Subbasal Nerve Plexus Changes in Type 2 Diabetes Mellitus Correlate with Tear Levels of IGFBP-3

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INTRODUCTION

In vivo confocal microscopy (IVCM) is a non-invasive clinical tool that allows for visualization of the corneal subbasal nerve plexus (SBNP, Fig. 1).[1] Growing evidence supports that IVCM can readily detect early nerve loss in patients with Type 2 Diabetic Mellitus (T2DM) prior to the development of diabetic peripheral neuropathy.[2,3] These findings suggest that changes in the subbasal nerve plexus may provide an early, surrogate marker for the onset of peripheral neuropathy.

Increasing studies are investigating the use of tear film proteins that correlate with corneal nerve changes as potential biomarkers in diabetic disease. Our prior studies have demonstrated that the primary insulin-like growth factor (IGF)-1 binding protein, IGF-binding protein-3 (IGFBP-3), is elevated in the diabetic tear film and is produced by corneal epithelial cells cultured in high glucose.[4]

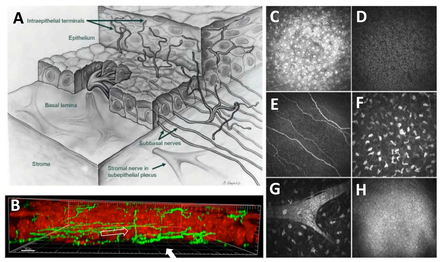

FIGURE 1: Anatomical structure of the cornea. (A) Schematic showing the location of the SBNP running just under the basal corneal epithelium. Intraepithelial terminals branch from the SBNP and run anteriorly toward the corneal surface.[5] (B) 3D reconstruction of the murine corneal epithelium (propidium iodide staining in red) and corneal epithelial nerves (β3-tubulin in green). Filled arrow indicates the SBNP, open arrow an intra-epithelial terminal.[6] (C-H) IVCM images of the human cornea: (C) surface epithelial cells; (D) basal epithelial cells; (E) SBNP; (F) stroma; (G) deep stromal nerve; and (H) corneal endothelium.

PURPOSE

The purpose of this study was to analyze tear levels of IGFBP-3 in patients with T2DM and healthy controls; and to determine if the level of IGFBP-3 could be used as a novel biomarker for monitoring corneal nerve damage in diabetes.

MATERIALS AND METHODS

A total of 40 patients were recruited into two study groups, detailed in Table 1. Each group was matched for age, gender and obesity status.

Table 1: Study Test and Control Groups

	Description	Inclusion Criteria
Group A	T2DM	Physician diagnosis
Group B	Control	No history of T2DM

Outcome measures:

- Review of medical history, including use of topical and oral medications
- Serology testing for HbA1c, lipid panel and hsCRP
- Anthropometric measurements including height, weight, neck, waist and hip circumference
- Ocular surface disease index (OSDI) questionnaire for assessment of dry eye
- Tear collection using glass microcapillary tubes
- Complete ocular examination, including dry eye testing and a dilated fundus exam
- Cochet Bonnet Aesthesiometry to assess corneal sensitivity
- *In vivo* confocal microscopic examination of the SBNP using a modified HRT II confocal microscope with a Rostock Cornea Module (Heidelberg Instruments, Heidelberg, Germany)[7]

RESULTS

Table 2: Patient Demographics

	Type 2 DM	Control	P value
Age (years)			
Mean ± SD	58.8 ± 10.2	53.3 ± 9.7	
Range	32 – 75	34 – 75	P=0.065
Gender			
Male	6 (33.3%)	10 (45.0%)	
Female	12 (66.7%)	12 (55.0%)	P=0.111
Smoking status			
Smoker	2 (11.2%)	3 (14.0%)	
Non-Smoker	16 (88.8%)	19 (86.0%)	P=0.669
BMI*			
Mean ± SD	33.5 ± 6.3	31.3 ± 4.5	
95% CI	30.3, 36.7	29.4, 33.2	P=0.222

*BMI: body mass index

Table 3: Dry Eye Test Results

	Type 2 DM	Control	P value
TFBUT (sec)§	5.5 (3.1 – 30.0)	4.8 (1.3 – 30.0)	P= 0.765
Schirmer's Score (mm)*	19.1 ± 8.2 9.6, 28.6	17.8 ± 7.9 10.1, 24.7	P= 0.510
NaFl Staining§	1 (0 – 9)	0.5 (0 – 7)	P= 0.881
OSDI Score§	10.4 (0 – 56.3)	3.2 (0 – 64.6)	P= 0.256

