dry spot on the cornea. Schirmer’s test was performed for 5 minutes with anesthesia and eyes closed. All tests were performed on both eyes by the same researcher.

In addition, 5 to 10 μL of tears were collected from both eyes. Tear samples were collected from the lower meniscus/marginal tear strip of the lower lid near the lateral canthus of both eyes by using 5-μL microcapillaries (Microcaps 5 μL; Drummond Scientific, Broomall, PA). Samples from the right eye were chosen. Sample microcapillary tubes were inserted into a nano-dispensing sampler, precalibrated to 0.5 μL. The 0.5-μL sample was then transferred into the small end of the sample cell (TearTip; Advanced Instruments) and inserted into the thermal chamber of the tear osmometer (model 3100; Advanced Instruments), to measure the tear osmolarity on the basis of the freezing point. The osmolarity of the samples was calculated in milliosmolar/kilogram H2O.

Patients’ signs and symptoms were analyzed for classification into the dry eye severity grade outlined by the DEWS report3 and the 2006 International Task Force Delphi Expert Consensus,2 by using the five selected criteria (OSDI, corneal staining, conjunctival staining, TFBUT, and Schirmer’s test with anesthesia). The DEWS classification table was modified with numeric ranges for classification of the disease state, rather than the descriptions by Asbell et al.1 (Table 1), and each patient was classified as having a dry eye severity on a scale of 1 to 4.

The modified DEWS table (Table 1) shows the descriptive definitions for dry eye disease severity with the ranges established for its classification. Mild dry eye disease, categorized as DEWS severity 1 and 2, and moderate-severe dry eye disease, which is DEWS severity 3 and 4, were distinguished by ranges of OSDI score, TFBUT, Schirmer’s test with anesthesia, corneal staining, and conjunctival staining.

Patient test results were rated on the numeric dry eye severity scale (Table 1) for each diagnostic test, and the patient was assigned an overall dry eye severity grade of 1, 2, 3, or 4, based on the mode and arithmetic mean of the individual severity grades for the selected criteria. (See Table 3 for de-identified patient data for the study.)

**Statistical Analysis**

Descriptive statistics for continuous variables are reported as the mean (SD). Box plots display the distribution of osmolality for each grade dry eye severity (Fig. 1). The correlation between tear osmolarity and each of the other variables under investigation was determined using Pearson’s correlation coefficient (r), if the other variable was normally distributed, or Spearman’s correlation coefficient (r_s), if the other variable was not normally distributed. A correlation was considered statistically significant at P < 0.05 for test of the null hypothesis that the correlation is 0. The contributions of each of these variables, adjusting for the other variables, in predicting tear osmolarity were analyzed by using multiple regression methods (SAS ver. 9.1; SAS Institute, Cary, NC).

**RESULTS**

OSDI, Schirmer’s test, and tear osmolarity were normally distributed and Pearson’s correlation coefficient was calculated to estimate the pairwise association between them. Schirmer’s test and tear osmolarity showed a significant negative correlation (r = −0.52; P = 0.02; Fig. 2).

Conjunctival staining, corneal staining, TFBUT, and DEWS severity grade were not normally distributed. Spearman’s correlation was used to assess association of these variables with tear osmolarity. DEWS severity grade and tear osmolarity correlated significantly (r_s = 0.47; P = 0.04).

Schirmer’s test results correlated significantly with tear osmolarity and remained a significant predictor of tear osmolarity when age was present in the multiple regression model (P = 0.02).

Statistical summaries of the signs and symptoms predicting dry eye disease severity are shown in Table 2. Data from subjects (n = 19) are in Table 3. There were 3 subjects in dry eye severity grade 1, 12 in grade 2, 2 in grade 3, and 2 in grade 4. Distributions of the tear osmolarity measurements for each dry eye severity grade are represented by the box plots in Figure 1.
Tear osmolarity has been reported to be elevated in dry eye patients when compared with that in normal control subjects. Past studies have been focused on comparing tear osmolarity in dry eye and normal patients, with less focus on differentiating for dry eye severity. In this study, tear osmolarity correlated with dry eye (DEWS) severity grade (Table 1).

