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ORIGINAL ARTICLE

Alterations of Tear Film and Ocular Surface in Children with Type 1 Diabetes Mellitus

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ABSTRACT

Purpose: To investigate whether diabetes mellitus (DM) affects ocular surface of children with well-controlled type 1 DM.

Methods: Sixty-five diabetic patients and 55 age-matched controls enrolled to study. Detailed ocular surface assessment including, ocular surface disease index (OSDI) questionnaire, tear film break-up time (TBUT) analysis, Schirmer test, and conjunctival impression cytologic analysis were performed.

Results: Schirmer test and TBUT results were significantly lower in DM group than controls ($p = 0.001$, for all). OSDI scores of all participants were within normal range. Impression cytology analysis showed grade 0 changes in all participants and there was no difference between groups for goblet cell density ($p > 0.05$). The TBUT results were significantly associated with duration of DM ($r = -0.309$, $p = 0.036$).

Conclusion: Diabetic children without symptoms, signs, and definite diagnosis of dry eye still had lower TBUT and Schirmer test results than controls; however, impression cytology analysis was similar in both groups.

Keywords: mellitus, dry eye, impression cytology, ocular surface, tear film

Diabetes mellitus (DM) is a chronic metabolic disease that has been reported to be associated with life-threatening or debilitating complications in a variety of organs including heart, kidney and eye.[1] Ocular complications such as transient refractive deviations, cataract, diabetic retinopathy (DR), neovascular glaucoma have been investigated extensively.[2–4] In recent years, attention has been drawn to ocular surface disorders, and the relationship between DM and dry eye has been documented.[5–8] Also, several clinical and experimental studies have reported structural, metabolic, and functional abnormalities in the conjunctiva and cornea of diabetic patients.[9–11] However, it is still to be debated how ocular surface abnormalities and tear film alterations are related to diabetes.

In this study, we performed ocular surface disease index (OSDI) questionnaire, tear film break-up time (TBUT) analysis, Schirmer test, and conjunctival impression cytologic analysis in children with well-controlled type 1 DM (glycemic control) and known durations of DM to investigate whether abnormal glucose metabolism in DM affects ocular surface and also compared the results with those obtained in healthy children.

PATIENTS AND METHODS

This prospective cross-sectional study was carried at the ophthalmology clinic of a tertiary referral eye

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hospital, at the pediatric endocrinology and metabolism clinic of a tertiary referral children's health and disease hospital and at histology and embryology department of the university. The study protocol was approved by the ethics committee and, study was carried out in accordance with tenets of Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of the patients prior to enrolment.

Type 1 DM patients who were referred to the ophthalmology clinic from the Pediatric Endocrinology and Metabolism Clinic for diabetic eye screening were included as DM group. Age- and sex-matched subjects who were admitted to the ophthalmology clinic for routine ophthalmologic examination and who did not have any systemic or ocular diseases were included as a control group. Only right eyes of the participants were analyzed in the study.

The inclusion criteria were no previous known corneal changes; best-corrected visual acuity (BCVA) equal to or greater than 20/20 according to Snellen chart; no ocular problems, other than spherical or cylindrical refractive errors ≤1.00 diopter (D); and no systemic disease, except for type 1 DM. All the patients with type 1 DM were ≤18 years. All were receiving insulin treatment and had a well-controlled type 1 DM without any signs of neuropathy and nephropathy. Only type 1 DM patients with information available on the duration of DM were included. In the study, the duration of DM referred the duration since the diagnosis of type 1 DM and this time period was used for analysis. The controls were also aged ≤18 years and had no ocular problems, other than spherical and cylindrical refractive errors ≤1.00 D.

Subjects with any of the following conditions were excluded: history of chronic ocular drug abuse; contact lens wear; topical medication; abnormalities in the cornea; conjunctiva or eyelid; strabismus; nystagmus; a history of previous ocular surgery or laser treatment; trauma or uveitis; secondary ocular and systemic diseases with dry eyes as a manifestation, bilateral dense cataracts; bilateral central corneal opacities; or any opacification in the media, fundus abnormalities including DR or diabetic maculopathy were excluded from the study. Patients who were not sufficiently cooperative for examinations were also excluded.

