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Analysis of basal and reflex human tear osmolarity in normal subjects: assessment of tear osmolarity

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Abstract

Purpose: The aim of this study is to evaluate the difference between reflex and basal tear osmolarity among healthy normal subjects.

Method: The right eyes of 20 healthy normal male subjects aged 20 to 40 years were recruited for this study. The inclusion criteria for the subjects were the Ocular Surface Disease Index questionnaire score of less than 12 and wetting length of the phenol red thread of more than 10 mm. Tear film osmolarity was assessed using TearLab osmometer. Basic tear osmolarity was measured normally without inducing any irritation to the eye. In order to stimulate reflex tear, subjects were asked to open their eye as long as they can till they feel ocular surface irritation (minimum 20 s).

Results: The mean score on the Ocular Surface Disease Index questionnaire was 5.5 ± 3. The mean value obtained from the phenol red thread was 21 ± 4.5 mm. There were no statistically significant differences between the osmolarity readings of basal and reflex tear osmolarity ($p > 0.05$). The mean value was 308 ± 12 and 306 ± 9 mOsm/l for basic and reflex tear osmolarity, respectively.

Conclusion: This study found that the osmolarity of the basal and reflex tears fell within the same range. The values found in this study are in agreement with published results for normal subjects.

Keywords: Osmolarity, Tear Film, TearLab Osmometer

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Introduction

Tear film is the outer most layer of the eye that provides lubrication, nutrition, and protection to the ocular surface. Adequate production and spreading of the tears are essential for tear film layer stability and ocular surface health.[1] Tear osmolarity is an index of normal tear film dynamic that provides a value of the balance of tear input and output.[2] It has been reported that tear osmolarity could provide a powerful tool for detecting dry eye syndrome.[3]

Tear hyperosmolarity and instability are believed to be the core factors in the mechanism of development of signs and symptoms of dry eye.[4] Tear hyperosmolarity has been shown to increase expression and production of pro-inflammatory cytokines and chemokines in the tears that stimulate ocular surface inflammation.[5] These ocular inflammatory events could lead to corneal epithelial cell death, goblet cell loss, and mucin expression defect.[6,7]

Different techniques have been used to measure osmolarity of tear sample. Colligative properties of tear sample such as freezing point depression and vapor pressure have been measured to estimate the osmolarity value of tear sample.[8,9] These techniques depend on the number of dissolved particles in the solution.

An electrical impedance spectroscopy technique has been used to measure tear film osmolarity. The concentration of ions of biological fluids

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plays an important role in the conductivity of fluids.[10] As fluids can be characterized by their ionic content, a change in composition and concentration within a fluid can change the electrical conductivity of fluids and consequently alter fluid osmolarity.[11] TearLab osmometer has been developed to measure tear film osmolarity using electrical impedance spectroscopy technique. This new technique required a very small sample (20 nL) in order to calculate tear osmolarity.[12] It provides rapid and reliable measurements for tear osmolarity in daily practice.[13]

Previous study has shown a significant correlation between tear osmolarity readings measured by the TearLab system and Clifton osmometer.[12] However, lack of agreement between these two techniques has also been reported. It has been found that that mean tear osmolarity values measured using TearLab osmometer were higher (305 mOsm/l) than those with the freezing point osmometer (293 mOsm/l).[14] Disagreement between these studies could be due to the fact that calibration of impedance osmometry is complicated.[15]

Variation in tear osmolarity due to different factors such as age,[16] diabetes mellitus,[17] dry eye, and other ocular disorders has been widely investigated.[18] It has been shown that dry eye could be diagnosed with a sensitivity of 81% when a cutoff value of 316 mOsm/l was used.[19] However, to date, the difference between basic and reflex tear osmolarity has received little or no attention.

