Interactive Effects of Obstructive Sleep Apnea and Type 2 Diabetes Mellitus on Corneal Nerves: Preliminary Findings

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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]

A prior study in the UK suggests a relationship exists between obstructive sleep apnea (OSA) and the severity of complications in patients with T2DM, including a higher prevalence of diabetic peripheral neuropathy.[4] Interestingly, the severity of diabetic neuropathy in this patient cohort was related to the severity of OSA and not the duration of diabetic disease.

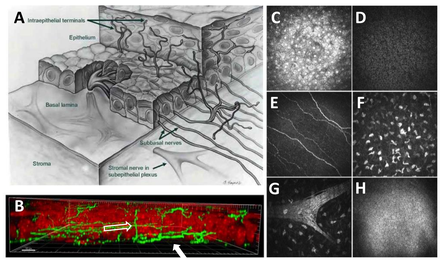


FIGURE 1: Anatomical structure of the cornea. (A) Schematic showing the location of the SBNP running just under the basal corneal epithelium. Intraepithelial terminals branch from the SBNP and run anteriorly toward the corneal surface. (B) 3D reconstruction of the murine corneal epithelium (propidium iodide staining in red) and corneal epithelial nerves (β3-tubulin in green). Filled arrow indicates the SBNP, open arrow an intra-epithelial terminal.[5] (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 is to use IVCM to examine changes in the corneal and retinal nerve fiber layer in patients with T2DM and OSA, compared to patients with either T2DM or OSA and normal, healthy controls. Early, interim results are reported.

MATERIALS AND METHODS

A total of 184 patients will be recruited into 1 of 4 study groups, detailed in Table 1. Each group will be matched for age, gender and obesity status.

Table 1: Study Test and Control Groups

	Description	Inclusion Criteria
Group A	OSA + T2DM	Group B and Group C inclusion criteria
Group B	OSA	Physician diagnosis AND an overnight polysomnogram within the last five years
Group C	T2DM	Physician diagnosis
Group D	Control	No history of OSA or T2DM

Outcome measures:

- Serology testing for HbA1c, lipid panel and hsCRP
- Questionnaires:
  - STOPBANG survey to assess risk for OSA
  - CPAP compliance survey (for OSA+ patients)
  - Ocular surface disease index (OSDI) survey for dry eye
- Complete ocular examination, including dry eye testing and a dilated fundus exam
- Cochet Bonnet Aesthesiometry to assess corneal touch thresholds
- *In vivo* confocal microscopic examination of the SBNP using a modified HRT II confocal microscope with a Rostock Cornea Module (Heidelberg Instruments, Heidelberg, Germany)
- Ocular coherence tomography (Spectralis, Heidelberg, Germany) of the retinal nerve fiber layer (RFNL) and macula

RESULTS

Table 2: Patient Demographics

	Group A	Group B	Group C	Group D	P value
Age (years)					
Mean ± SD	*65.0 ± 7.9	51.9 ± 16.2	59.4 ± 11.7	*50.1 ± 11.2	*P<0.05
range	54 - 79	26 - 73	32 - 75	34 - 74	
Gender					
Male	4 (50.0%)	**8 (88.9%)	6 (46.2%)	8 (47.1%)	**P<0.001
Female	4 (50.0%)	1 (11.1%)	7 (53.8%)	9 (52.9%)	
Race					
Black* **	2 (25.0%)	2 (22.2%)	4 (30.8%)	6 (35.3%)	
Caucasian* **	5 (62.5%)	7 (77.8%)	8 (61.5%)	9 (52.9%)	
Asian	0 (0%)	0 (0%)	0 (0%)	2 (11.8%)	***P<0.001
Native American	0 (0%)	0 (0%)	1 (7.4%)	0 (0%)	
Non-specified	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	
Smoking Status					
Smoker	****0 (0%)	1 (11.1%)	2 (15.4%)	2 (11.8%)	
Non-smoker	8 (100%)	8 (88.9%)	11 (84.6%)	15 (88.2%)	****P=0.002
BMI					
Mean ± SD	36.9 ± 7.4	36.8 ± 6.7	34.0 ± 6.3	31.1 ± 5.2	P=0.103

Table 3: Serological and Anthropomorphic Data (mean ± SD)