All participants underwent a comprehensive ophthalmic examination, including BCVA tests, using the Snellen chart; intraocular pressure measurements, using a pneumotonometer; slit-lamp biomicroscopy; and dilated fundus examination. Refraction measurements were performed by using the same automatic refractor-keratometer device (Canon RF-K2, Japan). High-quality color stereoscopic fundus photographs were also taken for diabetic children. Fundoscopic examinations and fundus photographs revealed that none of the children in the DM group had any sign of DR. Moreover, in the DM cases, blood samples were taken for preprandial blood glucose and glycosylated hemoglobin (HbA1c) levels on the day of ocular measurements. The duration of DM and levels of HbA1c were recorded.

The OSDI questionnaire, which has three subscales – ocular symptoms, vision-related function, and environmental triggers – was used in each subject to evaluate any existing complaint related to dry eye. Patients rate their responses on a 0–4 scale with 0 corresponding to "none of the time" and 4 corresponding to "all of the time." The OSDI score was calculated on the basis of this formula: OSDI = [(sum of the scores for all questions answered) × 100]/[(total number of questions answered) × 4].[12]

TBUT and Schirmer tear test (with topical anesthesia) were respectively carried out in all participants. TBUT analysis was done after waiting 3 min following topical anesthesia. A narrow (1 mm) fluorescein strip was wetted by a drop of physiological saline and touched the inferior fornix to provide a standard methodology.[13–16] Then, the subjects were instructed to blink naturally three times, look straight ahead and then to cease blinking until instructed. Despite the detrimental effects of topical anesthesia and fluorescein on tear stability,[17,18] since the study group consisted of pediatric patients, topical anesthesia and fluorescein were used in our study. The precorneal tear film was examined under blue-light illumination and the first-time break of this layer was noted. Consequently, the Schirmer test was performed after obtaining topical anesthesia with 0.5% proparacaine hydrochloride. Standard Schirmer strips were placed into the lower conjunctival sac at the junction of the lateral and middle thirds, avoiding touching the cornea, and the length of wetting strips in millimeters was recorded after 5 min.

Impression cytology of the conjunctiva was performed according to the Nelson method.[19] Eyes were topically anesthetized with 0.5% proparacaine hydrochloride. Small discs of cellulose acetate filter paper with a pore size of 0.025 mm were placed on the superior conjunctiva adjacent to the corneal limbus. Conjunctival impression cytology samples were obtained from the nonexposed conjunctival surfaces to eliminate the influence of environment-related factors such as light on the ocular surface.[20,21] The specimens were placed in a fixative solution mixture consisting of 95% ethanol and 1% formaline and were stained with the periodic acid–Schiff (PAS: Pas Staining Kit M101646.0001, Merck Millipore) and hematoxylin.

The specimens were examined with light microscope (Leica DM6000B) and images were transferred to a computer using a digital camera (Leica DC 500). Five random areas of each sample were photographed. The ratio of cytoplasm to nuclei, histological

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characteristics (size and shape) of the nucleus, the degree of eosinophilic staining of cytoplasm, the presence of intercellular linkages, the staining of goblet cells with PAS, the shape and counting of goblet cell nuclei were independently evaluated in a blinded fashion by two independent histologists under the 40 objective lens. Goblet cells were counted, and the goblet cell density in one high power field was represented as the number of cells per square millimeters. The degree of squamous metaplasia and goblet cell densities were graded from 0 to 3 according to the Nelson grading scheme.[19]

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. Measurements that need slit lamp were performed in a darkened room with the same slit lamp and by the same physician.

Statistical Analyses

An a priori power analysis using the PASS 11 calculation software (Power and Sample Size, version 11) revealed that at least 40 subjects must be enrolled from each group in the study. The study included 65 patients with DM and 55 control subjects, and accordingly, the power of the study was found as 90.5%. The study data were analyzed using the Statistical Package for Social Sciences (SPSS), version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as the mean ± standard deviations, frequency distributions, and percentages. Pearson's chi-square test and the one-sample chi-square test were used to analyze the categorical variables. The normal distribution of the variables was checked by visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk Test). An independent sample *t*-test was used for normally distributed data, and a Mann–Whitney *U*-test was used for non-normally distributed data, to compare the DM group and the control group. Pearson correlation analysis was used to examine any relationship among the measured variables. Levels of $p < 0.05$ were considered as statistically significant.