Electrolytes of the aqueous layer of tear film play an important role in the process of determining tear film osmolarity.[20] Variation in concentration of electrolytes in tear film with variation the tear flow has been well-documented.[21] Therefore, it would be expected to find an alteration in tear osmolarity values with changing tear flow rate that resulted by reflex lacrimation. In this study, the effect of inducing reflex tearing on tear osmolarity measurement was assessed. The aim of this study was to determine whether a difference exists between basic and reflex tear osmolarity in normal subjects.

Methods

This study required a single visit for comparison both basal and reflex tear secretion. Ethical approval was obtained from College of Applied Medical Sciences Ethics Committee (CAMS 110-37/38). All subjects were asked to sign a written consent form after explanation of the procedures of the study. The study was conducted according to the Declaration of Helsinki ethical principles. All tear parameters' assessments were carried out at the cornea and tear film research center at the College of Applied Medical Sciences, King Saud University.

A total of 20 normal healthy male subjects (mean age, 25.0 ± 7.0 years) were recruited for this study. Inclusion criteria were the Ocular Surface Disease Index (OSDI) questionnaire with a score of less than 12 and phenol red thread (PRT) wetting length of more than 10 mm. Tear osmolarity was evaluated using TearLab osmometer (OcuSense, Inc, San Diego, CA, USA). Tear samples were collected from the lower-temporal tear meniscus.

Basic tear film osmolarity was assessed normally without inducing any irritation to the eye. In order to stimulate reflex tear, subjects were asked to open their eye continuously without blinking (for minimum 20 s) until they experienced severe irritation to their ocular surface and start tearing excessively. Once the watering eyes due to excessive reflex tearing is observed, the measurement of tear osmolarity was then taken for reflex tears secreted by the subjects.

Normality was tested using the Kolmogorov–Smirnov test. Paired sample t test was used to explore the difference between basal and reflex tears' osmolarity measurements.

Results

Table 1 shows the mean ± SD of reflex and basal tear osmolarity values in addition to PRT and OSDI measurements. The mean ± SD score of the OSDI questionnaire was 5.5 ± 3 and the mean ± SD values obtained from the PRT were 21 ± 4.5 mm.

The mean tear osmolarity was 308 ± 12 mOsm/l for basic tears and 306 ± 9 mOsm/l for reflex tears (Figure 1). Statistical analysis showed that there is no statistical difference between basal and reflex tear osmolarity ($p > 0.05$).

Discussion

The aim of this study was to investigate whether any difference in tear osmolarity between basic

and reflex tears exists. The results of this study showed that osmolarity readings for both basic and reflex tears fall within the same range. Evidence from large epidemiological studies indicates that female sex increases the risk for dry eye due to hormonal changes.[22] Therefore, only male subjects were recruited for this study.

Tear osmolarity has been widely investigated to find out the normal range of osmolarity. It has been reported that the normal range varies between 293 and 318 mOsm/l.[1,23] Diurnal pattern of tear osmolarity has been studied. It has been found that mean tear osmolarity upon awakening was significantly lower (269 mOsm/l) compared with the reported normal range.[24] The same study has reported no significant difference between osmolarity measurements taken at different time points up to 8 h after awakening.[24] Contrarily, in another study, lower tear film osmolarity readings were recorded for normal subjects when the sample of tear collected afternoon was compared with morning measurements.[25] However, the same study found no significant diurnal variation in tear osmolarity among dry eye subjects.[25]

Table 1. Mean and standard deviation of tear parameters measured before and after inducing ocular surface irritation.

	Phenol red	OSDI	Osmo basic	Osmo reflex
Mean	21.30	5.52	307.80	306.35
Average	4.57	3.33	12.38	9.37

OSDI, Ocular Surface Disease Index.