	Group A	Group B	Group C	Group D	P value
Serology					
HbA1c	7.1 ± 0.6*	5.6 ± 0.4	7.7 ± 1.2*	5.8 ± 0.4	*P<0.001
hsCRP	11.9 ± 21.1	7.3 ± 11.2	9.1 ± 16.7	4.9 ± 5.2	P=0.893
Risk for OSA					
STOPBANG	5.9 ± 1.4**	4.7 ± 1.3**	4.3 ± 1.4	2.3 ± 1.5	**P<0.001
Anthropometric Measurements					
Neck Circumference (inches)	16.7 ± 2.4	16.8 ± 1.6	15.7 ± 1.2	15.5 ± 1.5	P=0.249
Waist Circumference (inches)	46.3 ± 10.6	44.3 ± 4.7	42.4 ± 6.9	39.8 ± 5.2	P=0.088
Hip Circumference (inches)	48.2 ± 8.9	46.4 ± 5.5	44.9 ± 4.1	43.6 ± 4.4	P=0.294
Waist to Height Ratio	0.69 ± 0.14	0.65 ± 0.09	0.65 ± 0.10	0.60 ± 0.08	P=0.199

Table 4: Dry Eye Test Results

	Group A	Group B	Group C	Group D	P value
TFBUT (sec)	8.6 ± 4.0	15.0 ± 26.4	10.1 ± 8.8	12.7 ± 19.1	P=0.776
Schirmers Score (mm)	17.6 ± 9.3	14.1 ± 7.8	18.9 ± 9.0	17.1 ± 8.2	P=0.557
NaFl Staining	1.5 ± 1.2	3.1 ± 3.5	2.14 ± 2.5	1.8 ± 2.4	P=0.346
LG Staining	5.7 ± 4.3	4.0 ± 3.4	2.7 ± 4.6	5.4 ± 4.7	P=0.172
OSDI score	12.2 ± 21.2	22.6 ± 26.5	18.4 ± 20.1	12.7 ±17.3	P=0.756

TFBUT: Tear Film Breakup Time measured in seconds; NaFl: sodium fluorescein; LG: Lissamine green; OSDI: Ocular Surface Disease Index questionnaire. Data represented as mean ± standard deviation.

DRY EYE STATUS

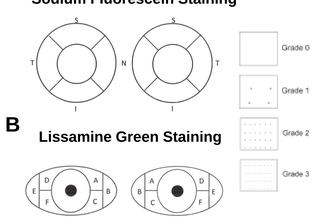


Figure 2: Dry eye exam. Patients were tested for clinical signs of dry eye by measuring tear film stability using fluorescein, aqueous tear production using Schirmers strips without anesthesia, and assessment of ocular surface damage using fluorescein and lissamine green staining. (A) Corneal staining was assessed in 5 different quadrants using a grading scale of 0 – 3. Scores from each quadrant were summed per eye. (B) Conjunctival staining was similarly assessed in 6 different quadrants.

CORNEAL NERVE STRUCTURE AND FUNCTION

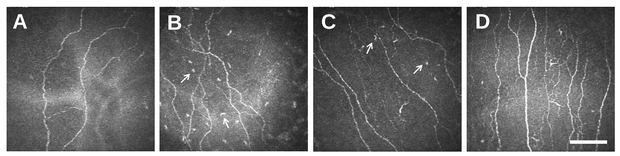


Figure 3: Representative IVCM images of the subbasal nerve plexus for each study group. Note the presence of dendritic cells in some images (arrows). Scale bar: 100 µm. (A) OSA + T2DM; (B) OSA only; (C) T2DM only; (D) control.

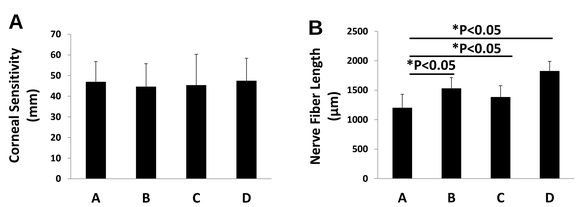
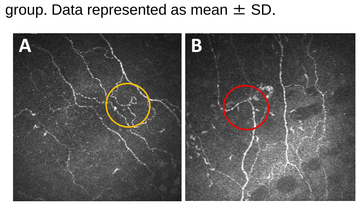


Figure 4: Corneal nerve structure and function. (A) Cornea sensitivity was assessed in the inferior mid-peripheral cornea, approximately 3 mm above the limbus. There was no detectable difference in corneal sensitivity between groups (P=0.891, One–way ANOVA). (B) Unlike sensitivity, nerve fiber length was reduced in all test groups compared to the control (P<0.001, One-way ANOVA, Dunn's post-hoc comparison test). The shortest nerve fiber length was detected in the OSA + T2DM group. Data represented as mean ± SD.

