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Variability of tear osmolarity in dry eye patients and controls

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Abstract

Importance—Knowledge about the variability of measurements using the TearLab™ osmolarity system is necessary when evaluating the clinical utility of readings.

Objective—To examine the variability of tear osmolarity, as measured by the TearLab™ system, in subjects with Sjogren's syndrome (SS), blepharitis and controls.

Design—Cross-sectional study.

Setting—Tertiary care, academic center.

Participants—Seventy-four eyes of thirty-seven subjects from a volunteer sample (18 SS, 11 blepharitis, 8 controls) were evaluated using the TearLab™ osmolarity system with three consecutive osmolarity measurements taken at one-minute intervals in a session, and fifteen of these subjects had the same measurements taken by the same examiner in two additional sessions on the same day (9–10am, 12–1pm, or 3–4pm).

The majority of SS and blepharitis subjects were on systemic or topical dry eye medications at the time of enrollment.

Main Outcome Measures—Mean osmolarity and its variability calculated from a linear mixed model for each disease group that accounts for the variations attributable to different subjects, eyes, and sessions, and measurement error specific to each disease group.

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Results—Mean tear osmolarity was 307, 304, and 301 mOsm/L within the SS, blepharitis, and control groups, respectively ($p=0.46$). The error associated with repeated measurements within a session in the non-dry eye subjects (10.5 [95% CI 9.0, 12.4] mOsm/L) was significantly lower than in the blepharitis (14.6 [12.5, 17.5] mOsm/L, $p=0.006$) or SS (15.8 [14.2, 17.8] mOsm/L, $p<0.0001$) subjects, but a difference in the error of repeated measurements between blepharitis and SS subjects was not identified ($p=0.46$).

Conclusions and Relevance—There was increased variability attributable to error in repeated measurements among SS and blepharitis subjects compared to controls. The high variability of TearLab™ osmolarity readings in all groups makes clinical interpretation of measurements unclear.

The prevalence of dry eye disease (DED), which can significantly impair visual function, workplace productivity, and quality of life¹ has been estimated to be 14.4% of the general population and increases with age.² The 2007 Dry Eye Workshop report defined DED as being a multifactorial disease that "is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface."³ Hyperosmolarity is thought to activate inflammatory pathways⁴ that lead to epithelial damage, tear instability, and ocular discomfort.⁵

Elevated tear osmolarity is considered by many as an objective marker of DED.^{6–15} However, despite evidence that tear osmolarity may be helpful in the diagnosis of DED, challenges still remain for implementation into clinical practice.^{16–18} Historically, tear osmolarity testing in the clinic was impractical due to the difficulty of tear collection and analytic procedures that required laboratory facilities. Recently, the TearLab™ Osmolarity System (TearLab Corp., San Diego, CA) has become available and is appealing because of its ease of use and ability to give quick in-office results. The system uses single-use test cards that simultaneously collect and analyze the osmolarity of 50 nanoliters of tears using electrical impedance.¹⁹ Unlike other methods for measuring tear osmolarity such as using freezing point depression, the TearLab™ system does not require transporting tear samples to a separate device which risks evaporation and concentration of samples.²⁰

However, the clinical utility of tear osmolarity as measured by the TearLab™ system has recently been called into question due to the variability of measurements and lack of correlation with symptoms or fluorescein staining of the cornea.^{16, 21} As with any instrument, it is important to understand the variability of measurements to allow for useful clinical interpretation of readings. Variability of measurements can be due to a variety of sources including the operator, instrument, patient, and the disease. Tear osmolarity has been reported to vary to a greater degree in subjects with DED than in those without DED.^{14, 5, 22} However, while a high degree of variability of TearLab™ osmolarity measurements has been reported in 5 subjects without DED,¹⁶ more information regarding the intra-session and inter-session variability in DED is needed.

