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Investigation of the repeatability of tear osmolarity using an I-PEN osmolarity device

Raied Fagehi[1]*, Abdulkareem B. Al-Bishry[1], Mana A. Alanazi[1], Ali Abusharha[1], Gamal A. El-Hiti[2], Ali M. Masmali[2]

Abstract:

PURPOSE: To investigate the repeatability of tear osmolarity in healthy Saudi subjects using an I-PEN osmolarity device.

MATERIALS AND METHODS: Thirty typical male subjects with healthy eyes (27.4 ± 4.9 years) participated in the study. Eye abnormalities were tested with a slit lamp, and eye comfort was determined with the surface disease index. Measurements of the tear break-up time and phenol red thread tests were used for as exclusion criteria. The tear osmolarity test, using an I-PEN osmolarity system, was performed three times in the right eye of each subject with a 5 min' gap between tests.

RESULTS: The average osmolarity test score was 303.8 ± 4.8 mOsm/L. Tear osmolarity measurements showed tear osmolarity of 280–299 mOsm/L, 300–309 mOsm/L, and 310–329 mOsm/L in 14 (46.7%), three (10%), and 13 (43.3%) subjects, respectively. Correlations among the three I-PEN measurements were significant (Spearman's correlation coefficient; r = 0.036, 0.501, and 0.603; P = 0.050, 0.006, and 0.001, respectively). The mean coefficient of variance among the three measurements was 4.4%.

CONCLUSION: The mean measurement of an I-PEN tear osmolarity was 303.8 ± 4.8 mOsm/L which is in agreement with the range of those reported for healthy subjects. The I-PEN is reliable and has the advantage of portability (hand-held) compared to the other osmolarity systems.

Keywords:

Dry eye, hyperosmolarity, I-PEN osmolarity system, phenol red thread test, repeatability

Introduction

Ocular tear film stability is vital for maintaining healthy eyes and ocular surfaces. Disturbances in the tear film lead to numerous vision problems in which eye dryness is the most common.[1] Eye dryness is associated with pain, inflammation, discomfort, and redness.[2] The tear film structure is complex, but primarily contains an outer lipid layer that covers an aqueous phase representing a bi-phasic structure rather than a tri-layered structure that contains lipid, aqueous and mucin phases.[2,3] The lipid layer plays an essential role in the stability of tear film. It spreads over the tear film during blinking, therefore preventing tear evaporation.[4,5] In addition to lipids, salts, proteins, and mucins play an essential role in maintaining the stability of tear film.

The stability of the tear film can be detected through the measurement of both the volume and quantity of tears. Various tests can be used for this purpose. However, correlations among such tests are weak.[6] Therefore, a combination of tests must be used to diagnose eye dryness.[7] The quantity of tears can be measured using phenol red

[1]*Department of Optometry,* [2]*Cornea Research Chair, Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

***Address for correspondence:**
Dr. Raied Fagehi, Department of Optometry, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia.
E-mail: rfagehi@ksu.edu.sa

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thread (PRT), Schirmer, tear miscues height tests, and tear ferning and tear osmolarity tests.[8-10] While the quality of tears can be measured using the noninvasive tear break-up time (NITBUT).[11,12] In addition, the ocular surface disease index (OSDI) sheet[13] and McMonnies dry eye index[14] can be used to detect the degree of discomfort felt by patients with dry eye.