Figure 5: Tortuosity of the SBNP. Tortuosity grading is classified as short term and long term tortuosity. Fig. A shows an example of short term tortuosity (yellow circle) and Fig. B shows long term tortuosity (red circle). Patients with OSA appear to have greater long term tortuosity than those without OSA.



RETINAL NERVE FIBER LAYER

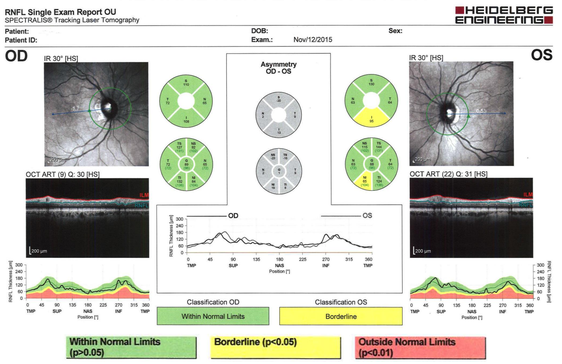


Figure 6: OCT measurement of the retinal nerve fiber. Representative scans of the right and left optic nerves using the Spectralis OCT.

Table 5: Retinal Nerve Fiber Layer Thickness (µm)

	OSA + T2DM	OSA	T2DM	CTRL	P value
Superior	108.9 ± 14.3	114.7 ± 16.4	114.7 ± 14.5	120.9 ± 18.8	P=0.138
Nasal	67.9 ± 11.6	70.2 ± 16.9	62.9 ± 16.2	69.5 ± 13.5	P=0.340
Inferior	118.1 ± 15.4	113.2 ± 21.9	120.7 ± 23.2	126.9 ± 23.5	P=0.194
Temporal	66.6 ± 17.2	68.8 ± 12.5	58.7 ± 18.4	66.8 ± 17.6	P=0.126
Global Average	90.4 ± 7.7	92.4 ± 10.7	90.1 ± 12.2	96.1 ± 11.7	P=0.193

Data represented as mean ± standard deviation. A power analysis indicates an additional 17 patients per group are needed for statistical significance with a power of 0.800, which will be reached following completion of enrollment.

- Hemoglobin A1C was significantly elevated in patients with T2DM (P<0.001).
- STOPBANG scores were statistically higher in patients with OSA (P<0.001).
- There were no differences in BMI, neck, waist or hip circumference, or waist to height ratios between groups. Linear regression analysis showed a relationship between neck circumference and STOPBANG score (R=0.579, P<0.001).
- SBNP nerve fiber length is shortest in patients with both OSA and T2DM, compared to all other groups.
- The SBNP in patients with OSA appeared more tortuous compared to T2DM and controls.
- Dry eye clinical findings were within normal range for all patients; thus dry eye does not account for any of the reported SBNP changes.
- While not yet significant, the changes in the RNFL are analogous to glaucomatous damage.

DISCUSSION

This is an early, interim report on the effects of OSA on T2DM ocular nerve damage. While the study is ongoing, results to-date indicate that the presence of comorbid OSA in patients with T2DM has deleterious effects on corneal sensory nerves and the retinal nerve fiber layer. A recent study in the UK analyzed the risk and severity of DPN in patients with OSA, and among 266 patients with T2DM, those with OSA had a higher prevalence of DPN.[4] Together, these findings support our hypothesis that OSA accelerates T2DM nerve damage in the eye. It is well established that loss of the SBNP precedes DPN. Thus, measurement of SBNP damage using IVCM represents an important early metric for assessing diabetic nerve damage.

There are two important considerations for this study. First, no patients presented with diabetic retinopathy. Taken together with HbA1c values, the T2DM cohort has relatively good glycemic control. Patients with poorly controlled disease will likely present with more severe corneal and retinal nerve fiber defects. Second, sample sizes per group are low as this is an early interim analysis. Although preliminary, emerging trends are evident with respect to both corneal and retinal nerves fibers. A larger analysis is needed following completion of the study to validate these findings.

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Supported by: NIH/NEI grants EY02443 (DMR), EY024546 (DMR), Core grant for Vision Research EY020799, and an unrestricted grant from Research to Prevent Blindness, New York, NY.