RESEARCH ARTICLE   Open Access

Dry eye disease in type 2 diabetes mellitus; comparison of the tear osmolarity test with other common diagnostic tests: a diagnostic accuracy study using STARD standard

Laily Najafi[1], Mojtaba Malek[1], Ameneh Ebrahim Valojerdi[1], Mohammad E Khamseh[1*] and Hossein Aghaei[2]

Abstract

Background: To determine the diagnostic performance of tear osmolarity in diagnosis of dry eye disease by using tear lab osmolarity system in people with type 2 diabetes, and to compare it with common diagnostic tests already available in clinical practice.

Methods: Two hundreds forty three people with type 2 diabetes were included. Tear osmolarity was measured with the tear osmolarity system. The 308 mOsm/L cutoff was used to diagnose dry eye disease. The following tests were also performed: Ocular Surface Disease Index (OSDI) questionnaire, Tear Film Break up Time (TFBUT), Schirmer I test, Rose Bengal and Fluorescein staining. The results of these tests were compared to the tear osmolarity measurement.

Results: The prevalence of dry eye disease detected by the tear osmolarity test was 27.7%. It was as follows for the other common diagnostic tests: OSDI (17.7%), Schirmer I test (33%), TFBUT (41%), Rose Bengal (11%), and Fluorescein staining (4%). Fluorescein staining had the highest specificity (97%). With the cutoff score >12, the positive likelihood ratio for the OSDI questionnaire was the highest (1.78). The sensitivity was poor for all common diagnostic tests. ROC curve analysis could not determine optimal cut offs for the common diagnostic tests.

Conclusions: The available common diagnostic tests underestimate the presence of dry eye disease in people with type 2 diabetes. Moreover, they could not discriminate tear hyperosmolarity from normal. Tear osmolarity could be considered as the best single test for detection of dry eye disease in people with type2 diabetes.

Keywords: Diabetes mellitus, Dry eye disease, Diagnostic accuracy Study, STARD

Introduction

In adult population, dry eye disease (DED) is a common ocular disease and has been recognized as an important public health problem in recent years [1-3].

Although our knowledge about its pathogenesis, classification, and characteristics has grown considerably over the past decade, there are still debates on diagnostic approach. This is mainly due to the lack of objective tests with sufficient sensitivity and specificity that could be applied easily in routine clinical care settings and at the same time be adequately reproducible [4,5].

Schirmer I test, tear film break-up time (TFBUT) analysis, Rose Bengal and fluorescein staining are the most well-known objective tests used for diagnosis of DED [6]. However, neither symptoms nor signs always match with the results of these tests [7].

Tear osmolarity measurement by freezing point depression technique has been proposed as the gold standard test for diagnosis of DED [8], however, the problems associated with the existing technologies hindered its use in clinical practice [9]. Moreover, it is costly, time-consuming; and requires tear volumes much higher than those collectable in several forms of dry eye disease, or inducing excessive reflex tearing during tear sampling.

* Correspondence: khamseh.m@iums.ac.ir
[1]Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences (IUMS), Firouzgar alley, Valadi St., Behafarin St., Karimkhan Ave., Vali- Asr Sq., Tehran 15937-48711, Iran
Full list of author information is available at the end of the article

Najafi et al. Journal of Diabetes & Metabolic Disorders (2015) 14:39

Page 2 of 8

Osmolarity microchip system appears to overcome these barriers. It measures tear osmolarity based on electrical impedance technique and the results correlate well with the gold standard freezing point depression technique [9].

Several previous studies, investigated the relationship between diabetes and DED [10]. It has shown that composition of tear proteins in people with diabetes is different from healthy subjects [11]. In diabetes mellitus corneal and conjunctival epithelial alterations, persistent epithelial defects, and potential visual impairment due to corneal scaring have been observed [12-18]. Damage to the microvasculature of the lacrimal glands accompanied with autonomic neuropathy could impair lacrimation in long standing diabetes [19].

The purpose of the present work was to determine the diagnostic performance of tear osmolarity test; used to diagnose DED in type 2 diabetes mellitus using tear lab osmolarity system as the reference standard and to compare it with the other diagnostic tests (index tests) already in use, specifically Ocular Surface Disease Index (OSDI) questionnaire, Schirmer I test, TFBUT, Rose Bengal and fluorescein staining.

Methods

We prospectively enrolled 243 consecutive diabetic patients at the Institute of endocrinology and metabolism between August 2011 and November 2012.

Exclusion criteria include use of medications or history of any other ocular or systemic disease that can affect tear production or quality, history of anterior segment surgery, Keratorefractive procedures (LASIK, LASEK, PRK) within one year prior to enrollment, trauma, contact lens wear, incomplete lid closure, entropion, ectropion, nasolachrymal drainage obstruction, punctual plugs placement, or cauterization; ocular allergy, glaucoma, pregnancy or lactation, and use of ocular medications or nutritional tear supplements.