RESULTS

This study included 120 eyes of 120 subjects: 65 of the subjects were in the DM group, and the remaining 55 were in the control group. The mean age of the patients with type 1 DM and that of the matched controls was 14.20 ± 3.12 (range: 8–18) years and 13.89 ± 2.42 (range: 10–18) years, respectively. There were no statistically significant differences in the ages and genders of the participants in two groups ($p > 0.05$). In the DM group, the mean duration of the disease was 5.46 ± 3.30 (min 1 and max 13) years, and the mean level of HbA1c was 6.26 ± 1.01% (min 5.7% and max 7.1%). Clinical characteristics of the study population are presented in Table 1.

Comparison of the results of OSDI, tear film, and ocular surface parameters between the two groups is shown in Table 2. OSDI scores of all participants were with in the normal range according to algorithm, and no significant difference was observed between groups in terms of OSDI scores ($p = 0.258$). TBUT was significantly shorter in the diabetic group (9.98 ± 3.97 s) than in the control group (12.13 ± 3.23 s) ($p = 0.001$). Schirmer test scores were 12.09 ± 5.73 mm in the diabetic group and 15.09 ± 4.01 mm in the control group. The differences between the two groups were statistically significant ($p = 0.001$). Slit-lamp biomicroscopy of the eyelid margins and conjunctiva did not reveal any coexistent blepharitis, meibomian gland disorder and there was no ocular surface staining with fluorescein in both groups.

Cytologic evaluation of the conjunctiva revealed grade 0 changes in all participants of both groups (Figure 1). Goblet cell count was 532.7 ± 72.3 cells/mm^2 in the diabetic group and 555.1 ± 65.6 cells/mm^{2}in the control group ($p = 0.098$).

Correlation analysis of results of OSDI, TBUT, and Schirmer tests with DM-related variables revealed that there was only statistically significant, moderate correlation between TBUT results with duration of DM (Table 3, Figure 2, $r = -0.309$, $p = 0.036$).

DISCUSSION

Dry eye is 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 sur-

TABLE 1. Demographics and clinical characteristics of participants.

	DM Group ($n = 65$)	Control Group ($n = 55$)	*p*-Value
Age, years (mean ± SD)	14.20 ± 3.12	13.89 ± 2.42	0.551*
Women/men (n/n)	29/36	30/25	0.278**
The duration of DM, years (mean ± SD)	5.46 ± 3.30	—	
The mean HbA1c, % (mean ± SD)	6.26 ± 1.01%	—	

DM: diabetes mellitus; HbA1c: glycosylated hemoglobin; SD, standard deviation.

*Independent samples *t*-test.

**Chi-square test.

TABLE 2. Comparison of OSDI results, Schirmer test, TBUT and goblet cell density among groups.

Tear film function tests and ocular surface parameters	DM group (n = 65)	Control group (n = 55)	p-Value
OSDI Mean ± SD	5.8 ± 6.3	5.2 ± 6.6	0.258*
Schirmer test (mm) Mean ± SD	12.09 ± 5.73	15.09 ± 4.01	**0.001***
TBUT (s) Mean ± SD	9.98 ± 3.97	12.13 ± 3.23	**0.001***
GCD (cells/mm^2) Mean ± SD	532.7 ± 72.3	555.1 ± 65.6	0.098*

OSDI: ocular surface disease index; DM: diabetes mellitus; TBUT: tear break-up time, GDC: goblet cell density; SD, standard deviation.

*Independent samples t-test.