Therefore, the purpose of this study was to examine the variability of tear osmolarity measurements using the TearLab™ osmolarity system in subjects with and without DED, including subjects with Sjögren's syndrome (SS) or blepharitis. Because the initiation of DED is thought to be due to a breakdown of compensatory mechanisms leading to transient

changes in tear stability,²³ we hypothesized that there would be increased variability among subjects with DED compared to those without. In addition, we set out to quantify the degree of variability that could be expected if repeated measurements were taken within the same session, and also throughout the day in a given subject.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at the University of Pennsylvania, was HIPAA-compliant, and informed consent was obtained from all participants prior to participation. This study also adhered to the tenets of the Declaration of Helsinki. Subjects who were 18 years of age or older were recruited from the ophthalmology and rheumatology practices at an academic center over five months. Blepharitis subjects were enrolled if they had a history of blepharitis and had clinical signs of at least moderate blepharitis (using an abbreviated grading system). SS subjects were diagnosed by the American-European Consensus Group (AECG) criteria or the American College of Rheumatology (ACR) criteria.^{24, 25} Controls were enrolled if they did not have dry eye symptoms (OSDI<13), and had no diagnosis of DED, SS, or blepharitis.

Exclusion criteria for all subjects included a history of any significant ocular surface disease (other than DED) or ocular inflammation, thyroid eye disease, diabetes, active ocular infection, active ocular allergy, ocular surgery within 1 year, punctal plug placement within 30 days, evidence of lid deformity or abnormal lid movement disorder, use of eye drops (excluding artificial tears), contact lens use within 1 month, pregnancy or lactation, birth control or hormone replacement therapy, abnormal nasolacrimal drainage, evidence of a systemic disease known to affect tear production (excluding SS in that subgroup) or use of artificial tears within 2 hours of enrollment. Subjects were also excluded if they had initiated or altered the dose of chronic systemic medication known to affect tear production within 30 days of testing.

For all subjects, demographic information, medical history and ocular history were obtained. Subjective symptoms of dry eye were assessed using the Ocular Surface Discomfort Index (OSDI) (Allergan, Inc., Irvine CA).

Tear Osmolarity Testing and Ocular Surface Exams

Tear osmolarity was measured in each eye with the TearLab™ osmolarity system (TearLab™ Osmolarity System, TearLab™ Corp., San Diego, CA).. To ensure proper functioning of the instrument, the system was calibrated at the start of each day of testing in accordance with the manufacturer's instructions. Using the same pen for all readings, three consecutive osmolarity measurements were taken at one-minute intervals in each eye of each subject (for all 37 subjects), always starting with the right eye first.

A subgroup of 15 subjects agreed to have three measurements per eye taken at each of 3 sessions throughout the day (9–10am, 12–1pm, 3–4pm) by the same examiner, to assess the inter-session variability over the course of the day. This resulted in a total of 402 individual tear osmolarity measurements from 37 participants.

All subjects also underwent a slit lamp exam, vital dye staining with fluorescein and lissamine green, tear break-up time (TBUT) assessment, and unanesthetized Schirmer testing.

Statistical Methods

Differences in ocular surface parameters between subjects with SS, blepharitis and controls were estimated using linear regression with general estimating equations (GEE) to account for inter-eye correlation.²⁶

Tear osmolarity was modeled using a hierarchical linear mixed effects model with disease group and age considered as a fixed effects, differences among subjects, eyes and sessions considered as successively nested random effects (i.e., subjects within disease group, eyes within subject, and sessions within eye), and differences among repeated measurements within a session considered as the residual variance, which was allowed to be of different magnitude for each of the three disease groups.²⁷ Model specification and output for the variance estimates are shown in eFigure 1. P-values for each random effect and residual variance estimate were calculated using Wald Z-tests, and p-values for comparisons between residual variance estimates of each disease group were calculated using Monte Carlo simulation with 10,000 samples.

Fixed effects represent factors that are associated with consistent differences in the true value of the tear osmolarity of an eye, such as an increased level among diseased groups or a decreased level among treated groups. Random effects represent factors that are associated with random differences for which the value of the difference is not of interest but the amount of variance is, such as the differences among subjects with the same disease, between a single patient's eyes, and among sessions in a single eye. Residual variance, or measurement error, represents the variance in the measurements after all the other factors in the model have been accounted for and may be due to causes such as moment-to-moment fluctuations in the value or limitations of the measurement tool.