All of the participants underwent a general physical examination and a thorough ophthalmologic exam. The visual acuity of both eyes was tested using Snellen's chart. Both eyes were examined first using the board beam of the slit lamp to know the condition of the ocular surface and adenexa, observing the tear film meniscus, tear film, corneal changes, conjunctival changes, and eyelids. They were also clinically evaluated with direct and indirect ophthalmoscopy to know the status of retina. The study population underwent tear osmolarity test (standard test) before the Ocular Surface Disease Index (OSDI) questionnaire, Schirmer I test, Rose Bengal and fluorescein staining, and tear film break-up time test (TFBUT) (index tests); all were performed on the same day. All of the tests were performed according to the randomization table for one eye.

OSDI questionnaire was administrated to the participants by a research associate trained by an expert ophthalmologist to score the questions and to follow the ambiguous information. Schirmer I test, Rose Bengal, fluorescein staining, TFBUT were performed for all of the participants by an expert ophthalmologist in consecutive sessions. Tear osmolarity test was done by a single specialist. The investigators were blinded to the patients' history and the obtained information.

To avoid diagnostic error, all of the examinations were performed in the same physical condition and in the morning to standardize the tests and to avoid possible diurnal variation. Assessments were made in a room controlled for enlightment (dim light), temperature, humidity, and airflow, to avoid ocular surface stress. The tests that need slit lamp were performed in a darkened room with the same slit lamp and by the same physician.

Tear osmolarity was measured using tear lab osmolarity system, (BON Co. Germany). The Tear lab instrument is based on a lab- on – a- chip technology working as both a collection device and an analytical system, in absence of any chemical reagent. This avoids the need for a capillary tube or absorbing acetate disc. The equipment consists of single use test cards containing microchannels to collect tear fluid, held by a pen designed to facilitate tear collection, and a portable reader unit which elaborates and displays the osmolarity results. A tear sample, approximately 50 nl, was collected from the inferior lateral tear meniscus of the ocular surface. To facilitate tear collection, patients were asked to position their head laterally for a few seconds before approaching the tip of the test card microchip: in this way, tears were driven laterally and collection made easier. Subjects had been requested not to wear makeup on their eyelids. Quality control procedures were applied at the beginning of each day of patient testing by using reusable electronic check cards (provided by the manufacture as a procedural quality control) to confirm the function and calibration of the TearLab osmolarity system. The 308 mOsm/L cutoff was used to diagnose DED [20]. At this diagnostic cutoff, osmolarity was found to have 88% specificity, 75% sensitivity in mild/moderate disease and 95% sensitivity in severe disease [20].The very rapid acquisition of tear samples by the TearLab would be less likely to be influenced by evaporation.

The OSDI questionnaire has a Likert design and assesses frequency of ocular subjective symptoms (soreness, blurred vision), difficulty with vision-related function (television, visual display unit, driving, reading) and discomfort due to environmental triggers (low humidity, high wind). The patients answer 12 questions, with higher scores representing greater disability [21].

Subjective symptoms of dry eye were graded on the basis of dry eye discomfort symptoms questionnaire

Najafi et al. Journal of Diabetes & Metabolic Disorders (2015) 14:39

Page 3 of 8

(OSDI) [9]. The score range was from 0–12 (no disability), 13–22 (light dry eye), to 23–32 (moderate dry eye), and 33–100 (sever dry eye) [9].

Schirmer I test and TFBUT were carried out as outlined in the DEWS report [4].

The Schirmer test is an invasive and indirect method to measure change in volume of the tears in the tear reservoir. This test involves insertion of a wick into the lower conjunctival sac and measurement of the wetting length over a set period of time. The Schirmer test uses filter papers to assess tear production. There are two commonly used variations of the Schirmer test: Schirmer I measures total tear secretion (reflex and basal tears) and Schirmer II utilizes anesthetic to measure basal secretions, although this has not been validated [22-24].

The strip was folded at the notch and placed at the junction of the middle and lateral thirds of the lower eyelids and allowed there to stay in place for 5 minutes [25].

A value of less than 5 mm wetting in 5 minutes is considered abnormal, more than 10 mm per 5′ seconds as normal and 6-10 mm per 5′seconds as borderline [4]. Medial and lateral placements of the paper have been described, as well as having the patient looking up, but no method has been deemed more reliable [26].

TFBUT is the standard test for estimating tear film stability. The results are explained as seconds. Patients with break-up time of more than ten seconds was consider as normal, those with less than ten seconds was labeled as unstable tear film, 6 to 10 seconds as moderate dry eye disease, and ≤5 sec as sever dry eye disease [9].