Data represented as:
*Mean ± standard deviation for normal distribution
95% CI: lower limit, upper limit
§Median (min – max) for non-normal distribution

Abbreviations:
TFBUT: tear film break up time
NaFl: sodium fluorescein
OSDI: ocular surface disease index

Table 4: Serological and Anthropometric Data (mean ± SD)

	Type 2 DM	Control	P value
Neck Circumference* (inches)	15.7 ± 1.2 15.1, 16.3	15.3 ± 1.5 14.7, 15.9	P=0.317
Waist Circumference* (inches)	43.0 ± 6.7 39.7, 46.3	39.4 ± 4.7 37.4, 41.4	P=0.055
Hip Circumference* (inches)	45.1 ± 4.9 42.7, 47.5	43.9 ± 4.1 42.2, 45.6	P=0.375
Waist to Height Ratio*	0.6 ± 0.1 0.66, 0.74	0.6 ± 0.1 0.66, 0.74	P=0.078
HbA1c (%)§	7.5 (5.9 – 9.9)	5.8 (4.8 – 6.3)	P<0.001**
hsCRP§	3.6 (0.4 – 63.9)	2 (0.2 – 18.1)	P=0.808
Cholesterol* (mg/dL)	177.8 ± 51.5 152.3, 203.3	200.3 ± 34.4 185.8, 214.8	P=0.126
HDL§ (mg/dL)	44 (30 – 81)	55 (32 – 119)	P=0.040**
Triglyceride§ (mg/dL)	169 (73 – 366)	86 (65 – 252)	P=0.004**
Systolic BP § (mmHg)	151 (115 – 220)	133 (61 – 125)	P=0.206
Diastolic BP* (mmHg)	90.1 ± 18.7 80.8, 99.4	89.8 ± 16.7 82.7, 96.9	P=0.839

Data represented as:
*Mean ± standard deviation for normal distribution
95% CI: lower limit, upper limit
§Median (min – max) for non-normal distribution

Reference ranges for our testing laboratory:
Cholesterol, total 125-200 mg/dL
HDL cholesterol > or = 40 mg/dL
Triglycerides <150 mg/dL
hsCRP levels 3.1 – 10.0 higher relative cardiac risk
HbA1c <5.7% no diabetes; 5.7% - 6.4% pre-diabetes or well-controlled; >=6.5% diabetes

***Mann-Whitney Rank Sum Test

CORNEAL NERVE STRUCTURE AND FUNCTION

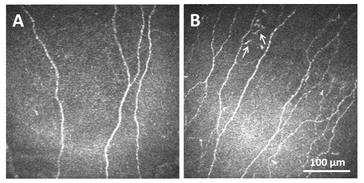

Figure 2: Representative IVCM images of the subbasal nerve plexus for each study group. Note the presence of dendritic cells in some images (arrows). Scale bar: 100 µm. **(A)** T2DM; **(B)** control.

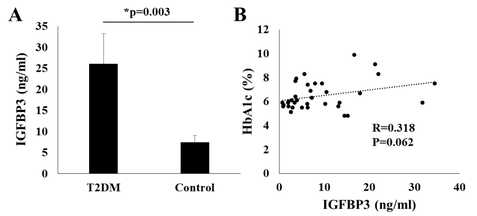

Figure 3: Tear levels of IGFBP-3. (**A**) ELISA analysis of basal tears showed an increase in tear levels of IGFBP-3 in patients with diabetes compared to non-diabetic controls (P = 0.003, t-test). (**B**) Linear regression analysis showed no correlation between HbA1c levels and tear concentration of IGFBP-3 (R = 0.318, P = 0.062).