There are many suggested a cut of values between normal and high osmolarity. Lemp and colleagues[26] have suggested that the most sensitive cutoff value between normal and hyperosmolarity is 308 mOsm/l, whereas the most specific was 315 mOsm/l. A cutoff value of 316 mOsm/l between normal and high osmolarity was reported by Tomlinson and colleagues.[12] Versura and colleagues[27] have suggested a value 305 mOsm/l as a cut of value for dry eye, and values of 309 and 318 mOsm/l were selected for moderate and severe dry eye, respectively. However, the same study has found an overlap in osmolarity readings for all groups and dry eye subgroups (mild, moderate, and severe dry eye).[27] Also, a low discrimination ability of TearLab device between dry eye and control subjects was reported. In this study, the mean tear osmolarity was 308 and 306 mOsm/l for basal and reflex tears which is slightly higher than previously published result by Masmali and colleagues[28](299 ± 7.6 mOsm/l) and Lemp and colleagues[26] (300 ± 7.8 mOsm/l). Another study has shown that the average of tear osmolarity in normal subjects is 301 mOsm/l (298 and 304 mOsm/l).[19] A previous study has collected serial tear osmolarity samples separated by 15 min and found that the average tear osmolarity is 304 mOsm/l.[29]

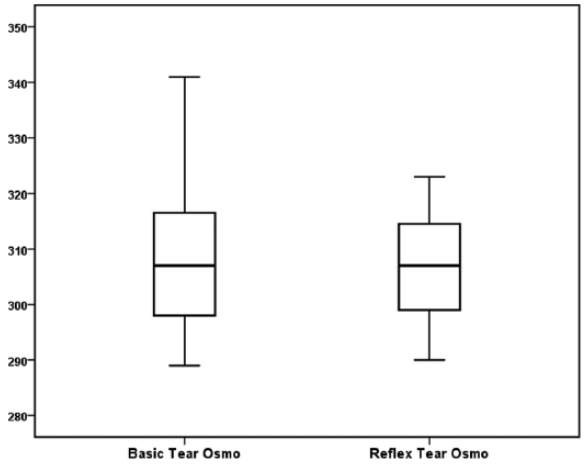

Figure 1. A box plot showing tear osmolarity (mOsm/l) measured with (reflex tear) and without (basal tear) ocular surface irritation. The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values. The line across the box indicates the median value.

Tear flow and evaporation are fundamental to dry eye and hyperosmolarity. Decreasing tear secretion and increasing evaporation decrease tear turnover rate and tear film thickness resulting in increased tear osmolarity.[30,31] Tear film hyperosmolarity could augment corneal nerve terminal impulses and act as an effective stimulus.[32]

Lacrimation process is essentially controlled by sensory stimulation of the cornea and conjunctiva. Ocular stimulations such as cooling and drying caused by tear evaporation are responsible for the basic tearing.[33] On the other hand, stronger stimulations of the anterior of the eye are required for reflex tearing.[33] Activation of sympathetic or parasympathetic nerves produces neurotransmitter that controls protein electrolytes and water secreted into the ocular surface by lacrimal gland. The parasympathetic neurotransmitters acetylcholine, vasoactive intestinal peptide (VIP), and sympathetic neurotransmitter norepinephrine played an important role in the regulation of tear secretion.[34] Several types of corneal and conjunctival afferents that regulate tear film production function have been identified. Reflex tear secretion is stimulated by activation the corneal polymodel nociceptors.[35,36] Less stimulation of tear secretion is found when corneal mechanoreceptors are activated. Activation of these two corneal afferents activates reflex tearing.[34] However, the neural mechanism for controlling and maintaining basal tear production is still undefined.

Understanding the mechanism responsible for regulating normal basal and reflex tears' production is very complex. Each type of tear secretion required different stimulations—a basic tear can be stimulated by stimulations such as cooling and drying of the ocular surface, whereas stronger stimulation of ocular and extraocular tissues is required to stimulate a reflex tear.[33,37] Secretion of water, electrolytes, and proteins from the lacrimal gland onto the ocular surface is controlled by neurotransmitters that are released from the stimulated parasympathetic and sympathetic.[38] The results in this study suggested that minimal irritation of ocular surface could not affect tear osmolarity measurement. Therefore, it does not matter whether basal or reflex tears are collected for the purpose of measuring tear film osmolarity.