The TFBUT was performed by applying a fluorescein strip after moistening it with a drop of sterile saline, to the lower tarsal conjunctiva without the use of topical anesthesia. The time lapse between the last blink to the appearance of the first random dry spot was taken as the tear film break up time [25].

Epithelial damage to the exposed surface of the eye can be demonstrated with vital and supra-vital stains. Fluorescein and Rose Bengal staining are the standard but invasive methods used to demonstrate ocular surface damage. This technique reveals surface damage on both the cornea and conjunctiva [27].

The eye is best viewed with anexciter (Wratten 47/47a; Edmund Optics, Barrington, NJ, USA) and barrier filter (Wratten 12/15) to assess staining on the cornea and conjunctiva.

Evaluation of staining is highly subjective, but the use of charts such as the Oxford grading scheme can help by enabling consistent recording of staining severity and is used to estimate surface damage in dry eye. The scheme has five panels, labeled A–E, with staining represented by punctuate dots that increase logarithmically between the panels [28]. The clinician compares the appearance of staining on the exposed interpalpebral conjunctiva and cornea with each panel and the closest match determines the grade. On a specially designed form, a grade between 0 and 3was given for staining on the cornea, based on the number of "dots" seen, which was to be added to a grade between 0 and 3 for the nasal and the same for the temporal conjunctiva. This gave a maximum possible total of 9 points. Three additional points were then allocated for fluorescein only if there was confluent staining (+1), staining in the papillary area (+1), or one or more filaments (+1), giving a maximum possible score of 12. This was performed for each eye. An abnormal score was considered to be 3 and above [21].

The study was approved by the local ethical committee of Tehran University of Medical Sciences and conducted in accordance with the ethical principles of the Declaration of Helsinki and all of the participants signed the written informed consent.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 18.0. Descriptive statistics were summarized as mean ± SD, or median and interquartile. Kolmogorov Smirnov was performed to assess normality for continuous variables.

An evaluation was made of the linear relationship between the tear osmolarity values and TFBUT, OSDI score, Schirmer I test and Rose Bengal and fluorescein staining. This was performed by using Spearman's correlation coefficient (rho). Student's t test and Mann–Whitney U test was applied for comparisons between two groups (significance $p < 0.05$).

Chi square test was used to compare discrete variables. Results were given with their 95% CIs.

Diagnostic accuracy tests was performed to analyze sensitivity, specificity, receiver operating characteristic (ROC) curves, positive likelihood ratio (LR+), and positive predictive values (PPV).

Results

From August 2011 until November 2012, we prospectively enrolled 243 people with type 2 diabetes at Institute of endocrinology and metabolism. Four patients were excluded from the study for the following reasons: LASIK within the 1 year prior to enrollment (2), contact lens wear (1), and glaucoma (1).

The data from 137 female and 102 male were used for the final analysis. The mean age was 55.8 ± 10.33 years old, and the mean duration of diabetes was 9.08 ± 7.9 years. The mean for fasting blood glucose was 152.4 ± 59.6 mg/dl, and for HbA1C was 7.55% ± 1.73%. Oral glucose lowering drugs (OGLDs) were used by 69.4% of the participants, while 10.5% were on insulin only, and 15.5% were on OGLDs plus insulin.

Najafi *et al. Journal of Diabetes & Metabolic Disorders* (2015) 14:39

Page 4 of 8

Tear osmolarity measurements were successfully performed at the first attempt in all participants; no reflex tearing was observed in any subjects. Considering of dry eye disease definition; two groups were identified: the dry eye disease (DED) group (67), and the normal group (172). All of the analyses were done considering this classification.

Table 1 illustrates clinical characteristics of the participants, according to the tear osmolarity test categorization.

The mean value for the tear osmolarity in all of the participants was 301.97 ± 13.52 mOsm/L. This value was 319.03 ± 7.92 mOsm/Lin the DED group and 295.38 ± 8.49 mOsm/Lin the normal group.

The prevalence of DED detected by the tear osmolarity test was 27.7% (10.2% in male, and 17.5% in female). The prevalence of DED detected by the other five diagnostic tests was as follows: OSDI (17.7%), Schirmer I test (33%), TFBUT (41%), Rose Bengal (11%), and fluorescein staining (4%).

Comparison of the results for all of the applied tests showed statistically non-significant difference between the two groups which is mentioned in Table 2.

We also compared the discriminative ability of the OSDI, Schirmer I test, Rose Bengal and fluorescein staining, and TFBUT tests results in Table 2 to detect DED compared to the tear osmolarity test.

Considering adverse events, only three patients reported eye discomfort following conjugate staining for a few hours.