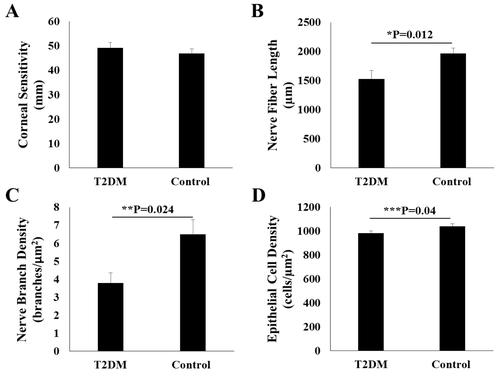

Figure 4: Corneal nerve structure and function. (**A**) Corneal sensitivity was assessed in the inferior mid-peripheral cornea, approximately 3 mm above the limbus. There was no detectable difference in corneal sensitivity between groups (P = 0.421, t-test). (**B**) Nerve fiber length was significantly reduced in the diabetic group compared to controls (P = 0.012, t-test). (**C**) Nerve branch density was also significantly reduced in the diabetic group (P = 0.024, Mann-Whitney rank sum test). (**D**) Basal corneal epithelial cell density showed a small, but significant reduction in the number of cells per µm²(P = 0.04, t-test).

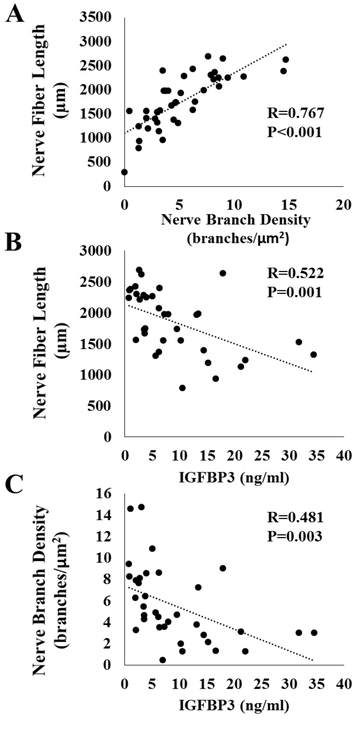

Figure 5 (left): The relationship between nerve fiber morphology and tear levels of IGFBP-3. (**A**) Regression analysis revealed a high correlation between nerve fiber length and nerve branch density (R = 0.767, P < 0.001). (**B**) There was a strong correlation between nerve fiber length and IGFBP-3 (R= 0.522, P = 0.001). (**C**) There was also a good correlation between nerve branch density and IGFBP-3 (R = 0.481, P = 0.003).

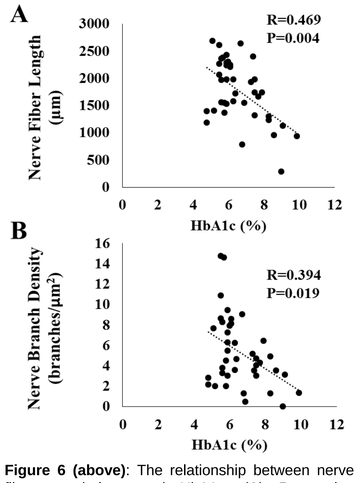

Figure 6 (above): The relationship between nerve fiber morphology and HbA1c. (**A**) Regression analysis showed a moderate correlation between nerve fiber length and HbA1c (R= 0.469, P = 0.004). (**B**) There was a weak correlation between nerve branch density and HbA1c (R = 0.394, P = 0.019).

- There were no differences in corneal sensitivity or dry eye parameters between groups (Table 3 & Fig. 4).
- Dry eye clinical findings were within normal range for all patients; thus dry eye does not account for the SBNP changes (Table 3).
- IGFBP-3 levels in tears from patients with T2DM were 3.5 times higher than controls (P<0.05, Fig. 3A).
- HbA1c was not correlated with IGFBP-3 (R=0.318, P=0.062, Fig. 3B).
- Tear levels of IGFBP-3 were correlated with nerve fiber length (R=0.522 P=0.001) and nerve branch density (R=0.481 P=0.003, Fig. 5B & C).
- IGFBP-3 was more tightly correlated with nerve changes than HbA1c (Fig. 6A & B).
- Consistent with our animal models, there was a decrease in corneal basal epithelial cell density in T2DM compared to controls (P=0.04, Fig. 4D).

DISCUSSION

This is the first report on the relationship between T2DM induced ocular nerve damage and tear levels of IGFBP-3. Importantly, this study demonstrates that tear levels of IGFBP-3 are higher in patients with T2DM and is associated with corneal nerve loss in diabetes. Changes in tear levels of IGFBP-3 were not due to tear changes induced by dry eye. These data suggest that tear levels of IGFBP-3 may represent a novel biomarker for assessing risk for diabetic complications in the eye. Further studies are need to stratify tear levels of IGFBP-3 with severity of disease and to test for correlations between tear and serum levels of IGFBP-3 and diabetic peripheral neuropathy.

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SUPPORT

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