The limitation of this study was the relatively small sample size due to the high cost of the disposable test chips. However, we were able to accomplish the goal of this study by taking 40 readings from normal healthy individuals.

Conclusion

This study has shown that short and minimal ocular irritation causes no effect on tear osmolarity. The current findings have suggested that no change in the concentration of tear electrolytes was resulted by inducing ocular irritation. A further study could assess the long-term effect of exposure to ocular irritation on tear osmolarity. Further research could also be conducted to determine the relationship between tear osmolarity and the concentration of tear electrolytes in basal and reflex tears.

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Compliance with ethical standards

All subjects were asked to sign a written consent form after explanation of the procedures of the study. Ethical approval was obtained from College of Applied Medical Sciences Ethics Committee. The study was conducted according to the Declaration of Helsinki ethical principles.

Conflict of interest statement

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References

1. Tomlinson A, Doane MG and McFadyen A. Inputs and outputs of the lacrimal system: review of production and evaporative loss. *Ocul Surf* 2009; 7: 186–198.
2. Stahl U, Willcox M and Stapleton F. Osmolality and tear film dynamics. *Clin Exp Optom* 2012; 95: 3–11.

3. Tomlinson A, Khanal S, Ramaesh K, *et al*. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci* 2006; 47: 4309–4315.
4. Lemp MA, Baudouin C, Baum J, *et al*. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the international Dry Eye WorkShop (2007). *Ocul Surf* 2007; 5: 75–92.
5. Yeh TN, Graham AD and Lin MC. Relationships among tear film stability, osmolarity, and dryness symptoms. *Optom Vis Sci* 2015; 92: e264–e272.
6. Argueso P, Balaram M, Spurr-Michaud S, *et al*. Decreased levels of the gel-forming mucin MUC5AC in tears of Sjogren's syndrome patients. *Invest Ophthalmol Vis Sci* 2001; 42: 2635.
7. Begley C, Liu H, Chen M, *et al*. A link between tear instability and hyperosmolarity in dry eye. *Invest Ophthalmol Vis Sci* 2008; 49: 1543.
8. Gilbard JP and Farris RL. Tear osmolarity and ocular surface disease in keratoconjunctivitis sicca. *Arch Ophthalmol* 1979; 97: 1642–1646.
9. Stahl U, Francis IC and Stapleton F. Prospective controlled study of vapor pressure tear osmolality and tear meniscus height in nasolacrimal duct obstruction. *Am J Ophthalmol* 2006; 141: 1051–1056.
10. Van Haeringen NJ. Clinical biochemistry of tears. *Surv Ophthalmol* 1981; 26: 84–96.
11. Ogasawara K, Mitsubayashi K, Tsuru T, *et al*. Electrical conductivity of tear fluid in healthy persons and keratoconjunctivitis sicca patients measured by a flexible conductimetric sensor. *Graefes Arch Clin Exp Ophthalmol* 1996; 234: 542–546.
12. Tomlinson A, McCann LC and Pearce EI. Comparison of human tear film osmolarity measured by electrical impedance and freezing point depression techniques. *Cornea* 2010; 29: 1036–1041.
13. Versura P and Campos EC. TearLab® osmolarity system for diagnosing dry eye. *Expert Rev Mol Diagn* 2013; 13: 119–129.
14. García N, Melvi G, Pinto-Fraga J, *et al*. Lack of agreement among electrical impedance and freezing-point osmometers. *Optom Vis Sci* 2016; 93: 482–487.
15. Benjamin WJ and Than TP. Impedance osmometry: standard solutions. *Invest Ophthalmol Vis Sci* 2012; 53: 562.
16. Craig JP and Tomlinson A. Effect of age on tear osmolality. *Optom Vis Sci* 1995; 72: 713–717.
17. Fuerst N, Langelier N, Massaro-Giordano M, *et al*. Tear osmolarity and dry eye symptoms in diabetics. *Clin Ophthalmol* 2014; 8: 507–515.
18. Szalai E, Berta A, Szekanecz Z, *et al*. Evaluation of tear osmolarity in non-Sjögren and Sjögren syndrome dry eye patients with the TearLab system. *Cornea* 2012; 31: 867–871.
19. Jacobi C, Jacobi A, Kruse FE, *et al*. Tear film osmolarity measurements in dry eye disease using electrical impedance technology. *Cornea* 2011; 30: 1289–1292.
20. Willcox MD, Argüeso P, Georgiev GA, *et al*. TFOS DEWS II tear film report. *Ocul Surf* 2017; 15: 366–403.
21. Johnson ME and Murphy PJ. Changes in the tear film and ocular surface from dry eye syndrome. *Prog Retin Eye Res* 2004; 23: 449–474.
22. Sriprasert I, Warren DW, Mircheff AK, *et al*. Dry eye in postmenopausal women: a hormonal disorder. *Menopause* 2016; 23: 343–351.
23. Gilbard JP, Farris RL and Santamaria J. Osmolarity of tear microvolumes in keratoconjunctivitis sicca. *Arch Ophthalmol* 1978; 96: 677–681.
24. Niimi J, Tan B, Chang J, *et al*. Diurnal pattern of tear osmolarity and its relationship to corneal thickness and deswelling. *Cornea* 2013; 32: 1305–1310.
25. Li M, Du C, Zhu D, *et al*. Daytime variations of tear osmolarity and tear meniscus volume. *Eye Contact Lens* 2012; 38: 282–287.
26. Lemp MA, Bron AJ, Baudouin C, *et al*. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol* 2011; 151: 792–798.e1.
27. Versura P, Profazio V and Campos E. Performance of tear osmolarity compared to previous diagnostic tests for dry eye diseases. *Curr Eye Res* 2010; 35: 553–564.
28. Masmali A, Alrabiah S, Alharbi A, *et al*. Investigation of tear osmolarity using the TearLab osmolarity system in normal adults in Saudi Arabia. *Eye Contact Lens* 2014; 40: 74–78.
29. Keech A, Senchyna M and Jones L. Impact of time between collection and collection method on human tear fluid osmolarity. *Curr Eye Res* 2013; 38: 428–436.
30. Khanal S, Tomlinson A and Diaper CJM. Tear physiology of aqueous deficiency and evaporative dry eye. *Optom Vis Sci* 2009; 86: 1235–1240.
31. Gaffney E, Tiffany J, Yokoi N, *et al*. A mass and solute balance model for tear volume and