Due to technical failure, we could not interpret the test results for Schirmer I test and TFBUT in ten subjects. In addition performing ophthalmic tattoos was the reason for indeterminant test results in two subjects.

Table 1 Clinical characteristics of the participants

	All participants	DED[d] group (n = 67)	Normal group (n = 172)	P-value
Age (yrs.)				
Mean ± SD	55.8 ± 10.33	56.50 ± 9.47	55.43 ± 10.66	0.48
Range	22-84	37-81	22-84	
Gender (female) n (%)	137 (58%)	42(64%)	95(56%)	0.28
BMI[a] (kg/m2)	29.2 ± 4.9	29.42 ± 5.36	29.36 ± 5.12	0.94
Diabetes duration (yrs.)	9.08 ± 7.9	9.73 ± 8.25	8.71 ± 7.69	0.32
FBS[b] (mg/dl)	152.4 ± 59.6	163.63 ± 61.27	147.44 ± 59.61	0.09
HbA1c[c] (%)	7.55 ± 1.73	7.94 ± 1.87	7.14 ± 1.66	0.054

[a]BMI, Body Mass Index.
[b]FBS, Fasting blood sugar.
[c]HbA1C, Hemoglobin A1C.
[d]DED, Dry eye disease.
Data are mean ± SD unless otherwise indicated.
Statistical method: Mann–Whitney test, Independent sample t-test and χ2 –test.

Table 2 Comparison of the OSDI, Schirmer I test, Rose Bengal staining, fluorescein staining, and TFBUT test results between the two groups

	All participants	DED group (n = 67)	Normal group (n = 172)	P-value
OSDI[a] (score)	2.08 (0, 8.3)	2.08 (0, 14.58)	2.08 (0, 8.3)	0.44
Fluorescein staining (score)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.70
Rose Bengal staining (score)	0 (0, 1)	0 (0, 1.25)	0 (0, 1)	0.13
Schirmer I test (mm)	5 (2, 10)	6 (2, 11)	5 (2, 10)	0.64
TFBUT[b] (secs)	8 (5, 12.25)	9 (5,13.25)	8 (5,12)	0.27

[a]OSDI, Ocular Surface Disease Index.
[b]TFBUT, Tear film break-up time.
For all variables, data are shown as Median (Interquartile), Kolmogorov Smirnov normality test distribution.
Statistical method: Mann–Whitney test.

The correlation coefficient for the measured tear osmolarity and the results of the other diagnostic tests were calculated in Table 3. No significant correlation was found.

The diagnostic performance for all of the diagnostic tests was determined. Data were summarized for sensitivity, specificity, positive predictive value (PPV), likelihood ratio (LR+), and area under the curve (AUC) from the ROC curve analysis in Table 4.

Fluorescein staining had the highest specificity (0.97), however its sensitivity was the lowest (0.05). With OSDI cutoff score of more than 12; the AUC was 0.558 which was the highest value compared to those found for the other tests. With this cutoff the positive likelihood ratio and PPV values were also the highest (1.78, and 0.4 respectively). The specificity value was also considerably high (0.85), behind that shown by the Rose Bengal staining (cut-off ≥ 3, specificity = 0.90) (Table 5).

We also tried to determine the cutoffs for all index tests with an optimal sensitivity and specificity in diabetic patients using ROC curve analysis in Figure 1, but we could not define any optimal cutoff value.

Discussion

As far as we searched the literature, this study was the first to compare the diagnostic performance of the tear osmolarity test to the other diagnostic tests used in clinical practice for detection of dry eye disease in people with type 2 diabetes mellitus. Our results revealed no significant correlation between tear osmolarity and the other diagnostic tests. OSDI had the highest AUC value with high LR+, PPV and specificity. However, it could not be suggested as a screening test due to its low sensitivity.

Najafi *et al. Journal of Diabetes & Metabolic Disorders* (2015) 14:39

Page 5 of 8

Table 3 DED detection rate by OSDI, Schirmer I test, Rose Bengal, fluorescein staining, and TFBUT compared to tear osmolarity test

	OSDI[a]	Schirmer I test	Rose Bengal staining	Fluorescein staining	TFBUT[b]
Dry eye disease (n = 67)	14(20.8%)	20(29.8%)	8(11.9%)	3(4.4%)	27(40.2%)
Normal (n = 172)	21(12.2%)	48(27.9%)	14(8.1%)	5(2.9%)	57(33.1%)

[a]OSDI, Ocular Surface Disease Index.
[b]TFBUT, Tear film break-up time.
Data are n. (%).

Dry eye disease is one of the most prevalent eye problems causing discomfort, deterioration in visual quality, and increased risk of infection [29]. Hosotani et al. [30], observed decreased tear production in diabetic patients. Goebbels M [31] suggested that amount of the reflex tearing is lower in the diabetics which can be due to diminished corneal and conjunctival sensations or may be due to neuropathy involving the lacrimal glands.