The measurement of tear osmolarity indicates the balance between tear secretion, evaporation, absorption, and drainage;[15] and measures electrolytes concentration within the mucoaqueous phase. Vapor pressure osmometry and freezing point depression were used in the past to measure tear osmolarity.[16,17] The vapor pressure technique depends on the correlation between osmolarity and the reduction in evaporation point, while the freezing point technique depends on the correlation between osmolarity and the reduction in freezing point.[18] However, it is difficult to decide when the frozen tears melt, and such a technique requires a large quantity of tears.[19,20] Most recent techniques to measure tear osmolarity involve the use of TearLab (TearLab Corporation, Escondido, CA, USA) and I-PEN (I-MED Pharma Inc., Dollard-des-Ormeaux, QC, Canada) osmolarity systems.[12,21] The tear osmolarity test is considered one of the most accurate dry eye diagnosis method.[22] It involves the use of a small quantity of tears (50 nL) and does not trigger any reflex tears.[23] Hyperosmolarity is suggested to be the most common cause of tear film instability that leads to dry eye syndrome.[24] Hyperosmolarity causes an increase in tear film osmolarity due to excessive tears evaporation. Few studies have been conducted using TearLab and I-PEN osmolarity systems to measure tear osmolarity.[12,25-27]

The I-PEN osmolarity system can be used as a valid and reliable technique to measure tear osmolarity.[25] Therefore, this study investigated the repeatability of tear osmolarity measurements in healthy-eye Saudi subjects using the I-PEN osmolarity system. The I-PEN osmolarity system is mobile, easy to handle, simple to use, and efficient *in vivo* using a disposable single-use sensor.

Materials and Methods

Participants

Thirty normal male subjects with healthy eyes and a mean (± standard deviation) age of 27.4 ± 4.9 years completed the study. The age of all subjects ranged from 20 to 37 years; in which 33.3% of the subjects (n = 10) ranged from 20 to 25 years; 53.3% of the subjects (n = 16) ranged from 26 to 30 years, and 13.4% of the subjects (n = 4) were older than 30 years. A slit-lamp was used to examine abnormalities in the eyelids, and subjects having abnormalities, recent ocular surgery, contact lens wearers, smokers, and subjects with diabetes, anemia, and thyroid disorders were excluded from the study. In addition, subjects with a high body mass index (above 24.9 kg/m^2), a high blood cholesterol level (above 4 mmol/L), Vitamin A and D deficiencies, and hypertension were excluded.

All measurements were performed by the same examiner at the Optometry Clinics of the College of Applied Medical Sciences between 08:00 and 11:00 a.m. under controlled conditions in terms of temperature (23°C) and relative humidity (<40%).[28] The OSDI sheet was completed first by all subjects, followed by the NITBUT, PRT, and I-PEN tear osmolarity tests with a 5-min interval between each test.

Ethical approval was obtained from the Ethics Committee at the College of Applied Medical Sciences, King Saud University (approval number: CAMS-036-3940), the subjects were treated in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before the commencement of the research.

The ocular surface disease index sheet

The OSDI sheet was completed first by each subject, and a score of <13 was considered a healthy eye.[13]

The noninvasive tear break-up time test

The NITBUT test was performed on the right eye of each subject using EASYTEAR view+ (Easytear SRL, Rovereto, Trento, Italy) without fluorescein drops. White illumination was used to create the corneal reflection to confirm regular mires and grid on the ocular surface. Each subject was asked to blink once; then, the time between the blink and the appearance of mires and grid distortion was calculated. The test was performed three times, and the average score was calculated for each subject. The tear break-up time provides information about the status of the eye in which longer time is an indication of healthy eyes. Healthy and normal eyes have a tear break-up time of more than 10 s.[10]

The phenol red thread test

The PRT test was performed on the right of each subject using PRT stripes (Zone-Quick, Showa Yakuhin Kako Co., Ltd, Tokyo, Japan). Each subject was asked to gaze at a primary position, and a 3 mm fold of the PRT thread was inserted gently into the lower lid conjunctival sac (one-third of the distance from the lateral canthus). The thread was removed after 15 s, and the length of the red portion was measured in millimeters. The length of PRT thread wetted portion is a measure of tear volume and large readings are characteristic for normal and healthy eyes. Healthy and normal eyes have a PRT reading of more than 10 mm.[8]