osmolarity in the normal and the dry eye. *Prog Retin Eye Res* 2010; 29: 59–78.

32. Parra A, Gonzalez-Gonzalez O, Gallar J, *et al.* Tear fluid hyperosmolality increases nerve impulse activity of cold thermoreceptor endings of the cornea. *Pain* 2014; 155: 1481–1491.
33. Hirata H and Meng ID. Cold-sensitive corneal afferents respond to a variety of ocular stimuli central to tear production: implications for dry eye disease. *Invest Ophthalmol Vis Sci* 2010; 51: 3969–3976.
34. Dartt DA. Dysfunctional neural regulation of lacrimal gland secretion and its role in the pathogenesis of dry eye syndromes. *Ocul Surf* 2004; 2: 76–91.
35. Müller LJ, Marfurt CF, Kruse F, *et al.* Corneal nerves: structure, contents and function. *Exp Eye Res* 2003; 76: 521–542.
36. Acosta MC, Peral A, Luna C, *et al*. Tear secretion induced by selective stimulation of corneal and conjunctival sensory nerve fibers. *Invest Ophthalmol Vis Sci* 2004; 45: 2333–2336.
37. Meng ID and Kurose M. The role of corneal afferent neurons in regulating tears under normal and dry eye conditions. *Exp Eye Res* 2013; 117: 79–87.
38. Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. *Prog Retin Eye Res* 2009; 28: 155–177.