The variance of a series of measurements is the sum of the disease-specific residual variance ("measurement error") plus the variance of any applicable random effects ("effects"). The variance of a difference between two independent measurements is the sum of the variability for each measurement. All variance estimates presented hereafter are transformed into standard deviations (SD) by taking the square root of the variance, and are referred to as "variability". The total variability from two or more sources are calculated by summing the their variances first followed by taking square root of total variance, instead of summing the SD directly

As suggested by TearLab™, a fixed effects analysis of variance (ANOVA) was also performed using only the maximum value of the two eyes from the first measurement of each eye at the first session for each patient. All statistical analyses were performed in SAS v9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of Study Subjects

Of the 37 subjects enrolled in the study, 18 subjects had SS (14 primary SS, 4 secondary SS), 11 had blepharitis, and 8 were classified as controls. Of the 15 subjects who had 3 sessions completed over the course of the day, 7 had SS (39% of SS group), 2 had blepharitis (18% of blepharitis group), and 6 were controls (75% of control group).

Table 1 summarizes the characteristics of study subjects. Subjects with SS or blepharitis were older than controls (Mean $\pm$ SD: 59 ± 12.6 years vs. 40.4 ± 12.7 years; Table 1). The majority of SS and blepharitis subjects were on systemic or topical dry eye medications at the time of enrollment. Table 2 summarizes the ocular surface parameters in all subjects. Groups differed on all of the characteristics except the Schirmer test. All control subjects had minimal to no dry eye symptoms (all OSDI <4). The majority of controls had modified DEWS severity grades of 0 in all but one parameter, with half having a grade of 0 in all parameters.

Comparison of Mean Osmolarity Among Groups of Subjects

Table 3 shows the estimates (mean [95% CI of mean]) of tear osmolarity by disease group. In the mixed-effects model, all 402 measurements (3 measurements per session per eye in 22 subjects, 9 measurements from 3 sessions per eye in 15 subjects) were used in estimating the mean values and their SD. A difference in the mean value among the three disease groups ($p=0.57$) was not identified. Similarly, when only the maximum value of the two eyes from the first measurement of the first session (1 measurement each from 37 subjects) was used, a difference in the mean value among the three disease groups ($p=0.60$) was not identified.

Sources of Variability

The estimated variability from each component is shown in Table 4. The within session measurement error in an eye of the controls (10.5 [95% CI: 9.0, 12.4] mOsm/L) was lower than that in the blepharitis (14.6 [12.5, 17.5] mOsm/L, $p=0.006$) or SS (15.8 [14.2, 17.8] mOsm/L, $p<0.0001$) subjects, but no difference in measurement error was found between blepharitis and SS subjects ($p=0.46$). The between-session random effect is 6.4 mOsm/L ($p=0.006$), which is an additional source of variability on top of the disease specific variability. The between-eye random effect was low compared to the other measurement errors and was not different from zero, and the between-person random effect is irrelevant to the discussion of variability within a patient; therefore these effects are not included in the analyses that follow. Figure 1 shows the individual tear osmolarity measurements from both eyes and all three sessions from a representative subject--a control subject with near-median within-patient variance.

DISCUSSION

When using all of the data and accounting for the experimental design with a hierarchical mixed model, the mean tear osmolarity level in subjects with blepharitis or SS was slightly higher than in non-dry eye controls, but not to a statistically significant degree. When the

maximum tear osmolarity value of the 2 eyes was used, as recommended by TearLab™, the differences among the estimated means for these groups were smaller (within 5 mOsm/L) and not statistically significantly different. One possible explanation is that the majority of SS and blepharitis subjects were on dry eye treatment at the time of enrollment, which may have improved their DED and decreased their osmolarity to a level that was similar to controls. There may have been a greater difference in mean osmolarity among the groups if none of the subjects were on treatment. However, this is unlikely because if treatment had improved their DED, we would have expected an improvement in all ocular surface parameters, not just in osmolarity.

