Evaluation of dry eye disease in patients with diabetic retinopathy

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Abstract

Introduction: Due to lack of correlation between symptoms and signs in dry eye disease, changes in tear film parameters and ocular surface health have shown wide variance across different studies worldwide. The present study evaluated the efficacy Schirmer, tear film break-up time (TBUT) and conjunctival impression cytology (CIC) in type 2 diabetics with retinopathy.

Materials and Methods: The scoring of Schirmer test, TBUT, and CIC was compared in 75 eyes of diabetics with retinopathy and 75 eyes of age and sex matched healthy controls. The diagnostic accuracy of these test for dry eye diagnosis was estimated using receiver operating curve (ROC) analysis.

Results: Dry eye indices showed a significant reduction ($P<0.001$) in diabetic retinopathy group as compared to healthy, age and sex-matched controls. Dry eye symptom severity (ANOVA, P=0.645), TBUT (ANOVA, P=0.478), Schirmer (ANOVA, P=0.676) and Nelson Grade (ANOVA, P=0.345) did not significantly differ between the stages of diabetic retinopathy. The diagnostic accuracy was CIC (AUC=0.982) >TBUT (AUC=0.948) >Schirmer (AUC= 0.864), respectively.

Conclusion: Tear film characteristics and ocular surface health in patients with type 2 diabetes mellitus with retinopathy include decreased tear production as well as tear film stability with worse Nelson grade (squamous metaplasia), respectively. Second, the accuracy of CIC to evaluate dry eye in patients with diabetes was higher as compared to TBUT and Schirmer. All diabetic patients should be examined for tear film and ocular surface changes irrespective of the stage of retinopathy.

Keywords: Conjunctival impression cytology, Dry eye, Receiver operating curve, Schirmer Test, Tear film break-up time, Type 2 retinopathy.

Introduction

Diabetes is a complex metabolic disorder of carbohydrate, protein and fat metabolism in which there is relative or absolute deficiency of insulin leading to sustained hyperglycemia. Although long term hyperglycemia often results in formation of cataract and development of changes in retina known as diabetic retinopathy, changes on ocular surface like dry eye syndrome have now surfaced as a common ocular symptom in diabetes and may also lead to reduction of vision.[1]

Recently, there has been a better understanding of dry eye pathophysiology; inflammation and increased tear film osmolarity have been found to be associated with dry eye disease process.[2,3] Ocular surface inflammation is associated with expression of pro-inflammatory cytokines, human beta-defensins (hBD) and markers like HLA-DR; this may lead to squamous metaplasia of conjunctival epithelial cells and reduction of conjunctival goblet cells.[4-6]

Recent research has found that obesity and inflammation play a role in pathogenesis of diabetes as well; inflammation leads to hypoxia and cellular death, activation of the nuclear factors, terminal kinase, interleukins, as well as immune cells.[7]

Ocular surface changes like superficial erosions to full thickness epithelial lesions, have been reported to occur in 47% to 64% of diabetic patients. Elevated expression and activity of matrix metalloproteinase (MMP)-10 and MMP-3 have been found in diabetic corneas.[8-9]

Most researchers are of the opinion that inflammatory responses in diabetics may contribute to the development of ocular surface lesions as well, since early conjunctival surface changes, tear film alterations, and meibomian gland alterations may be seen in early in diabetics.[10-19]

However, the diagnostic efficacy and accuracy of tests like Schirmer, TBUT and CIC for dry eye diagnosis in patients with diabetes have not been standardized. Second, the impact of diabetic disease process on ocular surface health, and conjunctival cytology needs to be studied in greater detail.

Conjunctival impression cytology is a minimally-invasive technique of harvesting cells from conjunctival surface; conjunctival cells are removed by application of strips cellulose acetate filter paper and then analyzed by microscopy after staining.

The present study evaluated the efficacy of routine tear film tests like Schirmer, TBUT and CIC for dry eye diagnosis in symptomatic patients with diabetic retinopathy as compared to age and sex matched healthy controls.