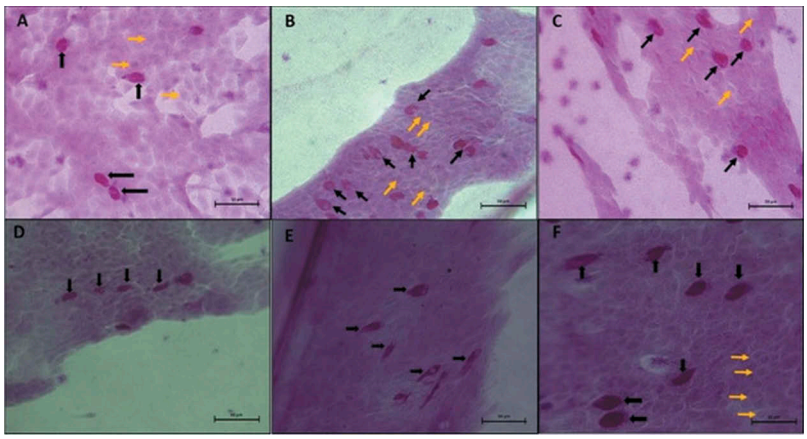

FIGURE 1. (A–F): Impression cytology specimens (periodic acid–Schiff hematoxylin staining, ×40). (A–C): Specimens from control subjects show abundant periodic acid–Schiff-positive oval-plum goblet cells (black arrows) and sheets of small, round nonsecretory epithelial cells (yellow arrows) with nucleocytoplasmic ratio 1:1 or 1:2 (squamous metaplasia grade 0). (D–F): Specimens from diabetic patients showconjunctival cytology of grade 0. Epithelial cells (yellow arrows) are small and round and have a normal nucleo⁄cytoplasmic ratio. Reddish pink goblet cells (black arrows) are abundant.

TABLE 3. Correlations between the duration of DM and HbA1c values with investigated parameters in diabetic patients.

	Duration of DM (years)	HbA1c values (%)
OSDI	r = 0.104, p = 0.331	r = 0.099, p = 0.534
Schirmer test (mm)	r = −0.177, p = 0.159	r = −0.024, p = 0.850
TBUT (s)	**r = −0.309, p = 0.036**	r = −0.076, p = 0.549

r: Pearson correlation coefficient.

Bold values indicate statistically significant correlations.

face inflammation and damage, and neurosensory abnormalities play etiological roles.[22] While alterations of tear function parameters in DM have been studied, the results remain controversial and few have focused on the ocular surface changes in children with type 1 DM. In this study, we assessed OSDI questionnaire, TBUT, Schirmer test, and conjunctival impression cytology in pediatric patients with type 1 DM without DR and compared the results obtained with those in healthy children to investigate the possibility of developing ocular surface changes in diabetic children. As children with type 1 DM without any signs of DR and neuropathy have a lower prevalence of comorbidities that may contribute to ocular surface changes, we thought that a study involving only children with type 1 DM may be better suited to evaluate ocular surface changes due specifically to the metabolic dysregulation of DM.[23]

There are limited number of studies evaluating ocular surface changes and dry eye in diabetic children in the literature.[24–26] Akinci et al.[24] performed TBUT and Schirmer tests with topical anesthesia as in our study and found a higher prevalance of dry eye in children with type 1 DM than controls based on TBUT and Schirmer test scores. They reported that there were no significant differences among subgroups divided according to HbA1c levels. However, they found TBUT and Schirmer test results were

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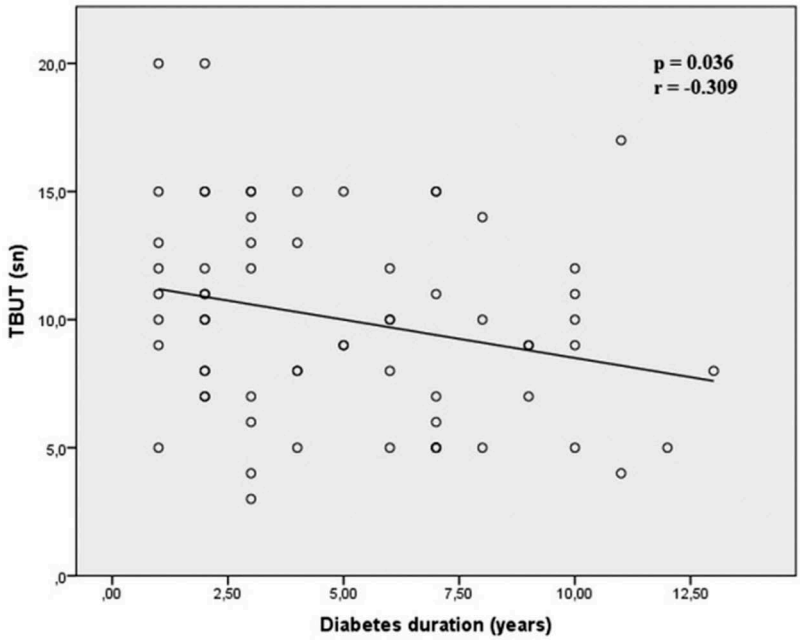