The I-PEN tear osmolarity test

Tear osmolarity was performed using the I-PEN osmolarity system 5 min after the PRT test. The I-PEN osmolarity system was used far away from electronic devices to ensure the accuracy of the readings. Each subject was asked to close their eyelids for 30 s gently, and then, the disposable single-use sensor was softly contacted with the palpebral conjunctiva from the lower eyelid at a 30° angle. By design, after a few seconds, the I-PEN beeps and displays an osmolarity reading on the screen.[25] Tear osmolarity was measured three times in the right eye of each subject with 5-min intervals between measurements. Based on the I-PEN tear osmolarity measurements, subjects were classified as a healthy eye (<290 mOsm/L), minor dry eye (290–310 mOsm/L), mild dry eye (310–330 mOsm/L), and moderate dry eye (330–350 mOsm/L).

Statistical analyses

The data were collected using Excel (Microsoft Office 2016, Microsoft Corp., Redmond, WA, USA) and was analyzed using the SPSS statistical package for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The data were not normally distributed (Kolmogorov–Smirnov test; $P < 0.05$) for the scores from the OSDI, NITBUT, and PRT measurements. For the osmolarity test, the data were normally distributed (Kolmogorov–Smirnov test; $P > 0.05$). Therefore, parametric tests (one-way repeated-measure analysis of variance) were used to analyze the osmolarity measurements. In addition, the intraclass correlation coefficient test among the three osmolarity readings was applied. A correlation coefficient (Spearman's correlation coefficient; r) was used to study the relationship among parameters.[29]

Results

The median scores (median interquartile range) obtained from the OSDI, NITBUT, and PRT measurements were 8.3 (6.4), 12.3 (4.5) s, and 25.0 (7.5) mm, respectively. For the osmolarity test, the average score was 303.8 ± 4.8. The tear osmolarity readings ranged from 277 to 337 mOsm/L in which 14 subjects (46.7%) had tear osmolarity of 280–299 mOsm/L, three subjects (10%) had tear osmolarity of 300–309 mOsm/L, and 13 subjects (43.3%) had tear osmolarity of 310–329 mOsm/L. The averages obtained from the scores of OSDI sheet and tear film tests, including tear osmolarity are summarized in Table 1.

There was no statistically significant difference among the three I-PEN osmolarity readings (Friedman test; $P = 0.786$). However, the standard deviation was high for some readings in which the average coefficient of variation was 4.4%, and the cohort ranged from 1% to 9% [Figure 1]. The intraclass correlation coefficient (average measures) was 0.745. The

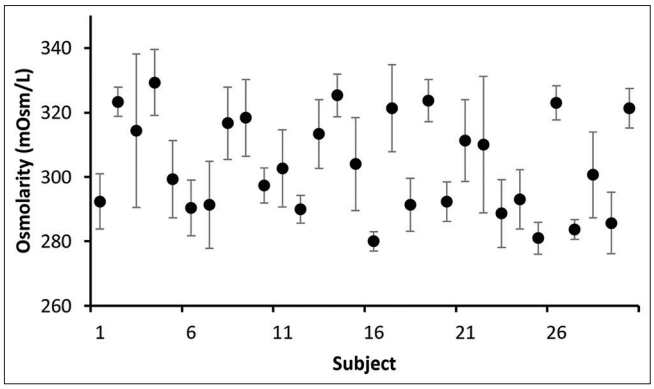

Figure 1: The mean ± standard deviation for the three I-PEN osmolarity measurements

Bland–Altman plots between three I-PEN measurements are shown in Figure 2. The difference between repeated measurements was to up to 40.7 mOsm/L. The correlations among the three tear osmolarity reading were strong ($r = 0.036$, 0.501, and 0.603; $P = 0.050$, 0.006, and 0.001, respectively). However, no correlations were found among the scores from the OSDI, NITBUT, PRT, and tear osmolarity measurements, expectedly, because each test detects a different parameter. The correlations between the scores obtained from different tests including OSDI are shown in Figure 3.

