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Differences of tear film osmolarity between two time-points of the day in healthy subjects

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Abstract
Purpose: *Tear film hyperosmolarity is considered one the core mechanism of the dry eye along with the tear film stability. Many tear physiological variables oscillate during the day. This study was designed to assess the differences in tear film osmolarity between morning and afternoon in a group of healthy subjects.*
Material and methods: *A total of 25 healthy subjects who fulfilled the study's inclusion criteria were enrolled for the study. Tear osmolarity was measured using the TearLab™ system in two separated sessions, at 9.30 am and 6.30 pm. A paired* t-test *and a Bland–Altman test were used to assess the differences between sessions.*
Results: *Tear osmolarity (mean ± SD) was 309.96 ± 9.00 and 296.48 ± 12.98 mOsm/l at 9.30 am and 6.30 pm, respectively, being significantly lower at 6.30 pm than at 9.30 am (mean difference ± SD = 13.48 ± 8.69 mOsm/l; paired* t-test; $p < 0.001$*).*
Conclusions: *Tear film osmolarity does appear to have some influence by the time of day in healthy patients.*

Keywords: *dry eye disease, osmolarity diurnal variations, tear film osmolarity, TearLab*

Introduction

Dry eye disease (DED) has recently been redefined by the Dry Eye Workshop II (DEWS II) as a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage and neurosensory abnormalities play aetiological roles.[1-4] Similar to the original DEWS report in 2007,[5-7] the DEWS II report reaffirmed that tear film instability and increased tear osmolarity are key mechanisms in DED, regardless of the underlying aetiology.[4,8] The inclusion of "homeostasis" in the new definition emphasizes that DED is not caused by any single factor but rather a fine balance of many different systems working in concert. It has been proposed that on normal or healthy subjects the tear film osmolarity value is near to 300 mOsm/l, while reaches to values up to 325 to 340 mOsm/l or

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higher on abnormal or dry eye subjects.[9-11] Thus, osmolarity measurement has been proposed as the gold standard in the dry eye diagnosis, being an easy and useful way to capture in a single parameter the status of the tear film status.[12]

Many physiological tear film and ocular surface variables change along the day, such as the corneal sensitivity, the tear pH or the tear film volume in the meniscus.[13-15] The possibility of diurnal variations in tear film parameter should be considered by the clinician, since the time of day tear film measurements are made can influence or be critical for a right diagnosis. A hallmark of DED is an unstable tear film, which is associated with variability in objective measures of sign and symptoms on this disease.[4,16,17] While repeated measurements over a period of time have been shown to be low and stable in normal subjects, DED subjects showed relatively elevated and unstable readings.[18-20] Indeed, the variability of osmolarity should be considered as an indication of the loss of tear film homeostasis that occurs with DED,[21] being recommended as a feature that clinicians should specifically be looking at diagnosis.[22] The aim of this study was to assess differences of tear film osmolarity between two time-points of the day, morning and afternoon, in a group of young healthy subjects.

Material and methods

Sample

A total of 25 participants (10 men, 15 women, mean age 21.5 ± 2.72 years), who fulfilled the study's inclusion established on a previous report,[23] were recruited from students and subjects attending the Optometry Clinic of the Optometry Faculty (USC, Spain). Subjects were excluded if they had a history of the conjunctival, scleral or corneal disease, prior eye surgery, glaucoma, diabetes mellitus, a thyroid disorder or wore contact lenses. Qualifying subjects were also administered a battery of dry eye tests (OSDI and McMonnies questionnaires, Schirmer test, phenol red test, tear meniscus height [TMH] and corneal staining) to rule out DED. Cut-off criteria were set at a score <13 for OSDI,[24] a score <10 for McMonnies,[25] >14.5 mm for both the Schirmer I test without anaesthesia and phenol red test,[26,27] a corneal staining grade ≤1 on the Oxford Grading Scale[28] and a central TMH without fluorescein ≥0.20 mm.[29,30] Subjects were excluded if they failed to fulfill more than two of these six inclusion criteria.[23] No participant was under any type of medication or used artificial tears at the time of the study. The study protocol was adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Santiago de Compostela.

Experimental procedure

Tear film osmolarity was measured using the TearLab™ (TearLab, San Diego, CA, USA).[31-35] During all protocols, the instrument and test cards used for both study parts were kept in the same humidity- and temperature-controlled room.[19] Quality control electronic check cards provided by the manufacturer was performed daily to verify the correct status of the system according to the given specifications (if reading was 334 ± 3, the pen was working correctly). In all procedures, the same test card lot number was used.