The lack of concordance between signs and symptoms in DED is a problem in the diagnosis of the disease. So, we need a reliable test or a group of tests that could be able to diagnose DED [9].

The current practical methods for diagnosing of DED such as corneal and conjunctival staining, Schirmer testing, and TFBUT are time-consuming and uncomfortable. Meanwhile, they could not predict symptomatic outcomes [32].

A new and reliable technology, tear osmolarity measurement can now is considered as a test suitable to be performed in clinical setting. The diagnostic accuracy of tear osmolarity test was found to be higher than the other tests [9].

Tear osmolarity, at a cutoff of more than 308 mOsms/L, achieved a 90.7% rate of proper diagnosis regarding severe dry eye patients in comparison with the other cutoff values (>314 mOsms/L; 86.7% and >311 mOsms/L; 89.3%), and also at the mentioned cutoff, a 73.2% rate of proper diagnosis was achieved regarding mild to moderate stage of dry eye. So, tear osmolarity is the most useful single objective test among the most commonly used tests to differentiate those with mild or moderate dry eye from those with sever disease. A cutoff threshold of more than

Table 4 Correlation coefficient of the tear osmolarity and other diagnostic tests

Variable	Correlation coefficients (r)	P-value
Rose Bengal staining	0.12	0.08
OSDI[a]	0.11	0.11
TFBUT[b]	0.08	0.26
Fluorescein staining	−0.06	0.41
Schirmer I test	−0.04	0.54

[a]OSDI, Ocular Surface Disease Index.
[b]TFBUT, Tear film break-up time.
Statistical method: Spearman's correlation coefficient (rho).

308 mOsms/L was found to be the most sensitive in differentiating normal from mild to moderate subjects [5].

In a comparison case- control study of diagnostic tests in keratoconjunctivitis sicca with a small sample size; tear osmolarity determination had 90% sensitivity and 95% specificity; while the Schirmer test yielded 25% sensitivity and 90% specificity [33].

In another study, tear osmolarity was found to have a 72.8% sensitivity and 92% specificity at a cutoff value of 312 mOsms/L, while corneal staining showed 54% sensitivity and 89.3% specificity, conjunctival staining 60.3% sensitivity and 90.7% specificity, TFBUT (cutoff value <10 secs) 84.4% sensitivity and 45.3% specificity, and Schirmer (cutoff value <18 mm) 79.5% sensitivity and 50.7% specificity [5].

In contrast with our findings; a diagnostic study in patients with Sjogren's syndrome revealed that the diagnostic usefulness of Schirmer's I test was inferior to that of TFBUT. The sensitivity and specificity were 80% and 53% respectively at a cutoff value of 5 mm in comparison with 88% and 35%, at a cutoff value of 10 mm [34].

The Schirmer test is a useful screening method for diagnosing lachrymal hyposecretion, but not for determination of the tear production threshold. Its sensitivity is between 10% and 30% [35,36]. In diabetes patients, the Schirmer test exhibits lower value than normal values [37,38]. In a series by Dogru [39], 22.7% of the eyes impregnated the strip less than 5.5 mm. In Gupta [25] and Ozdemir [40] series 9 and 34 percent of the eyes had Schirmer I value lower than 5 millimeters respectively.

The results of another study designed in six subgroups (Sjogern's syndrome, graft-versus-host disease, Graves orbitopathy, facial palsy, diabetes mellitus without proliferative retinopathy and glaucoma who chronically received topical drugs preserved with benzalkonium chloride) associated with dry eye disease were as follows: The most sensitive test was OSDI while the least accurate was lissamine green staining . The The best combination of tests to achieve the highest combined sensitivity (100%, C.I 95% 97.5–100), specificity (95%, C.I. 95% 75.1–99.9) and accuracy (99.3 C.I. 95% 96–99.9) for DED diagnosis was OSDI/TBUT/Schirmer test. The values (% and CI of 95%.) of sensitivity for the DED tests in diabetic patients for all tests were as follows: OSDI = 69.2 (38.6–90.9), Osmolarity = 78.2

Najafi *et al. Journal of Diabetes & Metabolic Disorders* (2015) 14:39

Page 6 of 8

Table 5 Diagnostic performance of all index tests

	Cut- off value	Sens[a] (%)	Spec[b](%)	AUC [c**](95% Confidence Interval)	LR+[d]	PPV[e] (%)
OSDI	>12 score	25.93	85.42	0.558 (0.464-0.651)	1.78	0.4
Fluorescein staining	≥3 score	5.17	96.64	0.509 (0.421-0.597)	1.54	0.37
Rose Bengal staining	≥3 score	13.79	90.60	0.522 (0.433-0.611)	1.47	0.36
Schirmer I test	≤10 mm	34.48	67.57	0.507 (0.415-0.598)	1.06	0.29
TFBUT	≤10 secs	46.55	61.49	0.535 (0.443-0.627)	1.21	0.32

[a]Sens: Sensitivity; [b]Spec: Specificity, [c]AUC: Area under the curve; [d]LR+: Likelihood ratio; [e]PPV+: Positive predictive value.
Statistical method: Diagnostic accuracy tests.
**No significant p-value in AUC was detected.