Discussion

Dry eye syndrome is a common problem that requires attention and immediate management to avoid damage of the ocular tear film. The diagnosis of eye dryness is achieved by a combination of conventional tests since no single test provides high accuracy. Tear hyperosmolarity is considered the primary cause of inflammation and discomfort among dry eye patients.[30] The measurement of tear film osmolarity is considered a gold standard for the diagnosis of dry eye.[31,32] The tear osmolarity test is superior to other dry eye diagenetic tests such as NITBUT and Schirmer tests.[23] The average tear osmolarity for a healthy-eye subject is 300.8 ± 7.8 mOsm/L based on measurements of 299 subjects (218 females and 81 males) using the TearLab osmolarity system.[23] *In vitro* measurements of tear osmolarity using electrical impedance is affected by temperature variation.[33] While the I-PEN or TearLab osmolarity systems are used only *in vivo* and therefore the temperature has no significant effect on tear osmolarity readings since the palpebral conjunctiva temperature remains steady at 36.2°C ± 0.6°C.[34] No significant differences were noted among the tear osmolarity readings measured at different times of day for 30 healthy controls.[35] In contrast, the tear osmolarity recorded on the TearLab osmolarity system among a small group of dry ($n = 10$) and healthy eye ($n = 10$) subjects differed by 21.9 ± 13.5 and

