Original Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com
OPEN ACCESS

A COMPARATIVE STUDY OF TEAR FILM FUNCTION AND TEAR SECRETION AMONG DIABETICS

Pai Shobha G[1], Pai Sheila R[2], Kini Jyoti[3], Nayak Madhurima K[1*] and Pai G Ajay[4]
[1]Department of Ophthalmology, [2]Department of Physiology, [3]Department of Pathology,
Kasturba Medical College, Manipal University, Mangalore, Karanataka, India.
[4]Grant Medical College, Gymkhana, Marine Lines, Marine Lines, Mumbai, Maharashtra, India.

Received for publication: May 27, 2014; **Revised**: June 12, 2014; **Accepted**: July 27, 2014

Abstract: The present work aims to study the changes in tear film function by studying tear film stability, amount of tear secretion and conjunctival impression cytology among diabetics and to compare with that in non-diabetics. In this prospective case control study, 100 eyes of 50 patients with type II Diabetes Mellitus and 100 eyes of 50 normal patients coming to OPD of a tertiary hospital were chosen. The tests done included Tear film Break Up Time, Rose Bengal staining, Schirmer's test and Conjunctival Impression Cytology (CIP). For CIP, samples were taken from nasal and temporal conjunctiva and smeared on slides and stained with Papanicolau stain and observed under light microscope of 400x magnification. The mean age among diabetics was 53.4±11.2 years and non-diabetics was 48.8±15.3years. The TBUT was significantly reduced in diabetics with a mean of 9.8±7.01 seconds and 12.8±5.71 seconds in non-diabetics ($p<0.005$). 30% patients had values less than 10 seconds. The Rose Bengal test did not show a statistically significant difference. Schirmer's test was positive in 26% in diabetics and 10% in non- diabetics. The mean was 7.7±3.9 in diabetics and 13.4±5.7 in non-diabetics ($p=0.003$). The mean grade of squamous metaplasia in diabetics was 1.58±0.62 and 0.52±0.57 in non-diabetics ($p=0.001$). Corneal sensitivity was found to be significantly lower in diabetics (29.17mm) when compared to controls (54.58mm). Dry eye is a significant ocular surface disorder in diabetes. The tear film parameters were significantly reduced in diabetics but Rose Bengal test did not show much difference.

Key Words: Diabetes, Schirmer's test, Dry eyes, Rose Bengal test, Impression cytology

INTRODUCTION

Diabetes Mellitus has many ocular manifestations in both anterior segment and posterior segment. They range from trivial blepharitis to blinding retinal detachments. Ocular manifestations such as acute orbital infections, glaucoma, refractive changes, cataract, diabetic papillopathy and retinopathy, and palsy of the III cranial nerve have been recognized. However, the awareness of corneal complications has occurred in recent years.

Diabetic patients commonly complain of symptoms like burning or foreign body sensation of the eyes, which can be even intolerable. These are attributed to dry eye state. Prevalence of dry eye in diabetic patients range from 20-55%.[1,2] Abnormal corneal nerve architecture in diabetics has been found to cause decreased corneal sensations.[3] Diabetic keratoepitheliopathy has been recognized and can by itself lead to quantitative and qualitative abnormalities in the tear secretion, poor adhesion of the regenerating epithelial cells and also decreased corneal sensitivity[4]. Poor metabolic control, laser photocoagulation and proliferative diabetic retinopathy have been found to be high risk factors for ocular surface disorder in diabetics.[5]

The purpose of this study is to describe the ocular surface disorder in diabetes by studying the tear film stability, amount of tear secretion and conjunctival impression cytology, which would give us a better insight into diabetes-related changes in the tear film function.

MATERIALS AND METHODS

This was a prospective case control study conducted at a tertiary care hospital in Mangalore, Karnataka. 100 eyes of 50 patients with Type II Diabetes Mellitus and 100 eyes of 50 healthy subjects coming to the out-patient department were recruited for the study. Both groups were age and sex matched. The diabetic status of the patient was assessed by the duration of the disease, the type, the staging of retinopathy by ETDRS criteria and metabolic control by glycosylated hemoglobin and fasting blood glucose levels.

Exclusion criteria included history of topical meditation within the past 6 months, laser treatment, contact lens wear, other ophthalmic surgical procedures and other ocular and systemic diseases that would alter the ocular surface.