A common method of quantifying tear secretion is Schirmer’s test. In this study, Schirmer’s test with anesthesia correlated negatively with tear osmolarity. This result affirms the prior postulations regarding dry eye’s etiology, as less tear secretion associated with higher tear osmolarity is consistent with the known mechanism of disease involving decreased tear production or increased evaporation rate. By showing a positive correlation between dry eye disease and tear osmolarity, this study affirmed what the DEWS regarded as the central mechanism in the damaging effects of dry eye.

Dry eye, or keratoconjunctivitis sicca, results from any disorder that increases tear evaporation or decreases tear secretion: evaporative dry eye and aqueous deficient dry eye, respectively. Present understanding of the pathogenesis of dry eye disease suggests an elevation in tear osmolarity with increasing dry eye severity, either as aqueous deficient dry eye or evaporative dry eye. The DEWS report highlights several possible mechanisms, citing deficient lacrimal gland secretion as the main cause of aqueous-deficient dry eye and several intrinsic and extrinsic conditions as causing evaporative dry eye, with the most predominant cause from diseases affecting the lid meibomian gland. Common mechanisms of dry eye etiology involve an intrinsic dysfunction, such as low blink rate, or an extrinsic problem, such as vitamin A deficiency and allergies.

The etiologies for the two dry eye disease categories are not exclusive of each other and as such are commonly discussed together. Vitamin A deficiency can effect changes in the ocular surface of the eye through unstable tear film or lacrimal damage, leading to increased tear osmolarity. Low blink rate is a noted mechanism in increased tear osmolarity, with several possible contributory factors. Contact lens wear is one such extrinsic factor that has been postulated to bring about increased tear osmolarity from a desensitization of the trigeminal nerve. Diabetes mellitus is another contributory factor in low blink rate, through the loss of sensory trigeminal pathways or changes in the lacrimal gland vascularization. Low blink rate can also occur, from carrying out tasks that require concentration (e.g., facing a computer screen) and from disorders such as Parkinson’s disease.

Changes in secretion from the lacrimal and meibomian glands can lead to increased tear osmolarity. Neurologic damage to the facial nerve (cranial nerve VII) would damage the motor pathway in lacrimal gland secretion, thereby bringing about dry eye and increased tear osmolarity because of hyposecretion from the lacrimal gland. Other causes of lacrimal gland hyposecretion have been identified, including: systemic drug medications, antihistamines, beta blockers, diuretics, and selective serotonin reuptake inhibitors. That such a broad array of factors contribute to the cause of dry eye disease underscores its importance for study.

This study’s focus on dry eye severity and tear osmolarity was made possible by technological advances. We used a tear osmometer that requires a minimal tear sample. Older tear osmometers that require a large sample are not practical for use in the clinical setting. In the past, there has been difficulty in demonstrating a difference in tear osmolarity between subjects with dry eye disease and those without, as prolonged ocular contact during the tear sample collection stimulates increased tear secretion, thereby decreasing tear osmolarity. The osmolarity of the tear sample during its transfer from the capillary tube to the osmometer chamber was deemed

### Table 1. Diag nostic Test Results

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<th>ID</th>
<th>Age (y)</th>
<th>Sex</th>
<th>OSDI</th>
<th>Conjunctival Staining (sum)</th>
<th>Fluorescein Staining (sum)</th>
<th>TBUT (s)</th>
<th>Schirmer (mm)</th>
<th>Eye</th>
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unchanged, as the sample was not stored between collection and measurement. Sample transfer in standardized osmolarity solutions by Yildiz et al.,11 who used the new osmometer, showed high accuracy on repeated testing, boosting confidence in the accuracy of tear osmolarity measurements in our study.

There are certain limitations to this study. The sample size was small and did not include normal patients, as the study was directed toward dry eye disease. Most of the subjects were women, although the disproportionate representation ought to be mitigated by findings in past studies that have shown more women than men to have dry eye.13 A large portion of the subjects in our study were rated as dry eye severity grade 2 but the study showed significant positive correlation between tear osmolarity and severe dry eye of grades 3 and 4 (Fig. 1). The dry eye severity grade distribution among the study patients limits the strength of our assertions that tear osmolarity can be a biomarker of disease severity. The age of the subjects in the study may be a confounder in establishing the dry eye severity grade, as age can affect the tear osmolarity through limited secretion. Age is not an established confounder, however.