(49.2–100), TBUT = 46.1 (19.2–74.8), Schirmer test = 54.5 (23.4–83.2), and fluorescein staining = 21.4 (4.67–50.1) [41].

Besides bias due to population characteristics, the wide ranges in tear osmolarity results could be attributed to technical characteristics of the instruments and to tear collection methods, which introduce a significant and uncontrolled variable in the measurement of tear osmolarity. Trying to collect the tear volumes needed for measurements (sample size of 0.5 to 1.0 µl or larger) often results in reflex tearing, which dilutes samples and invalidates the results. Moreover, many patients with dry eye may not be evaluated due to the impossibility to properly collection of enough tear samples [9].

The strengths of this study is being the first one conducted in people with type 2 diabetes and including adequate sample size to produce reliable conclusion and influenced the degree of generalizability of the results.

A common criticism for estimating sensitivity, specificity, and prevalence without a gold standard is that, without a gold standard, it is difficult to conceptualize sensitivity and specificity [42]. But here we used gold standard to lessen the problem of conceptualizing the truth.

Potential limitations of the study include the fact that we did not consider social and environmental context of the study population; race/ethnicity or season. It is not yet known the extent to which these variables affect the distribution of osmolarity in normal persons or dry eye disease patients.

Also we are aware that studies of diagnostic accuracy are not the only type of studies to evaluate diagnostic tests.

Conclusions

In conclusion, available common diagnostic tests for evaluation of dry eye disease underestimate the problem in people with type 2 diabetes. Moreover, they could not properly discriminate normal condition from tear hyperosmolarity and the severity of dry eye disease. So, direct measurement of the tear osmolarity could be considered as a suitable test for detection of dry eye disease in people with type 2 diabetes.
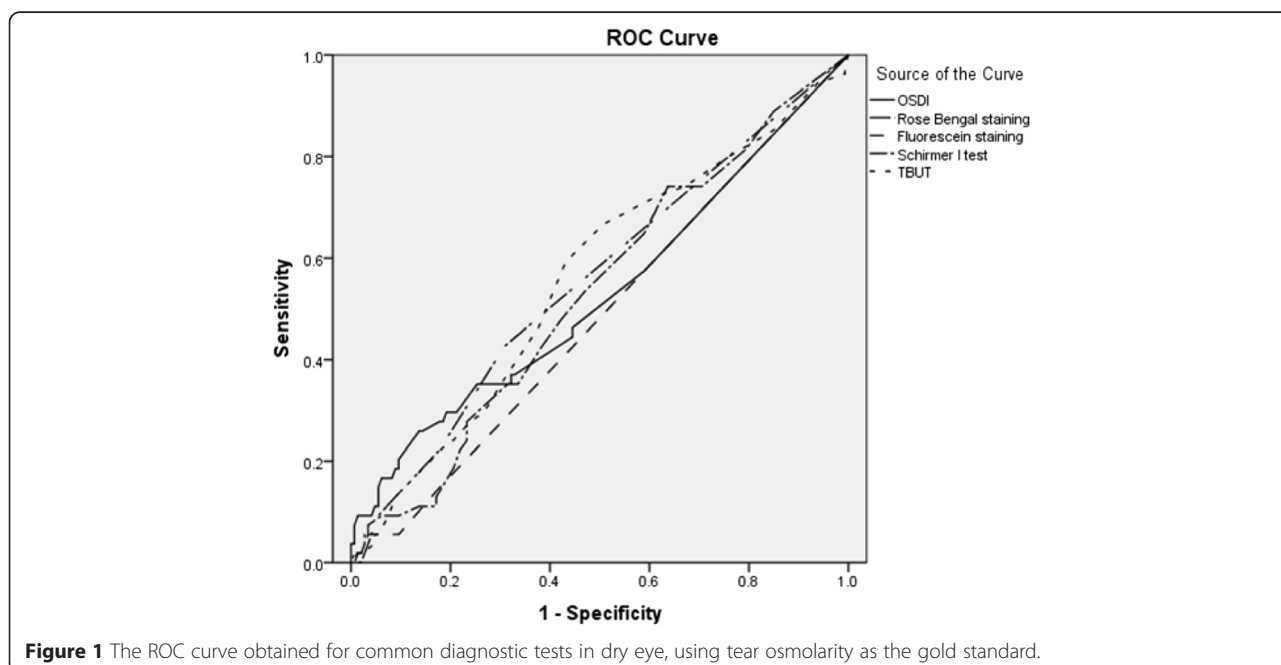
Figure 1 The ROC curve obtained for common diagnostic tests in dry eye, using tear osmolarity as the gold standard.