Materials and Methods

The present study was done at a tertiary care teaching medical college hospital in North India. Approval of institutional review board and the ethics committee was obtained prior to the study. All subjects were requested to sign a written consent and the research was carried out as per the tenants of the declaration of Helsinki.

Inclusion Criteria

Diabetes was diagnosed as per the suggestions of World Health Organization; fasting blood glucose ≥126 mg/dl and random blood glucose (RBG) ≥200 mg/dl on three separate occasions, respectively. Diabetic retinopathy was classified according to International classification of DR. Patients

were recruited based on their responses to a point-based system (Dry Eye Scoring System, DESS©) (Table 1).

Exclusion Criteria

Patients with type 1 diabetes, advanced diabetic eye disease (ADED), active herpes or past-history of ocular herpes disease were excluded. Conditions having potential to cause dry eyes such as history of prior laser in situ keratomileusis (LASIK), contact lens wear, computer usage>3h/day for more than 1 year were excluded. Those having psychiatric disorders, AIDS and hepatitis (B or C), punctual plugs users, those on anti-glaucoma medication, oral anti-coagulants, topical corticosteroids were also excluded.

Power Calculation (case control study)

This was made using one of the primary outcome measures. In the present study this was dry eye symptom score. The aim to calculate power was to detect clinical difference between the two groups. Considering leads from previous studies, power was calculated was done: Odds ratio = 9.6, exposed controls = 24%, one-sided alpha risk = 6%, controls / case ratio = 1.04, total exposed=57.3086%, Estimated power= 98.9999%.

Ophthalmic Examination

Scoring was done in response to answers to dry eye scoring system (DESS before doing tear film tests and CIC. DESS is an 18-point questionnaire. A higher score means dry eye of higher grade (Table 1). The point-based grading is as follows; a score of 0-6 represents mild, 6.1-12 moderate, and 12.1 to 18, severe symptoms in subjects.

Table 1: Dry eye questionnaire and scoring system (DESS©)

	Score (Maximum 18)			
Symptom	**Absent**	**Sometimes present**	**Frequently present**	**Always present**
	(0)	(1)	(2)	(3)
Itching or burning				
Sandy or gritty sensation				
Redness				
Blurring of vision				
Ocular fatigue				
Excessive blinking				

Scores of 0 to 6 were, 6.1 to 12 were moderate, and 12.1 to 18 indicated

Severely symptomatic dry eye.9-11 © Bhargawa R. Laser eye clinic, Noida, India

The subjects had an eye examination performed by an investigator who not a study participant. The general eye examination included recording best corrected vision, stereoscopic examination; assessment of lids, eye lashes, and meibomian glands for any blockage; retinal was examined with + 90D lens (dilated fundus examination). One eye was selected randomly.

TBUT was performed first to avoid eyelid manipulation; this could adversely affect the results. A moistened fluorescein strip was applied over lower conjunctiva after shaking off excess dye. The patient was advised not to squeeze the eyelids tightly. The pre-corneal film was observed on cobalt blue light of slit-lamp. The length of time between the blink and disintegration of tear film on the corneal surface was noted with a stop-watch. An average of three readings in succession was taken and then averaged. A TBUT of less than 10 seconds was the normal cut-off limit.

There was an interval of 30 minutes after doing TBUT measurement. Then, Schirmer's test (eyes closed) was done after anaesthetizing the eye with 4% xylocaine. The normal cut-off limit of Schirmer strip wetting was 6 mm at 5 minutes interval.

Impression Cytology

This was done using circular cellulate acetate filter paper (0.22micron) of 13 mm size. The paper was held with blunt forceps and placed over the inferior bulbar conjunctiva. Placing strip on non-exposed part of the conjunctiva eliminated environmental influence on ocular surface. The filter-paper was then pressed with blunt forceps and removed after 4-8 seconds. Ethyl alcohol, formaldehyde, and glacial acetic acid in a ratio of 20:1:1 were used for fixation of slide. Subsequently, the slide was stained with PAS and re-stained with hematoxylin and eosin. Light microscopic examination of the slide was done with 100x low power field. Cells were localized and then examined with 400x final magnification. For epithelial and goblet cells, at least ten high power fields were assessed. Goblet cells were counted per high power field. Density of goblet cells was calculated from the following proportion. Number of goblet cells per high power field divided by sampling area mm^2. Nelson's method for grading and scoring was applied.