FIGURE 2. The correlations among the TBUT and duration of DM (p = 0.036, r = −0.309).

significantly lower in patients with DM for more than 10 years. Similarly in our study, Schirmer test and TBUT results were significantly lower in diabetic children and there was only statistically significant correlation between TBUT and duration of DM. Despite Schirmer and TBUT tests are widely applied for both clinical and research purposes, diagnosis of dry eye is a challenging task due to the limitations of tests including TBUT, Schirmer and their lack of correlation with dry eye symptoms.[27] Moreover, they are not able to predict the extent of cytological changes in the ocular surface. For this reason, Gunay et al.[25] investigated the presence of dry eye and ocular surface characteristics of diabetic children with dry eye tests including conjunctival brush cytology. They found lower Schirmer scores, similar TBUT results and a significant decrease in goblet cell count in diabetic children as compared to controls. Although brush cytology is also a promising technique, impression cytology, which is also safe, relatively simple, minimally invasive biopsy method that may detect early dry-eye changes, allows a homogenous collection of a single epithelial cell layer and does not depend on the strength and duration of application on the conjunctiva.[28] Therefore, we performed conjunctival impression cytology. The Schirmer test scores were low similarly to Gunay's study; however, the TBUT results were low and there was no significant difference in the number and morphology of the goblet cells. Goebbels[29] compared 86 Type 1 diabetics (>30 years duration of DM) with retinopathy and 84 nondiabetic controls. They reported that DM group showed decreased Schirmer test readings (without topical anesthesia) and significantly more frequent and pronounced signs of conjunctival metaplasia. Different impression cytology results from our study and can be explained by the study population of Goebbels[29] with more than 30 years duration of DM. However, TBUT was found to be to be equivalent in diabetic and nondiabetic individuals in the study of Goebbels[29] They reported that even though no significant differences were found between diabetic and nondiabetic subjects regarding TBUT, it cannot be concluded definitively from these data that tear film stability does not actually differ between diabetics and nondiabetics. Since TBUT is a very rough test for the determination of tear film stability and large interindividual and intraindividual deviations can be found even when performed in a standardized procedure.[30]

It is still not clear why diabetic patients develop dry eye more often than healthy subjects.[31] Lipid, aqueous, and mucin which are the major components of the tear film, are at the risk of being adversely affected in diabetic patients. One possible explanation could be an exocrine dysfunction of the main lacrimal gland, which is responsible for the secretion of the aqueous portions of the tear film, in patients with DM. Advanced glycation end-products (AGE) modified proteins were found to be elevated in DM tears and increased expression of the AGE in the lacrimal gland is assumed to be a cause of lacrimal gland dysfunction in DM.[32,33] Other reasons might be the reduction of stimulatory signals from the ocular surface to the lacrimal gland as consequence of the reduced corneal sensation and the influence on regulatory systems and/or could be the development of additional unknown proteins in the tear that change

the composition of tear.[34] Our results can support these mechanisms by revealing low Schirmer scores in diabetic children.