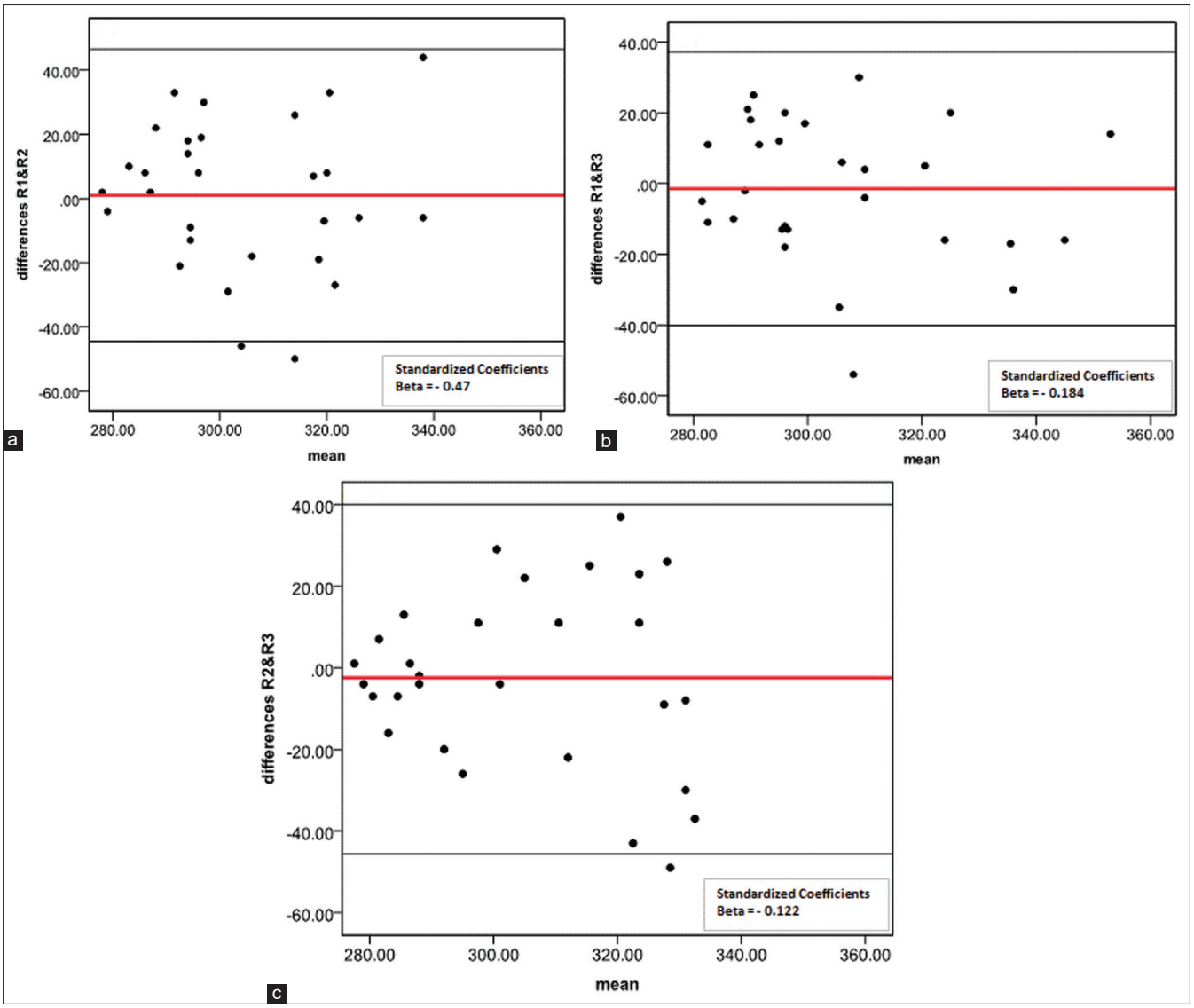

Figure 2: The Bland-Altman plots between: (a) first and second I-PEN measurements, (b) first and third I-PEN measurements, and (c) second and third I-PEN measurements

Table 1: The averages (mean±standard deviation or median (interquartile range)] for the ocular surface disease index, noninvasive tear break-up time, phenol red thread, and I-PEN tear osmolarity measurements

Test	Mean±SD or median (IQR)
Age (years)	27.4±4.9
OSDI	8.3 (6.4)
NITBUT (s)	12.3 (4.5)
PRT (mm)	25.0 (7.5)
Osmolarity (mOsm/L)	303.8±4.8

SD=Standard deviation, IQR=Interquartile range, OSDI=Ocular surface disease index, NITBUT=Noninvasive tear break-up time, PRT=Phenol red thread

21.0 ± 9.2 mOsm/L, respectively, over 8 h' period.[36] The average tear osmolarity for healthy and dry eye subjects was 298.0 ± 14.2 and 304.0 ± 10.8 mOsm/L, respectively.[36]

The reported average tear osmolarity measured using the I-PEN or TearLab osmolarity systems was 288.3–336.4 ± 7.6–22.0 mOsm/L.[12,25,37] In the current study, there were no significant differences among the three I-PEN readings. The average for the tear film osmolarity readings using the I-PEN osmolarity system was 303.8 ± 4.8 mOsm/L. That average was slightly higher than in some earlier studies and lower than others.[37] The I-PEN tear osmolarity from 65 readings recorded at 25.2°C ranged from 286.6 to 298.2 mOsm/L with a mean of 294.1 ± 2.3 mOsm/L and a coefficient of variation of 0.78% which is slightly lower compared to the mean (303.8 ± 4.8 mOsm/L) obtained for the current study.[25] In the current study, the difference between the repeated osmaolrity measurements was up to 40.7 mOsm/L, which is consistent with the literature.[27]