After a thorough history, complete external examination of the eyes was done with the help of a slit lamp, which included tear film examination – marginal tear meniscus, increase in mucous strands and

***Corresponding Author:**
Dr. Madhurima K Nayak,
Department of Ophthalmology,
Kasturba Medical College,
Manipal University, Mangalore,
Karnataka, India.

debris, corneal abnormalities like punctuate epitheliopathy, filamentary keratitis and mucus plaques staining with Rose Bengal dye. Special tests carried out included:

A. Tear Film Break-up Time (TBUT)

It is a measure of the time elapsed from a blink until the appearance of the first randomly distributed dry spot in the precorneal film. A TBUT value of <10sec was considered abnormal.

B. Rose Bengal Staining (RBS)

This dye has an affinity for dead and devitalized epithelial cells and mucus. The typical staining pattern in dry eye consists of two triangles with their bases at the limbus nasally and temporally. Corneal filaments and plaques are also shown up more clearly after instillation of the dye, van Bijsterveld scoring is then done:

3 zones are described namely:

a) Nasal bulbar conjunctiva -Mild
b) Cornea -Moderate
c) Temporal bulbar conjunctiva -Late

Grading is 0-3 in each zone, where 0 indicates no stain, 1= < 1/3 area, 2= 1/3 – 2/3 area and 3 = confluent stain >2/3 area.

If the total exceeds 3.5, it is positive for dry eye. Severity was scored by multiplying the area score by the density score. The product is an index of corneal surface damage.

C. Schirmer's Test

This measures the amount of wetting of a special (Whatman) filter paper which is 5mm wide and 35mm long.

Test I-Without an anaesthetic-Total secretion (Basal + Reflex)
Test II -With an anaesthetic – Basal secretion
Test III-After stimulation of nasal mucosa – Reflex secretion

A reading less than 5mm of wetting was referred to as dry eye. (Normal >15mm, Borderline 5-10mm, Dry eye < 5mm)

D. Conjunctival impression cytology (CIC)

Conjunctival impression cytology is a non-invasive and painless technique, easy to perform and provides conjunctival and corneal information, cellular morphology, cell to cell relationship, interaction between epithelial cells and other cellular components and allows 1-3 cell layers of conjunctiva and cornea to be removed. CIC is done to study squamous metaplasia of conjunctival epithelium. Samples were taken from nasal and temporal conjunctiva and smeared on slides. The slides were then stained with Papanicolaou method. This method consists of a nuclear stain and two counter stains. Following staining, the slides were seen under light microscope under a magnification 400x. The cytological features were staged according to the following staging system.[6]

Stage 0: Normal conjunctival epithelium, moderate number of goblet cells scattered among uniform non goblet epithelial cells which had blue green cytoplasm. N/C ratio 1:1.

Stage 1: Early loss of goblet cells without keratinization: decreased GCD: mild enlargement of non-goblet epithelial cell which had blue green cytoplasm: N/C ratio 1:2 to 1:3.

Stage 2: Total loss of goblet cells without keratinization no goblet cells observed: all epithelial cells moderately enlarged and flattened with cytoplasm blue green or ink in colour and N/C ratio1:4.

Stage 3: Early and mild keratinization: all epithelial cells markedly squamoid with metachromatic change of cytoplasm to pinkish color; some epithelial cells contain visible keratin filaments. N/C ratio is 1:6 due to flattening and mild pyknotic change of nucleus.

Stage 4: Moderate keratinization; in the midst of squamous and metachromatic large epithelial cells described above, more cells contained densely packed keratin filaments, keratohyalin granules, pyknotic nuclei: N/C ratio 1:8.

Stage 5: Advanced keratinization; more kertinized cells with shrunken cytoplasm and densely packed keratin filaments in which nuclei are markedly pyknotic, lytic, or enucleated and sometimes aggregated in keratinized debris.

RESULTS

This study compared 50 Diabetic with 50 Non-Diabetic patients. Both the groups were age and sex matched. Sex and age distribution in both the groups is depicted in figures 1 and 2. The mean age in diabetic group was 53.4±11.2 years and 48.8±15.3years non-diabetic group. Most patients belonged to the age group of 40-60 years with 38 patients in diabetics and 17 in non-diabetic group.