Najafi *et al. Journal of Diabetes & Metabolic Disorders* (2015) 14:39

Page 7 of 8

Abbreviations
DED: Dry eye disease; OGLDs: Oral glucose lowering drugs; OSDI: Ocular surface disease index; PRK: Photorefractive keratectomy; STARD: Standards for reporting of diagnostic accuracy; TFBUT: Tear film break-up time.

Competing interest
The authors declare that they have no competing interest.

Authors' contributions
1. Study conception/design: MEK, MM, HA, LN, AEV. 2. Data collection/analysis: LN, AEV. 3. Drafting of manuscript: MEK, MM, LN, HA, AEV. 4. Critical revisions for important intellectual content and administrative/technical/material support: MEK, MM. 5. Supervision: MEK, MM, LN, HA. 6. Statistical expertise: LN, AEV. All authors read and approved the final manuscript.

The place that study was performed
Endocrine Research Center (Firouzgar), Institute of Endocrinology & Metabolism, Iran University of Medical Sciences (IUMS).

Ethical code
This project was accepted by ethical committee of Tehran University of Medical Sciences; ethical code: 39661, 31/10/2011.

Author details
[1]Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences (IUMS), Firouzgar alley, Valadi St., Behafarin St., Karimkhan Ave., Vali- Asr Sq., Tehran 15937-48711, Iran. [2]Eye Research center, Rassoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