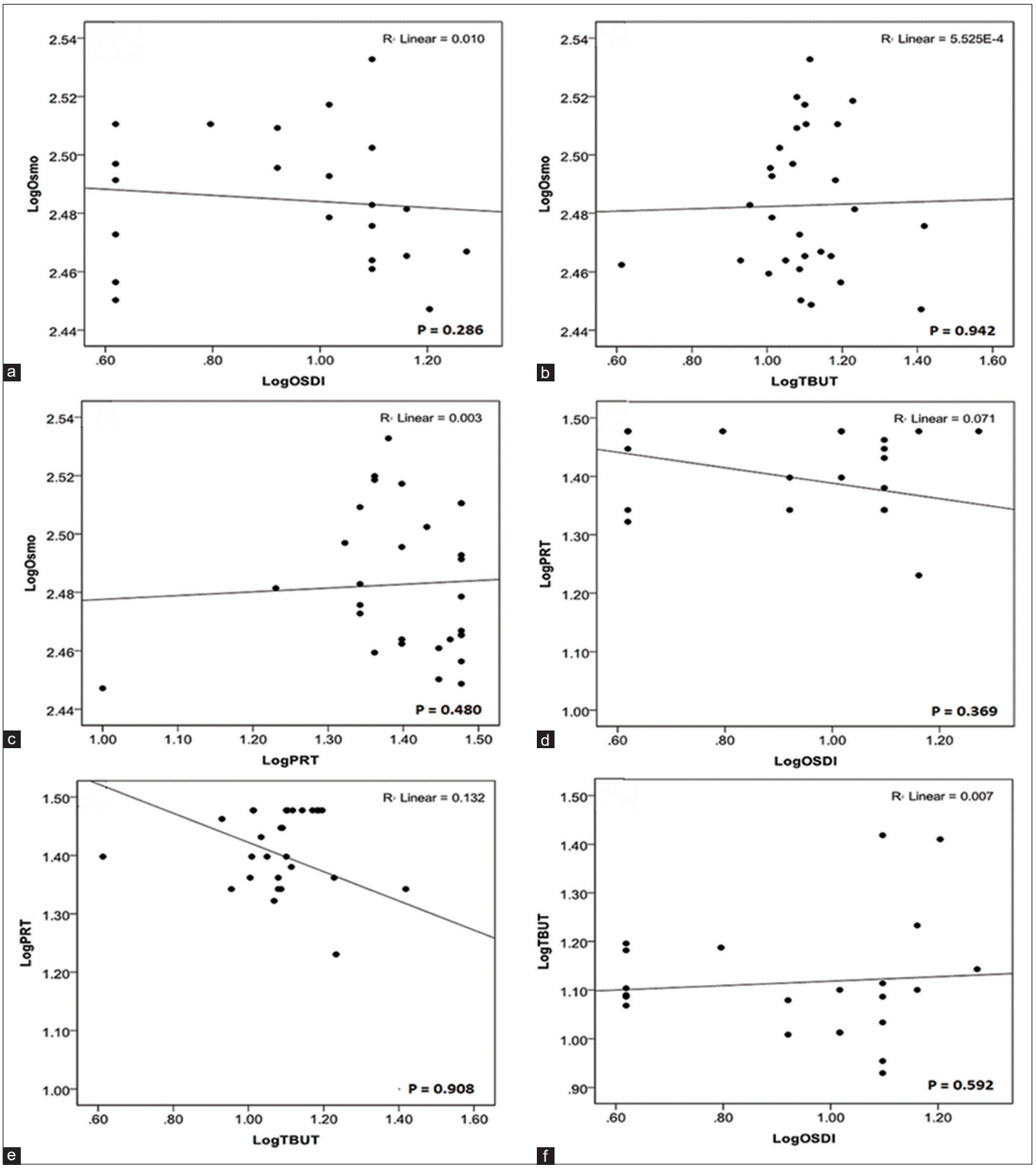

Figure 3: Correlations between (a) I-PEN and ocular surface disease index scores, (b) I-PEN and noninvasive tear break-up time scores, (c) I-PEN and phenol red thread scores, (d) phenol red thread and ocular surface disease index scores, (e) phenol red thread and noninvasive tear break-up time scores, and (f) noninvasive tear break-up time and ocular surface disease index scores

However, it is much higher (four-time) than that obtained using TearLab system.[10,27]

