In Vivo Ocular Surface Osmolarity in a Dry Eye Population

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Introduction

The lacrimal functional unit (LFU) is a system of anatomical, physiological and biochemical mechanisms working in concert to maintain homeostasis at the ocular surface.[1] When any component of this system becomes unstable or dysfunctional, the resultant cascade can affect ocular surface and if corrective measures are not taken, can cause significant discomfort, pain and visual disturbances for a given patient. Dry eye disease (DED) is the result of dysfunction of the LFU and affects between 5% to 30% of adults over age 50 according to the TFOS Dry Eye Workshop.[2]

Tear Osmolarity

In 1978, Dr. Jeff Gilbard wrote about tear osmolarity as a clinical indicator of dryness in a small study of 36 samples.[3] The concept of hyperosmolarity was eventually integrated into the clinical definition of DED in 2007 based on the TFOS Dry Eye Workshop.[2] Tear film hyperosmolarity has been used as a DED metric in numerous publications including landmark studies by Tomlinson (2008) and Lemp (2011).[4,5] At the time, the prevailing and sole commercial device available to measure osmolarity was TearLab® (TearLab Corp, San Diego, CA). This system measures the electrical impedance of a 50 ηL sample collected from temporal location of the meniscus of the tear film to quantify osmolarity *ex vivo*.[6]

A new osmometer known as the i-Pen® (I-MED Pharma Inc., Montreal QC), is now commercially available for clinical use. Like previous systems, it measures electrical impedance, but does so *in vivo* which results in the ability to assess ocular surface osmolarity more rapidly than current commercially available systems. Measuring tear osmolarity through the tissues of the palpebral conjunctiva is not novel and has been previously shown as an effective method of measuring osmolarity *in vivo*. A flexible conductimetric sensor fabricated using micro-electronic techniques is small and flexible enough to be placed on the peri-ocular surface to measure the electrical conductivity of tear fluid *in vivo*.[7] As with any new diagnostic device however, interpretation of a new measure comes from studying clinical use and outcomes. As such, this prospective observational case study sought to examine the meaning of ocular surface osmolarity through the lens of this new device in comparison to accepted metrics of the tear film and ocular surface: corneal staining, tear break-up time, Ocular Surface Disease Index (OSDI), Standard Patient Evaluation of Eye Dryness (SPEED) and Schirmer volume testing.

Methods

This prospective observational case series involved 48 randomly selected patients measured bilaterally (n=96) presenting to a referral center for ocular surface disease. Patients ranged from age 27 to 88, 28 (58%) females and 20 (42%) males (average age 59.2 ± 15.6).

Subjects of this study were excluded if contact lenses were used within 24 hours of testing, had an active ocular infection, had undergone ocular surgery or changed systemic medicine in the previous 30 days.

Subjects presented to the clinic for a single visit which involved the following diagnostic protocol based on common tests for dry eye disease[8]:

1. Ocular Surface Disease Index and Standard Patient Evaluation of Eye Dryness (SPEED)
2. Osmometric testing using i-Pen®
3. Schirmer (without anesthetic)
4. Tear break-up time (TBUT) using fluorescein (NaFl) (10 minutes after Schirmer)
5. Corneal and conjunctival staining (Oxford staining protocol)

Steps 1 through 3 were performed by a trained ophthalmic technician with steps 4 and 5 by the attending clinician who was blinded from the previous steps findings.

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Table I Cut-off values for Dry Eye Disease tests (Binary score of 1 or 0 were applied for values beyond cut-off)

DED Test	Cut-off Value
OSDI	>12
SPEED	>6
Oxford Staining	>I
TBUT	<10 s
Schirmer	<18 mm

Table III Sensitivity and specificity values of non-osmolarity tests

Test	Sensitivity	Specificity
TBUT	91.8%	5.9%
Oxford Staining	58.8%	62.5%
OSDI	100%	16.1%
SPEED	89.1%	22.3%
Schirmer	40.2%	53.1%

The study was designed to assess the sensitivity and specificity of ocular surface osmolarity against a composite of common tests for dry eye disease. As such, the investigator compared osmolarity against a composite of each metric in a binary format to determine the cut-off between a 'normal' subject and 'mild' DED as noted by the Dry Eye Workshop Scale[2] (Table I). Similar to Lemp's work in 2011, each metric was mapped to this binary score of 1 or 0 so as not to create more weight to any one metric depending on the disease severity.[5] Thus, disease severity and etiology were not considered in this study.

Subjects presenting with 3 or more subnormal test outcomes were classified as having at minimum mild DED (classified as the binary score of "1" for yes and "0" for no). The absolute osmolarity and an inter-eye osmolarity difference of > 8 mOsm/L were compared to the binary composite DED score. The > 8 mOsm/L value is based on the observation made by Lemp et al.[5]

Results

In this study's cohort, the prevalence of DED based on greater than 3 positive non-osmolarity test outcomes was 76.0%. Osmometric readings > 290 mOsm/L (which is the upper range of blood plasma and serum osmolarity) had 91.8% sensitivity and 71.4% specificity which was the greatest sensitivity and specificity range of all other tests (Table II). TBUT yielded 91.8% sensitivity with only 5.9% specificity, while Oxford staining yielded the lowest sensitivity at 58.8% (Table III).