Statistics

Statistics analysis was done with IBM, Statistical Package for the Social Sciences (SPSS) Statistics version 25, (IBM Corp., New York, NY). Independent t-tests were done to ensure group similarities at baseline. Means of the cases and controls were compared using t-tests. A one-way analysis of cross variance (ANOVA) was done when more than two groups were compared (symptom score and stages of DR). P value <0.05 was considered significant. The efficacy of Schirmer, TBUT and CIC was estimated by the area under the curve (AUC) in receiver operating curve (ROC).

Results

In the present study we compared dry eye in diabetics versus controls (n=75) which were matched for age and gender. The mean age did not differ significantly amongst diabetics and controls (paired t-test, P=0.345). The gender was comparable between the groups (Chi-square test, P=0.406). Dry eye symptoms, Schirmer, TBUT and Goblet cells differed significantly between the groups (t-test, P<0.001). Comparison of test values between diabetics and controls is shown in Table 2.

Table 2: Demographic and test values

Parameter	Diabetics	Controls	t test (P value)
Age (years)	51.8±3.4	52.4.9±3.1	0.345
Symptoms	8.0±3.5	1.6±1.6	<0.001
Schirmer (mm)	11.8±6.8	16.8±3.4	<0.001
TBUT (sec)	8.0±1.8	13.1±1.9	<0.001
Nelson Grade	1.7±1	0.4±0.3	<0.001
GCD (cells/mm^2)	290±104	634±124	<0.001

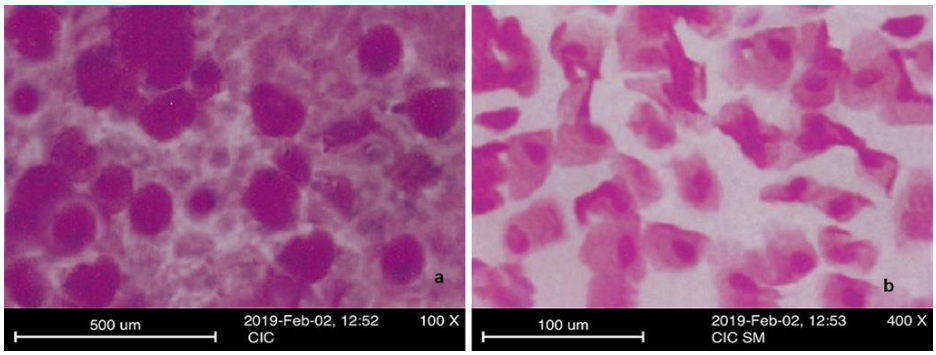

Fig. 1: Photomicrograph showing conjunctival impression cytology: a): Abundant goblet cells in control group; b) Goblet cell loss with squamous metaplasia (decreased nuclear cytoplasmic ratio) in type 2 diabetic retinopathy group with dry eye disease

In DR group, 20(20.67%) had mild, 30(40%) moderate and 25(33.33%) severe diabetic retinopathy, respectively. Severe dry eye symptoms were seen in 6 (7.5%), moderate in 40(53.33%), and mild in 25(33.33%) cases respectively. Four (5.33%) cases were free of dry eye symptoms. In contrast, 4(5.33%) controls had moderate, and 14(18.66%) mild dry eye symptoms, respectively. However, 57(76%) controls were totally free of dry eye symptoms.

In DR group having dry eye symptoms, 24(32%) had abnormal Schirmer, 30(40%) had abnormal TBUT and 17 (22.66%) had abnormal Nelson grade on CIC (Grade 2 and 3); amongst these, (12/70.58%) patients had Nelson grade 2 changes (Fig. 1).