Tear osmolarity of constructed tear solutions from electrolytes and proteins that have different osmolarity (297 mOsm/L for healthy eye tears and 342 mOsm/L for dry eye tears) was measured using a vapor pressure osmometer, TearLab osmolarity, and I-PEN osmolarity systems.[26] The average tear osmolarity for healthy and dry eye tears using the

three devices was 305.6 ± 4.0 and 352.2 ± 5.5 mOsm/L, 300.6 ± 3.7 and 341.4 ± 7.9 mOsm/L, and 336.4 ± 21.5 and 342.0 ± 20.7 mOsm/L, respectively.[26] Both the vapor pressure osmometer and the TearLab osmolarity system showed exceptional consistency and accuracy. However, I-PEN was less accurate in measuring contrived tears that have a known osmolarity.[26] Another study was conducted among healthy eye subjects (n = 20) in which tear osmolarity was measured five times using TearLab and I-PEN osmolarity systems.[27] The average tear osmolarity using the I-PEN osmolarity system was higher (319.4 ± 20.3 mOsm/L) compared to the average obtained using the TearLab osmolarity system 295.4 ± 8.6 mOsm/L).[27] Again, TearLab osmolarity was accurate in identifying all the subjects having healthy eyes, while, I-PEN showed exceptionally low accuracy (15%).[27] The tear osmolarity measurements in 25 subjects using the TearLab osmolarity system was found to be higher (305.2 ± 16.1 mOsm/L) than those obtained using the Fiske 210 osmometer (293.4 ± 12.2 mOsm/L).[38]

Dry eye subjects tend to have higher tear osmolarity (312.0 ± 16.9 mOsm/L) compared to healthy eye subjects (305.6 ± 9.7 mOsm/L) when using the TearLab osmolarity system.[39] There was an association between higher tear osmolarity and discomfort, higher OSDI, and conjunctival staining scores.[39] The average tear osmolarity among those subjects with Sjögren syndrome dry eye (n = 39) using the TearLab osmolarity system was 311.1 ± 16.4 mOsm/L.[40] The tear osmolarity was collated positively with both OSDI (r = 0.405; P = 0.011) and the ocular staining score (r = 0.592; P < 0.001) and negatively with the Schirmer I test score (r = –0.625; P < 0.001).[40] The mean tear osmolarity using the TearLab osmolarity system was 296.8 ± 16.5 mOsm/L in non-Sjögren syndrome dry eye subjects, 303.4 ± 17.2 mOsm/L in Sjögren syndrome dry eye, and 303.5 ± 12.9 mOsm/L in healthy eye subjects.[41]

A study conducted among healthy (n = 14) and dry eye (n = 74) subjects using the TearLab osmolarity system showed that plasma osmolarity was higher than tear osmolarity.[42] For healthy eyes, the mean for plasma osmolarity and tear osmolarity was 288.3 ± 6.6 and 293.1 ± 2.8 mOsm/L, respectively.[42] However, for dry eye, the mean for plasma osmolarity and tear osmolarity was 288.5 ± 9.4 and 293.4 ± 5.1 mOsm/L, respectively.[42] There was no correlation between plasma osmolarity and tear osmolarity since they are independent. The tear osmolarity measured among 30 healthy eye subjects using the TearLab osmolarity system ranged from 277 to 312 mOsm/L with a mean of 299.1 ± 7.7 mOsm/L with 0.80% as a coefficient of variation.[12] The osmolarity readings have a significant (P = 0.018) positive medium correlation (r = 0.429) with the scores from McMonnies questionnaire and a significant (P = 0.001) strong negative correlation (r = –0.587) with the NITBUT scores.[12] On the other hand, there was no significant correlation (r = –0.067; P = 0.725) between the osmolarity readings and the PRT scores.[12] Various osmolarity systems can be used to detect eye dryness and have acceptable repeatability and accuracy. The current study has some limitations, such as the use of a small size sample of male-only subjects from Riyadh City.

Conclusion

The mean for the I-PEN tear osmolarity was 303.8 ± 4.8 mOsm/L which is in agreement with the range of those reported for healthy subjects. The I-PEN osmolarity system is reliable and had the advantage portability (hand-held) compared to the other osmolarity system.

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Nil.

Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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