When removed from the analysis, inter-eye difference was shown to have a large impact on testing sensitivity, particularly at values over 290 mOsm/L (Table IV). The average inter-eye difference for values > 7 mOsm/L was 23.1 ± 16.5 and values < 8 mOsm/L was 3.1 ± 1.9. The sensitivity of an inter-eye difference > 7 mOsm/L alone was 84.9%. This is consistent with findings by Lemp.[5]

Table II Sensitivity and specifity of ocular surface osmolarity at specified cut-offs

Ocular Surface Osmolarity (mOsm/L)	Sensitivity	Specificity
286	93.2%	42.9%
290(i)	91.8%	71.4%
308	87.7%	85.7%
311	86.3%	100%

i = highest sensitivity and specificity combination between normal and DED patients

Table IV Sensitivity of ocular surface osmolarity without inter-eye difference consideration

Ocular Surface Osmolarity (mOsm/L)	Sensitivity
286	70.0%
290	57.5%
308	23.3%
311	15.1%

The mean osmolarity above the 290 mOsm/L cut-off was 307.8 ± 16.4 and below this cut-off the mean was 281.9 ± 9.0 mOsm/L.

Further analysis demonstrated a positive relationship between absolute inter-eye osmolarity difference and both standardized symptom measures, OSDI and SPEED with an R2=0.526 and R2=0.531, respectively however this was not statistically significant (p=0.05) (Figs. 1, 2).

As noted with sensitivity testing, inter-eye difference showed the strongest relationship to symptom severity when compared to average subject osmolarity (mean of OD and OS).

The osmolarity values found in this small observational study seem to be lower than those observed in previous studies, notably the frequently cited Lemp paper in 2011 which showed the value of 308 mOsm/L as being the intersecting osmolarity between normal and DED subjects.[5] The *in vivo* method of measurement and sample cohort are the two most plausible explanations for this difference, which will be further discussed below.

Discussion

Previous studies have reported a range in tear osmolarity cut-off between normal and DED patients from 304 to 316 mOsm/L depending on the study in question.[3,5] Based on the results from this cohort, and using a novel method of *in vivo* electrical impedance, findings suggest that ocular surface osmolarity cut-offs may be lower than previously reported when using the i-Pen® osmometer.

The results raise questions as to why this shift to lower osmolarity range is noted using an *in vivo* technique. Some of the possible differences could be related to the site of sampling. The i-Pen®, when used as directed, takes a

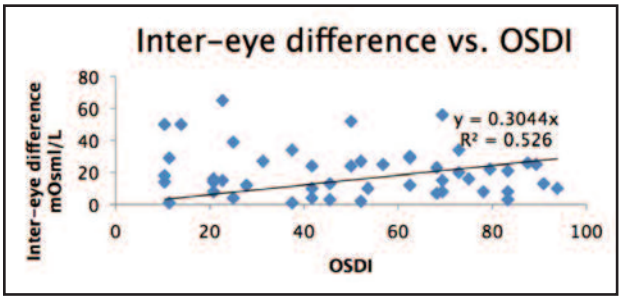

Fig. 1 The absolute inter-eye difference in osmolarity (mOsml/L) between OD and OS versus Ocular Surface Disease Index (OSDI)

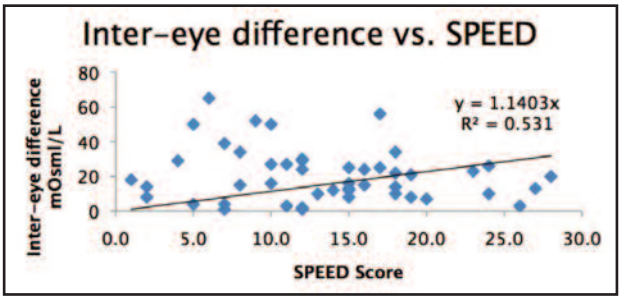

Fig. 2 The absolute inter-eye difference in osmolarity (mOsml/L) between OD and OS versus Standard Patient Evaluation of Eye Dryness (SPEED) score