The other explanation could be a reduction in numbers of goblet cells, main mucin-secreting cells, contributing to the tear film instability observed in DM.[35,36] The test of TBUT shows tear film instability which is not a deficiency of the aqueous layer of the tear film; rather, it is the inadequacy of the lipid and/or mucin component in the tear film produced by the meibomian glands and conjunctival goblet cells, respectively. In the present study, we found low TBUT values in the diabetic group while there was no significant difference in numbers and morphology of goblet cells between groups. Goblet cell loss in patients with diabetic peripheral neuropathy and poor metabolic control has been previously reported.[35] However, diabetic children with good metabolic control were included in the present study. This might be why we do not find a significant difference in the number of goblet cells among the groups. Low values of TBUT in the diabetic group might be due to dysfunction of meibomian glands, which are responsible for the secretion of the lipid portion of the preocular tear film, although meibomian gland dysfunction was not detected biomicroscopically. Two human studies have previously reported a compromised tear lipid layer in DM patients.[37,38] Lack of lipid layer is associated with tear film evaporation, one of the important factors in dry eye development.[39]

The cornea is the most densely innervated part of the human body and derives its innervation from the ophthalmic division of the trigeminal nerve. Animal studies suggested that decreased trophic effects of trigeminal sensory nerves on the conjunctiva and cornea could be a reason of decreased tear secretion.[40] Hosotani et al.[41] revealed that corneal sensation is impaired within 3 months in diabetic rats and it was reported that in vivo corneal confocal microscopy can be useful for early detection of diabetic neuropathy.[42] Besides this intense innervation of the ocular surface, meibomian glands are innervated by parasympathetic fibers with a smaller contribution from sympathetic and sensory neurons.[43] In the study of Misra et al.[44] comparing tear film metrics in patients with type 1 DM and healthy controls and investigating the association between peripheral neuropathy and ocular surface quality, it was shown that DM group exhibited significantly reduced tear film stability, secretion, and lipid layer quality relative to the age-matched control group. They also found negative correlation between tear film parameters and total neuropathy score suggesting that ocular surface abnormalities occur in parallel with diabetic peripheral neuropathy. Decreased innervation of lacrimal and meibomian glands due to subclinical neuropathy may be the underlying cause of low TBUT and Schirmer scores detected in diabetic children.

In our study, the similarity in OSDI scores may be due to the fact that the study group is composed of a pediatric population. In the study of Han et al.,[45] it was shown that children with dry eye conditions may have fewer symptoms than adults with similar dry eye conditions. It may be explained that children may have less experience of discomfort or pain, thus have a poorer ability to identify discomfort caused by ocular surface compromise.[46]

One of the important limitations of the study was lack of meibography, *in vivo* corneal confocal microscopy and tear film osmolarity data of participants. With the future studies including all these tests, we can better understand the underlying causes of dry eye in diabetic patients. Another limitation was that the cross sectional nature of the study which might limit the generalizability of the results. However, the strengths of the study are its strict inclusion criteria and its power of >90%.

In conclusion, our study indicates that diabetic children without the symptoms, signs and definitive diagnosis of dry eye still had lower TBUT and Schirmer test results than healthy children. The only disease related variable, which was found to be associated with alterations in tear film was the duration of DM. Therefore, we advise screening for dry eye among children with Type 1 DM, especially in cases with long duration of disease.

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DECLARATION OF INTEREST

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article. The authors alone are responsible for the content and writing of the article.

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REFERENCES

1. Smith-Palmer J, Brändle M, Trevisan R, Orsini Federici M, Liabat S, Valentine W. Assessment of the association between glycemic variability and diabetes-related complications in