There were differences among the three patient groups in the magnitude of measurement error with the non-dry eye subjects having lower measurement error, or variability, than the two dry eye groups. This is consistent with previous reports that subjects with DED exhibit greater tear instability and thus greater variability in osmolarity,^{14, 6, 22} and that non dry-eye subjects have less variation of tear osmolarity over the course of the day.²⁸

Previously, Khanal and colleagues found a high degree of variability in osmolarity measurements with typical differences of 10–15 mOsm/l for three consecutive osmolarity measurements in an individual non-dry eye subject, with variations of up to 35 mOsm/l.¹⁶ Our study showed similar variability in osmolarity measurements, but included both dry eye and non-dry eye subjects, as would commonly be evaluated in clinical practice.

When a single tear osmolarity measurement is obtained, the variability in the measurement needs to be taken into account for clinical interpretation. For a non-dry eye patient, the 95% confidence interval for a single measurement is 1.96 times the standard deviation (SD) ($SD_{\text{controls}} = 10.5$) for measurement error or $(1.96 * 10.5 = 20.5)$, ± 20.5 mOsm/L. For example, with a tear osmolarity reading of 310 mOsm/L in a non-dry eye subject, the 95% confidence interval is (289, 331). Because the measurement error is larger for blepharitis ($SD_{\text{blepharitis}} = 14.6$) and SS subjects ($SD_{\text{SS}} = 15.8$), the confidence intervals would also be larger: $(1.96 * 14.6 = 28.6)$, ± 28.6 and $(1.96 * 15.8 = 31.0)$, ± 31.0 mOsm/L, respectively. However, regardless of disease group, the confidence interval for a single measurement spans the range considered normal and the range considered abnormal, which has been described to be within the range of 305–316 mOsm/L.^{8, 12, 14}

When a single measurement is taken on the same eye at each of 2 sessions, such as before and after a new treatment, the measurement error for each reading as well as the variability in tear osmolarity between sessions needs to be taken into consideration. Continuing the example of measurements on a non-dry eye subject, the difference in two measurements on the same eye between 2 sessions would be associated with a SD of 17.4 mOsm/L (calculated as the square-root of twice the sum of the squares of the between-session random effect and the measurement error of the control group), resulting in confidence interval of ± 34.0 mOsm/L. If the measurement at the first session was 310 and the measurement at the second session was 290, the confidence interval on the difference of -20 would be $(-54.1, 14.1)$. Thus even with a large apparent decrease in tear osmolarity, the true difference in tear osmolarity could be 0 or actually even an increase. Among blepharitis and SS subjects, the

variability is even higher so that even larger changes are required to be confident that the tear osmolarity of an eye has actually changed.

This high degree of variation could explain the results reported recently by Amparo and colleagues who found that changes in tear osmolarity did not correlate significantly with changes in symptoms or corneal fluorescein staining.²¹ Our results are also consistent with those of Eperjesi et al who found that a level of 33mOsm/L was the clinically relevant level of change in healthy subjects and that any change smaller than this could be attributed to measurement noise.²²

TearLab™ recommends using the maximum of the patient's right and left eyes when measuring the tear osmolarity for a patient. However, the variability of a maximum of two measurements is no less than the variability of a single measurement, so the confidence intervals for the maximum from a single session or for the difference in the maximum between sessions would only be the same or larger than those cited above.

The control subjects in our study were younger than the SS and blepharitis subjects; however, because it has been shown that DED increases with age², we would expect this age difference to lead to a bias towards lower tear osmolarity compared to our dry eye subjects. We adjusted for age in our model and sensitivity analyses revealed no change in our results. Studies utilizing age-matched controls would be helpful in examining this further.

It is also possible that some of our controls had undiagnosed, asymptomatic dry eye. While all control subjects had minimal to no symptoms as evidenced by an OSDI of less than 4, some controls had minimal positive exam findings. However, the majority of controls had modified DEWS severity grades of 0 in all but one parameter, with half having a modified DEWS severity grade of 0 in all parameters. We found that it was rare to find control subjects with absolutely no symptoms or signs of dry eye, and therefore these represent "real-world" patients who we would be evaluating everyday in the office. Finally, sensitivity analyses by excluding control subjects with a single ocular surface parameter greater than modified DEWS severity grade 0 showed similar results.

A final limitation was our relatively small sample size. However, because our goal was to assess the variability in individual patients, we were able to accomplish this by taking multiple measurements in a small number of subjects.