Participants were seated with the chin tilted upward and eyes directed towards the ceiling. The first eye to be measured was randomly selected. The instrument probe (housing the disposable microchip) was then placed on the lower tear meniscus until a beep is emitted indicating the tear sample has been collected. Measurements are directly made on the tear meniscus using the probe, which takes up the sample through capillary action. Only a 0.05-µl tear sample is needed. The TearLab converts the electrical impedance of the sample into osmolarity (mOsm/l), which is displayed on the device screen. Device measurement range goes from 275 to 400 mOsm/l. Measurements were performed in two separate sessions, at 9.30 am and 6.30 pm.[23] Only the right eye was examined because of induced excess tearing in the second eye and to avoid overstating the precision of statistical estimates.[36] Throughout the study, laboratory conditions of temperature, light and humidity were kept constant (temperature 20-23°C, humidity 50-60%).

Statistical analysis

SPSS statistical software, v. 19.0 for Windows (SPSS Inc., Chicago, IL), was used for data analysis. Significance was set at a $p \leq 0.05$ for all the analyses. Previous to analysis, the normal distribution of the data was checked using the Kolmogorov–Smirnov test; osmolarity data for both sessions data showed a normal distribution (both $p \geq 0.153$);[37] hence, parametric tests were used.

Bland–Altman procedures were used[38] to compare intra-day differences in osmolarity obtained in each patient's eye on both sessions. Those differences

Table 1. Descriptive statistics, differences (paired t-test) and 95% CI between measurements recorded in the two sessions

Session	Mean ± SD	Mean difference ± SD	*p*	95% LoA	
				Minimum	Maximum
9.30 am	309.96 ± 9.00	13.48 ± 8.69	0.001	−3.55	+30.51
6.30 pm	296.48 ± 12.98				

All data expressed on mOsm/l. $n = 25$
95% CI: 95% confidence interval; 95% LoA: 95% limits of agreement; SD: standard deviation

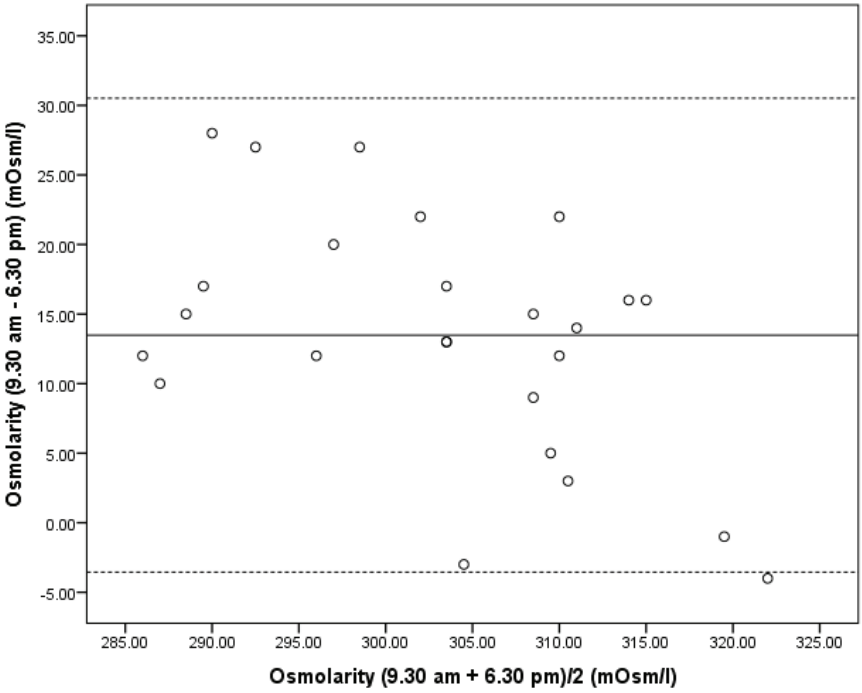

Fig. 1. Mean versus differences between the values obtained in the two sessions (9.30 am vs. 6.30 pm). The solid line indicates mean difference and dashed lines indicate the 95% LoA (Mean difference ± 1.96 × SD differences). SD = standard deviation. n = **25.**

between both osmolarity measurement sessions were assessed using a paired *t*-test for related samples. Also, 95% limits of agreements (LoA) were calculated (mean difference ± 1.96 × SD differences). In addition, a Bland–Altman plot representing averages versus differences was generated.

Results

Tear osmolarity (mean ± SD) was 309.96 ± 9.00 mOsm/l (values from 292 to 323) and 296.48 ± 12.98 mOsm/l (values from 276 to 324) at 9.30 am and 6.30 pm, respectively (Table 1). Results were significantly lower at 6.30 pm than at 9.30 am (paired *t*-test; $p < 0.001$), indicating better tear film quality in the afternoon than in the morning on healthy subjects (Table 1).

Figure 1 provides a Bland–Altman plot of means against the differences between the osmolarity values obtained in each time-point. As could be seen, dots were spread and there a was wide bias according to the 95% confidence

interval, showing high differences between the osmolarity values obtained in both morning and afternoon sessions.