In control group with dry eye symptoms, 5 (27.7%) had abnormal Schirmer, and 13(72.3%) had abnormal TBUT However, none of the controls had abnormal cytology. Nelson grading of controls was Grade 0 in 70(93.3%) and Grade 1 in 5 (6.7%), respectively. Dry disease parameters did not significantly differ amongst different stages of patients with diabetic retinopathy (ANOVA, P=0.478).

Patients with non-proliferative and proliferative DR did differ significantly with respect to DESS score (ANOVA, P=0.645), TBUT (ANOVA, P=0.478), Schirmer (ANOVA, P=0.676) and Nelson Grade (ANOVA, P=0.345), respectively.

The area under the curve (ROC) was uses to assess the efficacy of Schirmer, TBUT and CIC, respectively. According to it, the accuracy was CIC (AUC=0.982) >TBUT (AUC=0.948) >Schirmer (AUC= 0.864), respectively (Fig. 2,4 &4).

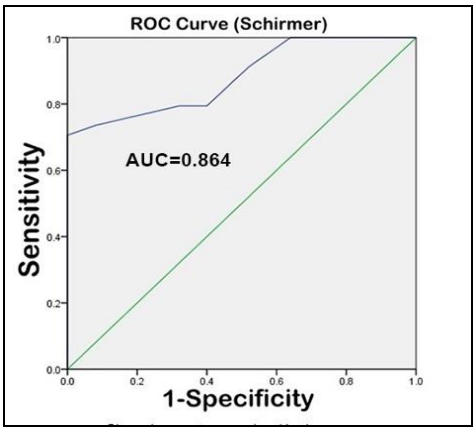

Fig. 2: Receiver operating curve analysis. Area under the curve for schirmer test

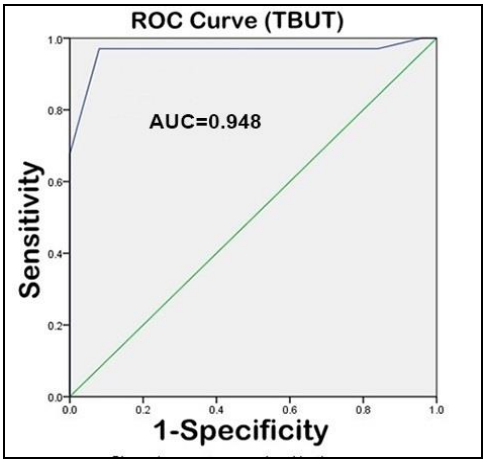

Fig. 3. Receiver operating curve analysis. area under the curve for tear film break up time

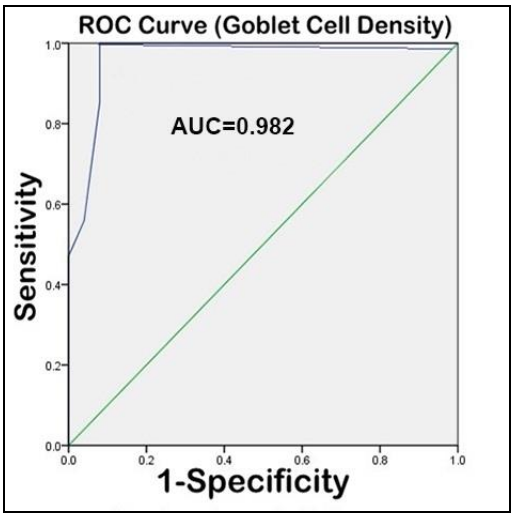

Fig. 3: Receiver operating curve. Area under the curve for goblet cells

Discussion

Diabetes is an important systemic risk factor for dry eye disease. The recognition of the role of inflammation in both dry eye and diabetes has led to a better understanding of the two conditions; researchers are of the opinion that sustained hyperglycemia initiates an inflammatory sequence involving enzymes and other signalling pathways and causes hyperosmolarity of tears, important observation in dry eye disease. Similarly, generation of inflammatory cytokines, interleukins, and tumor necrosis factor has also been implicated in the pathogenesis of DES.[20-21]