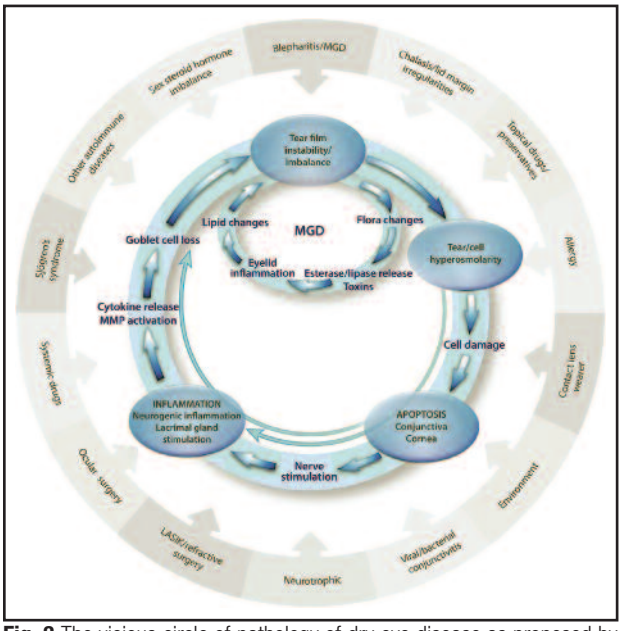

Fig. 3 The vicious circle of pathology of dry eye disease as proposed by Badouin (2016).[15]

measurement of the central palpebral conjunctival surface, while the TearLab® system takes samples peripherally. While looking at this question, it is important to consider a small single cohort from a focused demographic both geographically and pathologically may influence the results. Given that the patient base was from a referral dry eye clinic however, one would expect findings to skew to higher osmolarity measures rather than the lower shift noted in the results. The lower cut-off (290 mOsm/L) found in this study may be explained by the measuring method (*in vivo*) compared to previous studies (*ex vivo*).

The purpose of this paper is to consider a hypothesis based on homeostasis of the ocular surface as it relates to human reference fluid, namely blood plasma. Human plasma has an osmolarity range from 275-295 mOsm/L and the osmolarity of human serum has been reported to be 289 mOsm/L (281-297 mOsm/L).[9,10] There is a direct link between blood (plasma and serum) osmolarity and tear film osmolarity as observed in a recent publication which studied the effect of hemodialysis on tear osmolarity. It was observed that the tear osmolarity decreased in a statistically significant manner (p=0.0001) from 314 to 301 mOsm/L from before to immediately after hemodialysis.[9,11]

Tear hyperosmolarity has been shown to trigger a breakdown in ocular surface homeostasis at levels above 300 mOsm/L. The method of collection and testing for many of the aforementioned studies however uses the TearLab® system, which relies on an aliquot of 50 ηL from the tear meniscus which is then transferred to a testing chip *ex vivo*. This being the first study examining findings from osmolarity measures taken *in vivo*, it is possible that these measures are a closer representation of ocular surface osmolarity due to the elimination of variables like sample transfer, temperature variations, location of sampling, sample volume and humidity variations, which are factors using *ex vivo* systems. *Ex vivo* systems like TearLab® have been critiqued mainly due to high variability of the readings that could be instrument and/or sampling technique dependent.[12,13]

Corneal nociceptors, particularly those involved in sensing evaporation and hyperosmolarity have a pivotal role to play in the homeostasis of the LFU. The cornea contains three basic types of nociceptors which include mechano-receptors, polymodal receptors and cold receptors. Polymodal receptors make up 70%, and are stimulated by mechanical forces, pH, osmolarity and heat.[14] A plausible question is at what threshold do the polymodal nociceptors signal the dry eye alarm as postulated by Rosenthal?[14] Considering the human blood plasma/serum range of 275-297 mOsm/L, it may be that triggering of this alarm occurs when the ocular surface osmolarity differs sufficiently from serum osmolarity so as to signal apoptotic stress. The trigger of hyperosmolar stress could vary from person to person depending on the difference between blood and tear osmolarity. Certainly, further

studies would be needed to draw this conclusion, however this theory could explain the range in hyperosmolarity we see in prospective studies and meta-analyses such as Lemp and Tomlinson.[4,5]

Continuing with this hypothesis, the observed cut-off between normal and mild DED noted in this study's cohort of 290 mOsm/L may be lower due to the lack of variables with the i-Pen® *in vivo* method of measurement and therefore being more representative of true ocular surface osmolarity. With sensitivity of 91.8% and specificity of 71.4%, it would seem that this lower threshold is in fact reasonable and can be explained by current understanding of the interaction between ocular surface osmolarity and corneal nociceptor stimuli. What is consistent with previous studies is that the inter-eye osmolarity variability has significant sensitivity at 84.9% independent of average eye osmolarity. In line with polymodal nociceptor stimulation, if triggering the dry eye alarm requires an osmolar point-of-reference, then the inter-eye difference in osmolarity could also serve as that reference.

In anticipation of the DEWS II report, a newer understanding of the immunophysiology and biochemistry of DED are evolving. Badouin has postulated his 'Vicious Circle' theory (Fig. 3), in which we start to see the cyclical nature of dry eye, rather than the conventional Aqueos Deficient Dry Eye (ADDE) versus Evaporative Dry Eye (EDE) binary pathways in the past.[15] What remains constant however is the role of tear and cell hyperosmolarity as an influencer in this vicious circle which drives the cycle forward.

The findings of this report warrant further multicenter trials to shed a new light on this familiar metric. This paper does not in any way suggest that the data results should become a new standard, but rather a signal to study the unifying measure of ocular surface osmolarity sufficiently and with a wider lens, using this new *in vivo* technique, perhaps evolving what has previously been accepted as fact. ❑

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