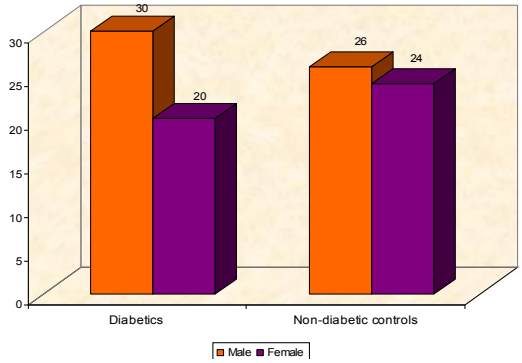

Figure 1: Sex distribution in the diabetic and the control group.

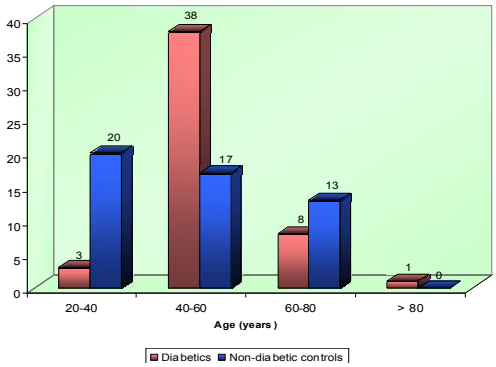

Figure 2: Age distribution in the diabetic and the non-diabetic group. Maximum patients belonged to the age group of 40-60 years with 38 patients in diabetics and 17 in non-diabetic group.

Most of the patients were diagnosed to have moderate NPDR (30%), followed by PDR with high risk characteristics (22.5%), mild NPDR (17.5%), PDR (12.5%), severe NPDR (10%), no diabetic retinopathy (7.5%) and very severe NPDR (0%).

The tear film break up time of the was found to significantly reduced in the Diabetic group with 15 (30%) patients showing less than 10 seconds as compared to 7 patients (14%) in the non-diabetic group. [Fig. 3] The overall mean TBUT in the diabetic group was found to be 9.8±7.01 seconds. The average mean of the non-diabetic group was 12.8±5.71 seconds. This difference was found to be statistically significant using student t-paired test (p-value<0.05). Chi square test showed a significant association of 3.79. Fischer test showed a significant difference of tear film break up time in various grades of diabetic retinopathy. (F=41.84, p<0.00)

The Rose Bengal test did not show a statistically significant difference in both the groups. It was found to be positive in 2 patients (4%) and negative in 48 patients (96%) in both the groups. [Fig. 4] Also, it was not statistically different in various groups of diabetic retinopathy. (p=0.848)

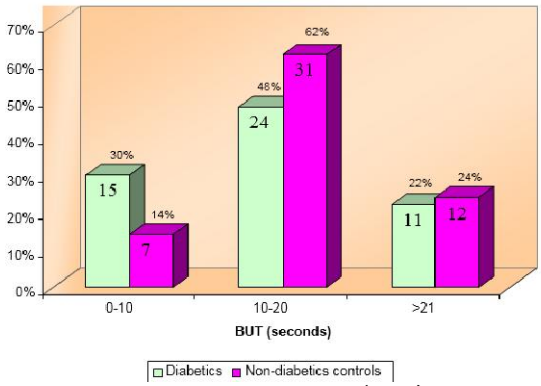

Figure 3: Tear film breakup time (BUT) in diabetics and non-diabetic controls. The overall mean of the diabetic group was found to be 9.8±7.01 seconds. The average mean of the non-diabetic group was 12.8±5.71seconds. (Significant)

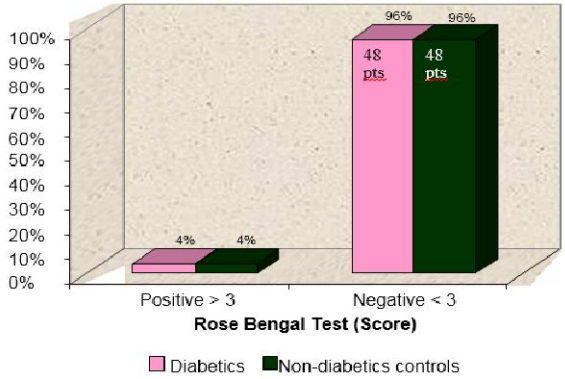

Figure 4: The Rose Bengal test did not show a statistically significant difference in both the groups.