Discussion

Diurnal variations in tear film variables have not been clearly established yet. The present findings indicate that tear film osmolarity does appear to be influenced by the time of day in healthy subjects: osmolarity readings indicated an improvement (lower osmolarity) in the afternoon (Fig. 1). As in previous reports,[39,40] two time-points were used (9.30 am-6.30 pm), which represent the start and the end of a normal work timetable in Spain.[40] In addition, it is important to note that the inclusion criterion for the present study was strict in order to use only really healthy patients.

Tear osmolarity is considered a global indicator of the DED.[9-11,41,42] Elevated tear osmolarity induces apoptosis, serve as a pro-inflammatory stress and reduce the ability of mucin-like molecules to lubricate the ocular surface, which can permanently damage the ocular surface.[41,43,44] It was reported that osmolarity is the single best marker of disease severity as an objective numerical measure for diagnosing, grading severity and managing treatment of DED.[12,45] However, to date, there is still controversy over the best cut-off for osmolarity between normal and DED subjects. Most studies have examined the threshold for DED diagnosis, and were recommended values that vary from 308 to 320 mOsm/l.[9-11,45] Using a cut-off of 312 mOsm/l, tear osmolarity have a 72.8% sensitivity and 92.0% specificity in separating DED from normal eyes.[9,45,46] While in the present study, a battery of specific dry eye diagnostic test was made as an inclusion criteria (OSDI, McMonnies, Schirmer, phenol red test, TMH and corneal staining).[24-30] It is important to note that the mean osmolarity in the first session was near to this cut-off criteria value (mean ± SD = 309.96 ± 9.00 mOsm/l). On the other hand, normal eyes tend to vary by ±7 mOsm/l, whereas DED can vary ≥11 mOsm/l between eyes or and tests but generally a difference of ≥8 mOsm/l between eyes indicates tear film instability.[4,9,16] In the present study, a mean difference ± SD between morning and afternoon of 13.48 ± 8.69 was found, higher than those diagnostic values. One reported reason for variability in tear osmolarity threshold values is tear film instability, a hallmark characteristic of the disease.[19,16] Indeed, the variability of osmolarity or increasing variation with increasing value is a statistical characteristic called heteroscedasticity and might be considered as a clinical indication of the loss of tear film homeostasis that occurs with dry eye.[4,16,19,21,45] It has been reported that consecutive measurements of the tear film osmolarity in short periods of time showed a lower variability, contrary to dry eye patients.[18,20] Tears of individuals with DED demonstrated increasing variation due to a combination of chaotic or incomplete mixing between blinks and spatially variable tear

film break-up, leading to a stochastically increased evaporation rate.[4,19]

In addition to the cut-off limitation or differences between measurements, diurnal variations of that parameter should be assessed and established in order to minimize possible diagnosis misleading. Previous studies have also used the TearLab osmometer to assess the osmolarity diurnal variation in healthy patients.[31-35] Some of those studies also reported no variations on tear film osmolarity along the day,[31,34,35] while other shows variations in some points of the day.[33] The same results were found in studies where osmometers based on freezing point depression were used, where variation[47,48] and no variation[40,49] was found between osmolarity measured at some different points of the day in healthy patients. Despite the little variations found in some measurement points on these studies, all of them concluded that osmolarity has a near to stable profile along the day in healthy subjects. In addition, although there is some controversy over diurnal tear film osmolarity, this variable has been observed to differ between healthy individuals and those pathological.[33,35] Also, as eye closure during sleep generates a hypoosmotic environment due to the reduction in tear film evaporation, production, and drainage, it has been hypothesized by previous authors that osmolarity is in its lower values upon eyelid opening.[37] Then, in the afternoon as the eye responds to the relative variations in the surrounding conditions that could enhance the evaporation process,[51] osmolarity rises to normal values.[31-35] Patients have reported that symptoms worsened over the day within 2 hours of getting up in the morning and at the end of the day, suggesting an environment- or task-related aetiology for dry eye symptoms.[52]

These differences between studies, both the daily variation and the relationship between healthy and pathological subjects, may reach from different error sources. The first one is the different criteria to choose the session day-time, with a wide range of day points from 6.00 am[49] to 7.00 pm.[40] The second could be the different devices or principles used in the different studies, while some studies have been reported a poor correlation between different principle osmometers.[53,54] Finally, the last source of error may be the different number of subjects in the studied groups (very small in some cases),[55] or the variations between age, sex or symptomatology. A larger study population, both healthy and pathological, may be required to detect a true daily osmolarity pattern and the differences between the tear osmolarity of dry eye subjects and that of healthy individuals.

In summary, while the osmolarity general profile follows a near to stable pattern along the day, tear film osmolarity does appear to be influenced by the time of day in healthy patients.

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