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type 1 and type 2 diabetes. *Diabetes Res Clin Pract*. 2014;105 (3):273–284. doi:10.1016/j.diabres.2014.06.007.
2. Reddy VS, Reddy GB. Role of crystallins in diabetic complications. *Biochim Biophys Acta*. 2016;1860(1):269–277. doi:10.1016/j.bbagen.2015.05.009.
3. Yarbağ A, Yazar H, Akdoğan M, Pekgör A, Kaleli S. Refractive errors in patients with newly diagnosed diabetes mellitus. *Pak J Med Sci*. 2015;31(6):1481–1484. doi:10.12669/pjms.316.8204.
4. Yokota S, Takihara Y, Takamura Y, Inatani M. Circumpapillary retinal nerve fiber layer thickness, anterior lamina cribrosa depth, and lamina cribrosa thickness in neovascular glaucoma secondary to proliferative diabetic retinopathy: a cross-sectional study. *BMC Ophthalmol*. 2017;17(1):57. doi:10.1186/s12886-017-0456-9.
5. Najafi L, Malek M, Valojerdi AE, Khamseh ME, Aghaei H. 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. *J Diabetes Metab Disord*. 2015;14:39. doi:10.1186/s40200-015-0157-y.
6. Beckman KA. Characterization of dry eye disease in diabetic patients versus nondiabetic patients. *Cornea*. 2014;33 (8):851–854. doi:10.1097/ICO.0000000000000163.
7. Gekka M, Miyata K, Nagai Y, et al. Corneal epithelial barrier function in diabetic patients. *Cornea*. 2004;23:35–37.
8. Fuerst N, Langelier N, Massaro-Giordano M, et al. Tear osmolarity and dry eye symptoms in diabetics. *Clin Ophthalmol*. 2014;10(8):507–515.
9. Chang SW, Hsu HC, Hu FR, Chen MS. Corneal autofluorescence and epithelial barrier function in diabetic patients. *Ophthalmic Res*. 1995;27(2):74–79. doi:10.1159/000267600.
10. Hashemi H, Asgari S, Mehravaran S, Emamian MH, Fotouhi A. Five-year changes of anterior corneal indices in diabetics versus non-diabetics: the shahroud eye cohort study. *Curr Eye Res*. 2019; 44(1):30–33. doi: 10.1080/02713683.2018.1521977.
11. Bikbova G, Oshitari T, Tawada A, Yamamoto S. Corneal changes in diabetes mellitus. *Curr Diabetes Rev*. 2012;8:294–302.
12. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol*. 2000;118:615–621.
13. Mooi JK, Wang MT, Lim J, Muller A, Craig JP. Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability. *Cont Lens Anterior Eye*. 2017;40:170–174. doi:10.1016/j.clae.2017.01.004.
14. Pult H, Riede-Pult BH. A new modified fluorescein strip: its repeatability and usefulness in tear film break-up time analysis. *Cont Lens Anterior Eye*. 2012;35(1):35–38. doi:10.1016/j.clae.2011.07.005.
15. Kim KT, Kim JH, Kong YT, Chae JB, Hyung S. Reliability of a new modified tear breakup time method: dry tear breakup time. *Graefes Arch Clin Exp Ophthalmol*. 2015;253 (8):1355–1361. doi:10.1007/s00417-015-3080-5.
16. Korb DR, Greiner JV, Herman J. Comparison of fluorescein break-up time measurement reproducibility using standard fluorescein strips versus the Dry Eye Test (DET) method. *Cornea*. 2001;20:811–815.
17. Pfister RR, Burstein N. The effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium: a scanning electron microscope study. *Invest Ophthalmol*. 1976;15:246–259.
18. Lemp MA, Hamill JR Jr. Factors affecting tear film breakup in normal eyes. *Arch Ophthalmol*. 1973;89:103–105.
19. Nelson JD. Impression cytology. *Cornea*. 1988;7:71–81.
20. Kumar P, Bhargava R, Arora YC, Kaushai S, Kumar M. Conjunctival impression cytology versus routine tear function tests for dry eye evaluation in contact lens wearers. *J Cytol*. 2015;32:261–267. doi:10.4103/0970-9371.171242.
21. Zuazo F, López-Ponce D, Salinas-Toro D, et al. Conjunctival impression cytology in patients with normal and impaired OSDI scores. *Arch Soc Esp Oftalmol*. 2014;89:391–396. doi:10.1016/j.oftal.2014.04.013.
22. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf*. 2017;15 (3):276–283. doi:10.1016/j.jtos.2017.05.008.