In the present study efficacy of Schirmer, TBUT and CIC was assessed patients with DR suffering from dry eye disease. Ninety four percent patients with diabetic retinopathy do experience dry eye symptoms sometime during the disease. In contrast, only (24%) of controls do. However, symptom severity did not differ between the stages (non-proliferative, pre-proliferative and proliferative) of retinopathy (ANOVA, P=0.645). In DR group, tear film stability and tear production were significantly compromised (P<0.001). Moreover, conjunctival cytology was significantly worse in comparison to controls. Conjunctival impression cytology was the most efficacious test to diagnose DED and ocular surface health in diabetic patients.

Many researchers have previously evaluated Schirmer, TBUT and CIC in diabetics, but the results remain poorly standardized due to several factors including different inclusion criteria, sequence of and timing of performing the tests and prior medications.

In a case control study, Kesarwani et al dry eye disease in Indian diabetics. Eighty eyes of 53 diabetics were compared with 50 eyes of 30 healthy controls. Out of these, 42 eyes had diabetic retinopathy. The authors found that as compared with the healthy controls, diabetics showed significantly reduced Schirmer, TBUT measurements and higher rose bengal staining scores. On CIC, reduction of goblet cells and decreased nuclear-cytoplasmic ratio was observed in diabetics. Deranged TBUT, Schirmer and abnormal cytology in this study agreed to the present study.[22]

Yu et al studied the ocular surface in patients suffering from with diabetic retinopathy. The tests studied were TBUT, Schirmer, rose Bengal staining, total tear protein detection, tear sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and corneal topography. The authors found that there was a significant difference in test values between controls, non-proliferative and proliferative diabetic retinopathy groups, respectively. On Pearson's correlation analysis, dry eye symptoms correlated significantly with TBUT and Schirmer test. Fluorescein staining scores and surface asymmetry index also showed a positive and significant correlation (r=0.480). In our study, the severity of dry eye disease did not correlate with the severity of DR. The probable explanation for these observations could be that dry eye signs and symptoms do not go in tandem; not all patients having dry eye symptoms have abnormal tear function tests. A clue to the above-mentioned fact stems from the by the observation in the present study that amongst diabetics, an abnormal Schirmer was observed in 32%, abnormal TBUT in 40 and abnormal cytology in 22% cases despite having dry eye symptoms.[23-24]

Yoon and co-workers studied 94 eyes of patients with type 2 diabetes and 60 eyes of healthy controls. Schirmer, TBUT, CIC and some other tests were evaluated. The authors found that DR group had abnormal test results. The difference was significant between controls and proliferative DR group, controls and non-proliferative DR groups, respectively.[25] In our study, all tear function tests were abnormal in the DR group, but these abnormalities were not related the stage of DR. In contrast, Saito et al reported that tear production did not correlate with the stage of DR, a finding like the one observed in present study.[26] In another study, Dogru et al found that diabetics with poor metabolic control had abnormal TBUT Schirmer. However, this decrease was not related to the duration of diabetes or the stage of retinopathy.[27]

Nepp and co-workers conducted a study to evaluate whether a correlation exists between disease severity in diabetic retinopathy and dryness of eyes. One hundred and forty-four eyes were assessed for Schirmer, TBUT, Rose bengal staining and CIC. The correlation between test parameters was positive (r=0.24) but not significant statistically.[28]

In conclusion, our study indicates that tear film and ocular surface changes in patients with type 2 diabetes mellitus with retinopathy include reduced tear production, tear film stability, and goblet cells with decreased nuclear cytoplasmic ratio. Second, CIC is the most efficacious test to evaluate dry eye in patients with diabetes. The need of the hour warrants studies on a large sample size of patients to establish a direct relationship between diabetes and dry eye disease. All diabetic patients should be examined for tear film and ocular surface changes irrespective of the stage of retinopathy.

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Conflict of Interest: None.

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