Schirmer's test values were found to be significantly reduced in diabetic group. It was positive in 13 patients (26%) in the diabetic group and 5 patients (10%) in non-diabetic group. The mean for the diabetic group was 7.7±3.9 mm. The mean for the non-diabetic group was 13.4±5.7 mm. This difference was found to be statistically significant using student t-paired test. (p=0.03) and Chi square value 4.34 showing significant association. [Fig. 5]

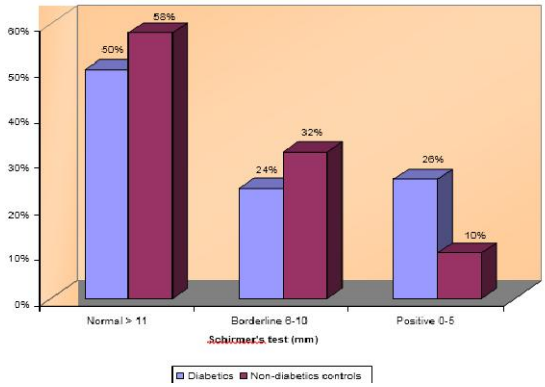

Figure 5: Schirmer's test values significantly reduced in diabetic group. It was positive in 13 patients (26%) in the diabetic group and 5 patients (10%) in non-diabetic group.

Fischer test showed a significant difference in Schirmer test in various grades of retinopathy. (F=63.832, p<0.00)

The conjunctival impression cytology study showed more frequent and pronounced signs of squamous metaplasia. The mean grade in diabetics was 1.58±0.62 and that in non-diabetics was 0.52± 0.57 .The p value was 0.001 which showed highly significant association, although there were no statistically significant differences among various grades of diabetic retinopathy. (p=0.392) This suggests that squamous metaplastic changes and goblet cell deficiency is more common in diabetics.

Mean corneal sensitivity was 29.17mm in diabetics whereas it was 54.58mm in non-diabetics and this difference was statistically very highly significant. (p<0.001) Also, corneal sensitivity decreased as the severity of diabetic retinopathy increased and this was found to be statistically significant. (F=60.411, p<0.001) [Table 1]

Table 1: Correlation of corneal sensitivity to severity of diabetic retinopathy

Type of diabetic retinopathy	Mean corneal sensitivity (in mm)	Std deviation
No diabetic retinopathy	37.833	4.4903
Mild NPDR	36.713	4.268
Moderate NPDR	33.458	3.764
Severe NPDR	25.500	2.563
PDR	21.700	3.198
PDR with high risk character	20.500	2.5

F=60.411, p<0.001

The various tests for dry eye were correlated to each other and the results are shown in table 2. Among the cases, Schirmer test correlated positively with BUT (r=0.779, p=0.00) and corneal sensitivity (r=0.804, p=0.00), whereas there was a negative correlation to Rose Bengal test and this was insignificant. (p=0.291) The Rose Bengal test correlated negatively to impression cytology scores. (r=-0.476, p=0.00) The BUT correlated positively to corneal sensitivity and negatively to Rose Bengal test, however there was no statistical significance.

Table 2: Correlations among various tets for dry in cases and controls

Group			TBUT	RBS	Impression cytology	Corneal sensitivity
Study	Schemer	R	.779**	-.120	.019	.804**
		P	.000	.291	.865	.000
		N	80	80	80	80
	TBUT	R		-.093	-.083	.681**
		P		.413	.463	.000
		N		80	80	80
	RBS	R			.476**	.057
		P			.000	.617
		N			80	80
	Impression cytology	R				.051
		P				.656
		N				80
Control	Schirmer	R	.721**	-.087	-.172	-.094
		P	.000	.445	.127	.408
		N	80	80	80	80
	TBUT	R		-.166	-.148	.005
		P		.142	.192	.964
		N		80	80	80
	RBS	R			.347**	.028
		P			.002	.803
		N			80	80
	Impression cytology	R				-.013
		P				.912
		N				80

**. Correlation is significant at the 0.01 level (2-tailed).

Among the controls, Schirmer test correlated positively to BUT with statistical significance (r=0.721, p=0.00).

DISCUSSION

We assessed TBUT, keratoepitheliopathy scorings Schirmer I test for tear secretion and conjunctival impression cytology in NIDDM patients and compared the results with those in the normal subjects.

The tear film parameters were significantly reduced in the diabetic patients, whereas the Rose Bengal test did not show much significance when compared with non-diabetic controls. This indicates that dry eye in diabetic patients is a significant feature of diabetic ocular surface disorder.