Our study has shown that measurements of tear osmolarity taken with the TearLab™ system in both dry eye and non-dry eye subjects are highly variable, replicating and extending the results of previous studies of the TearLab™ system.^{16, 21} Consistent with previous studies, we found a higher degree of variability in DED subjects compared to those without dry eye. This variability is likely due to a combination of the variability of the osmolarity in the tear film itself, the sampling of a small volume of tears, and the variability in the measurement process. However, whatever the cause of the variability, the interpretation of the values after a single measurement is unclear when the 95% confidence interval includes both the normal and abnormal range. In clinical practice, this means that in a given patient, a single tear osmolarity measurement cannot be used to distinguish dry eye patients from normal patients. In addition, the interpretation of differences in values between visits is ambiguous when the

95% confidence includes both large increases and large decreases. Because of this large degree of variation, tear osmolarity may not be useful to follow patients over time. In conclusion, the high variability among TearLab™ osmolarity readings calls into question its clinical utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of interest:

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Vatinee Y. Bunya, MD had full access to all of the data in the study and takes responsibility for integrity of the data and the accuracy of the data analysis.

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Figure 1. Individual tear osmolarity measurements from a representative control patient (with near-median within-patient variance)

Right and left eyes are in the right and left columns within each session, respectively, and numbers 1, 2, and 3 represent the 1st, 2nd, and 3rd measurements taken in the same session of an eye.

Table 1

Characteristics of Subjects with Sjogren's Syndrome, Blepharitis, and Controls

		Control (n=8)	Blepharitis (n=11)	Sjogren's syndrome (n=18)
Mean age (SD)		40.4 (12.7)	65.2 (12.7)	55.2 (11.2)
Gender				
	Female	8 (100%)	8 (73%)	16 (89%)
Race				
	White	7 (88%)	11 (100%)	12 (67%)
	African American	1 (13%)	0 (0%)	4 (22%)
	Other	0 (0%)	0 (0%)	2 (11%)
Artificial Tear Use (Yes)		0 (0%)	8 (73%)	17 (94%)
Topical Cyclosporine (Yes)		0 (0%)	2 (18%)	2 (11%)
Fish Oil (Yes)		0 (0%)	5 (45%)	6 (33%)

* Fisher's exact test for categorical variables, t-test for age. P-values are not calculated for DED-specific characteristics.

Table 2

Clinical ocular surface parameters of subjects with Sjogren's syndrome, blepharitis, and Controls

	Median (25th quartile, 75th quartile)		
	Controls (n=16 eyes)	Blepharitis (n=22 eyes)	Sjogren's syndrome (n=36 eyes)
OSDI	0.83 (0, 2.5)	20 (10, 31)	37 (25, 47)
Schirmer (mm/5 min)	18.5 (13, 33)	10.5 (7, 15)	11 (7, 17)
TBUT (sec)	8 (7.33, 9.67)	2.67 (2, 4.33)	2 (1, 4)
Fluorescein staining	1 (0, 1)	2 (1, 6)	3 (1, 5)
Lissamine Green staining	0 (0, 0.5)	3 (0, 4)	6 (1.5, 9)

* Linear regression with general estimating equations* (GEE) to account for between-eye correlation

Table 3

Comparison of mean tear osmolarity among groups of subjects

Group	Mixed Effects Model of all Osmolarity measurements		Model using the maximum value between eyes from the first measurement of the first session	
	Mean (95% Confidence Interval)	p-value	Mean (95% Confidence Interval)	p-value
Control	302 (293, 310)	0.57	312 (299, 326)	0.60
Blepharitis	303 (295, 312)		307 (296, 325)	
Sjogren's syndrome	307 (301, 312)		314 (306, 321)	

Table 4

Variability components of tear osmolarity: random effects and measurement error

Variability Components	Variability of Measurement (mOsm/L)	p-value *
Measurement error		
Control	10.5	<0.0001
Blepharitis	14.6	<0.0001
Sjogren's	15.8	<0.0001
Random Effects		
Person	9.6	0.003
Eye	2.8	0.29
Session	6.4	0.006

* p-value from the mixed effects model for testing whether the variance attributable to the source is different than 0.