There are some methodological points to consider. An overall dry eye severity grade was assigned based on the mode and arithmetic mean of the dry eye severity categorizations of the individual diagnostic tests: OSDI questionnaire, corneal staining with fluorescein, conjunctival staining with lissamine green, TFBUT, and Schirmer’s test. The OSDI questionnaire is based on questions pertaining to ocular discomfort, vision symptoms, and environmental triggers. Although the DEWS report classification is based on ocular discomfort levels and visual blur, the OSDI questions on environmental triggers and their scores were included for assigning a dry eye disease severity grade in this study, because its validity in discriminating between dry eye disease severity grades has been shown.14 OSDI ranges for dry eye severity grade were set on the basis of the OSDI dry eye assessment chart developed by Allergan, Inc. (Irvine, CA). Using tear osmolarity to diagnose severe dry eye in patients who present with ocular discomfort and extensive surface staining may not be necessary. However, tear osmolarity may play a role in establishing objective metrics for the grading of dry eye severity.

The new DEWS classification (Table 1) facilitated grouping patients for clinical trials and for the study of dry eye change. The staining grade ranges in the modified Ashwell scale were set to facilitate the grading of dry eye severity, on the basis of the judgment of an experienced clinician. Although there is the limitation in DEWS dry eye severity grading that the numeric cutoffs were not empirically established, its facilitation of comparing the results of accepted diagnostic tests with tear osmolarity is a strong case for its utilization in this study.

We looked at the right eye’s tear osmolarity and standard diagnostic test results (Schirmer’s test, corneal staining with fluorescein, conjunctival staining with lissamine, TFBUT, and OSDI). The tear osmolarity sample was taken once and placed in the tear osmometer promptly for reading. In a study in which multiple samples were collected throughout the day, tear osmolarity was found to differ significantly between morning and afternoon in normal subjects.15 Although similar differences in dry eye patients were only speculative because of a wider variation in measurements, time of day for tear sampling was thought to influence tear osmolarity.15 Nonetheless, single measurements of tear osmolarity have been shown to have high sensitivity and specificity for dry eye.

Although we sampled only once, the study goal was to compare tear osmolarity with diagnostic test results and dry eye severity grades in assessing its use as an objective metric for dry eye disease severity, not to establish benchmark tear osmolarity values for dry eye disease severity grades. As tear osmolarity is regarded to be elevated in patients with dry eye disease when compared with that in normal patients, tear osmolarity measurements from one sampling were used. Tear osmolarity of the eyes in patients with dry eye has been noted to vary more greatly than that in normal patients,16 but we examined only right eye tear osmolarity, as the left eye sample was saved for use in a study of cytokine content. Evaluating dry eye severity in an individual on the basis of a single sample from one eye ought to be approached with caution. This study is limited in assessing the true state of dry eye severity in individuals, as only one eye was studied, and there may be variability in osmolarity between the two eyes. As the goal of this study concerned comparing tear osmolarity with dry eye disease severity grades and the diagnostic tests, this methodology was considered appropriate.

Despite the limitations of this pilot study, it is unique in determining the association of tear osmolarity and dry eye severity grade. The only diagnostic test under evaluation was tear osmolarity; the other diagnostic tests—corneal staining with fluorescein, conjunctival staining with lissamine, TFBUT, and Schirmer’s test—are all accepted metrics for the diagnosis of dry eye. The small number of subjects in this study and the majority classification into dry eye disease severity grade 2 (12 of 19 subjects) limits the significance of this study in establishing tear osmolarity as a biomarker for dry eye disease severity. However, the findings support further investigation into using tear osmolarity to monitor dry eye disease.

In conclusion, our study offers support to the ramification of dry eye disease that involves progressively elevated tear osmolarity with worsening disease severity. Although it is important to keep in mind that tear osmolarity cannot be used as the sole indicator of dry eye disease, its positive correlation with dry eye severity grade lends support to considering the use of tear osmolarity as a biomarker for dry eye disease severity. Further data will help to establish the current methodology of evaluating tear osmolarity as a minimally invasive, objective metric that will be useful in classifying persons with dry eye disease as well as in identifying change in dry eye disease over time. Such a quantitative metric would be useful in assisting in the care of patients and merits further investigation.

References