Schirmer test values are significantly reduced in diabetic group. Also, Schirmer test correlated to the tear film break up time and corneal sensitivity

significantly. Schirmer test is rather a rough screening test for the detection of tear hypo-secretion. The sensitivity could be shown to be as low as 10-30%. But, when performed in a standardized procedure, the findings of a statistically significant difference between the Schirmer test values of 2 groups may provide valuable information on the amount of stimulated tear secretion.[7,14]

TBUT is the only direct test of tear film stability available and is extremely valuable when performed accurately. Studies have shown that patients with poor control and diabetes have lower TBUT scores when compared to patients with good diabetic control but it did not relate to the duration of diabetes or status of retinopathy (69.3% poor TBUT scores). The decrease in TBUT showed the marked instability of the tear film in diabetes. Reduction in goblet cell number may account for the shortening of the TBUT and the instability of the tear film as a result of decreased mucin production because goblet cells are the major source of mucin in the tear film. TBUT has a sensitivity of 82% and a specificity of 86%. TBUT is an invaluable and direct test of tear film stability when performed carefully.[8] Low tear production seen in some diabetic patients is related to dysfunction of the ANS.[9] Beaver Dam Eye study showed Odds ratio of 1.38 towards prevalence of dry eye syndrome in diabetes.[9]

Conjunctival epithelium can be observed by Rose Bengal staining. Rose Bengal is a vital stain, staining degenerating cells. It is an important diagnostic aid and is sensitive indicator for the level of protection of the ocular surface. RBS, though subjective, is one of the most specific tests (95%) when testing in specificity control subjects along with impression cytology (98.8%). [10] Therefore increased RBS correlates with decreased goblet cell density and mucosal epithelial deficiency.[11]

Mucin is a glycoprotein that covers and protects mucosal surfaces and ocular surface epithelium. Mucin is secreted by goblet cells and some exogenous glands.[4.12] In the eye, conjunctival goblet cells are thought to be the main source of tear mucin which plays an important role in increasing the tear film stability and wettability of the ocular surface. The goblet cell population of the conjunctiva reflects the degree of differentiation or maturation of the conjunctival epithelium, which in turn reflects the overall health of the ocular surface.[13]

Epithelial damage will deteriorate the tear film stability by accelerating the process of drying of the ocular surface. The accurate and extended examination of the ocular surface by means of CIC appears to be useful to identify those "marginally" dry eyes, which at the Schirmer test show theoretically "normal" values with a wet ocular surface and which are often misdiagnosed.[14]

Staging of squamous metaplasia of conjunctival epithelium enables us to estimate the severity of disease involvement and evaluate the progression or improvement of the disease. Because CIC is rather simple, relatively non-invasive and repeatable, it can be applied to clinical use.[6] Nelson et al (1983) stated that average goblet cell densities lower than 500 cells/sq.mm of inferior palpebral epithelium would suggest that there was an ocular surface disorder.[11] Impression cytology is a better predictor of mild to moderate dry eye syndrome than tear ferning.[15] Our study demonstrated a significant negative correlation between Rose Bengal test and the grade of squamous metaplasia. Hence the keratoepitheliopathy may be due to reduced sensation of cornea.

Our study showed a significant decrease in corneal sensation among the diabetics when compared to non-diabetics. Also, a significant decrease in corneal sensation was noted with severity of retinopathy. Decrease in the nerve fibre bundle count has been proposed for reduced corneal sensations.[16] Decreased corneal sensations correlate with severe polyneuropathy. [16] This seems to be a limitation in our study, since we did not record neuropathy for correlation.

All the tests for dry eye done in this study were correlated with the severity of diabetic retinopathy. Schirmer test, tear film break up time and corneal sensations were significantly different for various grade of diabetic retinopathy. The values of Schirmer test, BUT and corneal sensations decreased as the severity of retinopathy increased. Older studies have shown no correlation between the tear secretion volume and stage of retinopathy, although corneal sensation has been negatively correlated with degree of severity of diabetic retinopathy. [17,8]

This study shows that patients with Non-Insulin Dependent Diabetes have decreased tear stability and secretion, keratoepitheliopathy with conjunctival squamous metaplasia suggesting an ocular surface disease.

The tear film parameters were significantly reduced in diabetics though Rose Bengal staining did not show much difference. This suggests that dry eye in diabetics is a significant feature of ocular surface disorder.

Another limitation of our study is that we did not evaluate the subjective symptoms in patients and

thus cannot correlate our results to the amount of discomfort experienced by the patient.