References
1. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol. 2000;118:1264–8.
2. Moss SE, Klein R, Klein BE. Incidence of dry eye in an older population. Arch Ophthalmol. 2004;122:369–73.
3. Najafi L, Malek M, Valojerdi AE, Aghili R, Khamseh ME, Fallah AE, et al. Dry eye and its correlation to diabetes microvascular complications in people with type 2 diabetes mellitus. J Diabetes Complications. 2013;27(5):459–62.
4. Bron AJ, Smith JA, Calonge M. Methodologies to diagnose and monitor dry eye disease. In: 2007 report of the international Dry Eye workshop (DEWS). diagnostic methodology subcommittee of the international Dry Eye workshop. Ocul Surf. 2007;5(2):108–23.
5. Lemp MA, Bron AJ, Baudouin CH, Benítez Del Castillo JM, Geffen D, Tauber J, et al. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol. 2011;151(5):792–8.
6. Figueroa-Ortiz LC, Jiménez Rodríguez E, García-Ben A, García-Campos J. Study of tear function and the conjunctival surface in diabetic patients. Archivos de la Sociedad Española de Oftalmología. 2011;86(4):107–12.
7. Narayanan S. Osmolarity. A diagnostic test for dry eye. Review of ophthalmology. 2011; http://www.revoptom.com/content/d/dry_eye_report/i/1378/c/26521/. Accessed 4/12/2013
8. International Dry Eye Workshop. The definition and classification of dry eye disease. In: 2007 report of the international dry eye workshop (DEWS). Ocul Surf. 2007;5(2):75–92.
9. Versura P, Profazio V, Campos EC. Performance of tear osmolarity compared to previous diagnostic tests for dry eye diseases. Curr Eye Res. 2010;35(7):553–64.
10. Manaviat MR, Rashidi M, Afkhami-Ardekani M. Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients. BMC Ophthalmology. 2008;8:10.
11. Grus FH, Sabuncuo P, Dick HB, Augustin AJ, Pfeiffer N. Changes in the tear proteins of diabetic patients. BMC Ophthalmology. 2002;2(1):4.
12. Alves Mde C, Carvalheira JB, Módulo CM, Rocha EM. Tear film and ocular surface changes in diabetes mellitus. Arq Bras Oftalmol. 2008;71(6 Suppl):96–103.
13. Azar DT, Spurr-Michaud SJ, Tisdale AS, Gipson IK. Altered epithelial basement membrane interactions in diabetic corneas. Arch Ophthalmol. 1992;110(4):537–40.
14. Chung H, Tolentino FI, Cajita VN, Acosta J, Refojo MF. Reevaluation of corneal complications after closed vitrectomy. Arch Ophthalmol. 1988;106(7):916–9.
15. Taylor HR, Kimsey RA. Corneal epithelial basement membrane changes in diabetes. Invest Ophthalmol Vis Sci. 1981;20(4):548–53.
16. Kaji Y. Prevention of diabetic keratopathy. Br J Ophthalmol. 2005;89(3):254–5.
17. Foulks GN, Thoft RA, Perry HD, Tolentino FI. Factors related to corneal epithelial complications after closed vitrectomy in diabetics. Arch Ophthalmol. 1979;97(6):1076–8.
18. Hyndiuk RA, Kazarian EL, Schultz RO, Seideman S. Neurotrophic corneal ulcers in diabetes mellitus. Arch Ophthalmol. 1977;95(12):2193–6.
19. Nielsen NV, Lund FS. Diabetic polyneuropathy, corneal sensitivity, vibratory perception and Achilles tendon reflex in diabetes. Acta Neurol Scand. 1979;59:15–22.
20. Foulks GN, Lemp MA, Berg M, Bhola R, Sullivan BD. TearLab™ osmolarity as a biomarker for disease severity in mild to moderate dry eye disease. Am Acad Ophthalmol. 2009;PO382.
21. Mc Ginnigle S, Naroo SA, Eper F. Evaluation of Dry Eye. Surv Ophthalmol. 2012;57(4):293–316.
22. Clinch TE, Benedetto DA, Felberg NT, Laibson PR. Schirmer's test: a closer look. Arch Ophthalmol. 1983;101:1383–6.
23. Stuchell R, Feldman J, Farris R, Mandel ID. The effect of collection technique on tear composition. Invest Ophthalmol Vis Sci. 1984;25:374–7.
24. Van Bijsterveld O. Diagnostic tests in the sicca syndrome. Arch Ophthalmol. 1969;82:10–4.
25. Gupta I, Mengi RK, Bhardwaj S. Tear secretion and tear film function in diabetics. JK SCIENCE. 2010;12(4):172–4.
26. Loran D, French C, Lam S, Papas E. Reliability of the wetting value of tears. Ophthal Phisiol Opt. 1987;7:53–6.
27. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. IOVS. 2006;47(10):4309–15.
28. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea. 2003;22:640–50.
29. Graham JE, Moore JE, Jiru XU, Moore JE, Goodall EA, Dooley JS, et al. Ocular pathogen or commensal: A PCR-based study of surface bacterial flora in normal and dry eyes. Invest Ophthalmol Vis Sci. 2007;48:5616–23.
30. Hostani H, Ohashi Y, Yamada M, Tsubota K. Reversal of abnormal Corneal epithelial cell morphologic Characteristics and reduced corneal sensitivity in diabetic patients by aldose reductase inhibitor, CT −1 12. Am J Ophthalmol. 1995;19(3):288–94.
31. Goebbels M. Tear secretion and tear film function in insulin depended diabetes. Br J Ophthalmol. 2000;84:19–21.
32. Sullivan BD. Osmolarity Testing Enables Accurate In-office Diagnosis of Dry Eye Disease. Refractive eye care. http://www.helloworldbox.com/~refracti/2012/01/osmolarity-testing-enables-accurate-in-office-diagnosis-of-dry-eye-disease/. January 2012; Accessed 9/5/2015.
33. Lucca JA, Nunez JN, Farris RL. A comparison of diagnostic tests for keratoconjunctivitis sicca: lactoplate, Schirmer, and tear osmolarity. CLAO J. 1990;16(2):109–12.
34. Danjo Y. Diagnostic usefulness and cutoff value of Schirmer's I test in the Japanese diagnostic criteria of dry eye. Graefes Arch Clin Exp Ophthalmol. 1997;235(12):761–6.

Najafi *et al. Journal of Diabetes & Metabolic Disorders* (2015) 14:39

Page 8 of 8

35. Lamberts DW, Foster CS, Perry HD. Schirmer test after topical anesthesia and the tear meniscus height in normal eyes. Arch Ophthalmol. 1979;97:1082–5.
36. Mackie I, Seal D. The questionably dry eye. Br J Ophthalmol. 1981;65:2–9.
37. Lemp MA, Hamill JR. Factors affecting tear film breakup in normal eyes. Arch Ophthalmol. 1973;89:103–5.
38. Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface. Dry eye states. Arch Ophthalmol. 1983;101:1869–72.
39. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmology. 2001;108:586–92.
40. Ozdemir M, Buyukbese MA, Cetinkaya A, Ozdemir G. Risk factors for ocular surface disorders in patients with diabetes mellitus. Diab Res Cl Pract. 2003;59:195–9.
41. Alves M, Reinach PS, Paula JS, Vellasco e Cruz AA, Bachette L, Faustino J, et al. Comparison of diagnostic tests in distinct well-defined conditions related to dry eye disease. PLoS One. 2014;9(5), e97921.
42. Alonzo TA, Pepe MS. Using a combination of reference tests to assess the accuracy of a new diagnostic test. Stat Med. 1999;18:2987–3003.