23. Song SH. Complication characteristics between young-onset type 2 versus type 1 diabetes in a UK population. *BMJ Open Diabetes Res Care*. 2015;3(1):44. doi:10.1136/bmjdrc-2014-000044.
24. Akinci A, Cetinkaya E, Aycan Z. Dry eye syndrome in diabetic children. *Eur J Ophthalmol*. 2007;17:873–878.
25. Gunay M, Celik G, Yildiz E, et al. Ocular surface characteristics in diabetic children. *Curr Eye Res*. 2016;41 (12):1526–1531. doi:10.3109/02713683.2015.1136421.
26. Akil H, Buluş AD, Andiran N, Alp MN. Ocular manifestations of Type 1 diabetes mellitus in pediatric population. *Indian J Ophthalmol*. 2016;64(9):654–658. doi:10.4103/0301-4738.194336.
27. Savini G, Prabhawasat P, Kojima T, Grueterich M, Espana E, Goto E. The challenge of dry eye diagnosis. *Clin Ophthalmol*. 2008;2:31–55.
28. Egbert PR, Lauber S, Maurice DM. A simple conjunctival biopsy. *Am J Ophthalmol*. 1977;84:798–801.
29. Goebbels M. Tear secretion and tear film function in insulin dependent diabetics. *Br J Ophthalmol*. 2000;84:19–21.
30. Vanley GT, Leopold IH, Gregg TH. Interpretation of tear film breakup. *Arch Ophthalmol*. 1977;95:445–448.
31. Seifart U, Strempel I. The dry eye and diabetes mellitus. *Opthalmologe*. 1994;91:235–239.
32. Alves M, Calegari VC, Cunha DA, Saad MJ, Velloso LA, Rocha EM. Increased expression of advanced glycation end-products and their receptor, and activation ofnuclear factor kappa-B in lacrimal glands of diabetic rats. *Diabetologia*. 2005;48(12):2675–2681. doi:10.1007/s00125-005-0010-9.
33. Zhao Z, Liu J, Shi B, He S, Yao X, Willcox MDP. Advanced glycation end product (AGE) modified proteins in tears of diabetic patients. *Mol Vis*. 2010;16:1576–1584.
34. Grus FH, Sabuncuo P, Dick HB, Augustin AJ, Pfeiffer N. Changes in the tear proteins of diabetic patients. *BMC Ophthalmol*. 2002;2:4. doi:10.1186/1471-2415-2-4.
35. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. *Ophthalmology*. 2001;108:586–592.
36. Tseng SC, Hirst LW, Maumenee AE, Kenyon KR, Sun TT, Green WR. Possible mechanisms for the loss of goblet cells in mucin-deficient disorders. *Ophthalmology*. 1984;91:545–552.
37. Inoue K, Kato S, Ohara C, Numaga J, Amano S, Oshika T. Ocular and systemic factors relevant to diabetic keratoepitheliopathy. *Cornea*. 2001;20:798–801.
38. Inoue K, Okugawa K, Amano S, et al. Blinking and superficial punctate keratopathy in patients with diabetes mellitus. *Eye (Lond)*. 2005;19(4):418–421. doi:10.1038/sj.eye.6701497.
39. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci*. 1997;74:8–13.
40. Marfurt CF, Echtenkamp SF. The effect of diabetes on neuropeptide content in the rat cornea and iris. *Invest Ophthalmol Vis Sci*. 1995;36:1100–1106.
41. Hosotani H, Ohashi Y, Kinoshita S, Matsumoto T, Awata T. Effects of topical aldose reductase inhibitor CT-112 on corneal sensitivity of diabetic rats. *Curr Eye Res*. 1996;15:1005–1007.
42. Hossain P, Sachdev A, Malik RA. Early detection of diabetic peripheral neuropathy with corneal confocal microscopy.

Lancet. 2005;366(9494):1340–1343. doi:10.1016/S0140-6736(05)67546-0.
43. Chung CW, Tigges M, Stone RA. Peptidergic innervation of the primate meibomian gland. *Invest Ophthalmol Vis Sci*. 1996;37:238–245.
44. Misra SL, Patel DV, McGhee CN, et al. Peripheral neuropathy and tear film dysfunction in type 1 diabetes mellitus. *J Diabetes Res*. 2014;2014:848659. doi:10.1155/2014/848659.
45. Han SB, Yang HK, Hyon JY, Hwang JM. Children with dry eye type conditions may report less severe symptoms than adult patients. *Graefes Arch Clin Exp Ophthalmol*. 2013;251(3):791–796. doi:10.1007/s00417-012-2097-2.
46. Greiner KL, Walline JJ. Dry eye in pediatric contact lens wearers. *Eye Contact Lens*. 2010;36(6):352–355. doi:10.1097/ICL.0b013e3181f8bc25.

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