Diabetic patients should be examined for tear film and conjunctival surface disorder along with routine fundus examination. The severity of dry eye correlates to tear film secretion and volume as demonstrated by decreased Schirmer and BUT; well with the severity of retinopathy. Hence, we advocate Schirmer test and Tear film break up time tests to be routinely done for diabetic patients and especially those with symptoms. Conjunctival impression cytology being a simple and better predictor of mild to moderate dry eye syndrome should be incorporated into our clinical practice. Rose Bengal test was not found to be significantly different among the two groups in our study.

REFERENCES

1. Manaviat MR, Rashidi M, Afkhami-Ardekani M, Shoja MR. Prevalence of Dry Eye Syndrome and Diabetic Retinopathy in Type 2 Diabetic Patients. BMC Ophthalmology 2008; 8:10.
2. Kaiserman I, Affiliations Department of Ophthalmology, Hadassah University Hospital, Jerusalem. Inquiries to Dr. Igor Kaiserman, Department of Ophthalmology, Hadassah University Hospital, P.O.B. 12000, IL-91120 Jerusalem, Israel; fax: (+972) 26416242
3. Kaiserman N, Department of Ophthalmology, Hadassah University Hospital, Jerusalem
4. Nakar S, Department of Family Medicine, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv Department of Family Medicine, Clalit Health Services, Central District, Rehovot, Israel
5. Vinker S. Dry Eye In Diabetic Patients. Am J Ophthalmol 2005; 139: 498-503.
6. Rosenber ME, Tervo MT, Immonen IJ, Müller LJ, Riska CG, Vesaluoma MH. Corneal Structure and Sensitivity in Type 1 Diabetes Mellitus. Invest Ophthalmol Vis Sci 2000; 41:2915-21.
7. Yoon KC, Im K, Seo MS. Changes Of Tear Film And Ocular Surface In Diabetes Mellitus. Korean J Ophthalmol 2004; 18:168-74.
8. Ozdemir M, Buyukbese M.A, Cetinkaya A, Ozdemir G. Risk Factors For Ocular Surface Disorders In Patients With Diabetes Mellitus. Diabetes Res Clin Pract 2003; 59:195–9.
9. Tseng SCG. Staging Of Conjunctival Squamous Metaplasia by Impression Cytology. Ophthalmology 1985; 92:6.
10. Goebbels M. Tear Secretion and Tear Film Secretion In Insulin Dependent Diabetes. Br J Ophthalmol 2000; 84:19-21.
11. Dogru M, Katakami C, Inoue M. Tear Function and Ocular Surface Changes In Non-Insulin Dependent Diabetes Mellitus. Ophthalmology 2001; 108:586-92.
12. Liu J, Shi B, He S, Yao X, Willcox M, Zha Z. Changes to Tear Cytokines of Type 2 Diabetic Patients with or Without Retinopathy. Clin Exp Rheumatol 1994; 12:375-80.
13. Nelson JD. Impression Cytology. Cornea 1988; 7:71-81.
14. Rivas L, Rodriguez JJ, Alvarez MI, Oroza MA, Castillo JM. Correlation between Goblet Cell Deficiency and Tear Function Parameters in Sjogren's syndrome. Acta Ophthalmologica 1993; 71:353-9.
15. Dogru M, Katakami C, Inoue M. Tear Function and Ocular Surface Changes In Non-Insulin Dependent Diabetes Mellitus. Ophthalmology 2001; 108:586-92.
16. Kinoshita S, Kiorpes TC, Friend J, Thoft RA. Goblet Cell Density In Ocular Surface Disorder: A Better Indicator Than Tear Mucin. Arch Ophthalmol 1983;101:1284-7
17. Rolando M, Terrarna F, Giordano G, Calabria G. Conjunctival Surface Damage Distribution In KCS. Ophthalmologica 1990; 200:170-6.
18. Jackson JA, Perrigin JA, Relationship of Impression Cytology and Tear Ferning to Reports of Dry Eye. J Am Optom Assoc 1999; 70:187-92.
19. Rosenberg ME, Tervo TMT, Immonen IJ, Müller LJ, Riska CG, Vesaluoma MH. Corneal Structure and Sensitivity in Type 1 Diabetes Mellitus. Invest. Ophthalmol. Vis. Sci. 2000;41(10):2915-21
20. Akira S, Kyoko S, Jun S, Mitsuru S. Study of tear secretion volume and corneal sensitivity in Diabetes Mellitus. Journal of Nihon University Medical Association 2004; 63 (10):446-9.

Source of support: Nil
Conflict of interest: None Declared