

# Corneal Confocal Microscopy in Type 1 Diabetes Mellitus: A Six-Year Longitudinal Study

Stuti L. Misra<sup>1</sup>, James A. Slater<sup>1</sup>, Charles N. J. McGhee<sup>1</sup>, Monika Pradhan<sup>1</sup>, and Geoffrey D. Braatvedt<sup>2</sup>

<sup>1</sup> Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Auckland, New Zealand

<sup>2</sup> Department of Endocrinology, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand

Correspondence: Stuti L. Misra, Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. e-mail: [s.misra@auckland.ac.nz](mailto:s.misra@auckland.ac.nz)

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**Purpose:** The current study describes corneal nerve morphology using in vivo confocal microscopy (IVCM) in patients with type 1 diabetes (T1D) who were followed up for 6 years, and it examines the relationship between corneal parameters and metabolic control of glucose and peripheral neuropathy.

**Methods:** Sixty-two participants (37 with T1D and 25 control participants) were assessed in 2011 and 2017. Participants with bilateral cataract surgery or controls who developed diabetes were excluded. All underwent HbA<sub>1c</sub>, IVCM, and central corneal sensitivity measurements at both time points in the eye previously examined. A modified total neuropathy score was obtained.

**Results:** Participants were age and sex matched. The mean duration of diabetes was  $32.1 \pm 12.0$  years at the follow-up visit. The sub-basal nerve density in participants with T1D was lower than that of the controls and did not change (mean  $\pm$  SD,  $11.07 \pm 4.0$  to  $11.41 \pm 4.1$  mm/mm<sup>2</sup>;  $P = 0.71$ ), but it showed a marginal change in controls ( $19.5 \pm 3.7$  to  $21.63 \pm 4.03$  mm/mm<sup>2</sup>;  $P = 0.06$ ). The corneal sensitivity in T1D did not change ( $1.3 \pm 1.5$  to  $1.4 \pm 1.0$  mbar;  $P = 0.8$ ), and it declined in the controls ( $0.2 \pm 0.3$  to  $0.6 \pm 0.3$  mbar;  $P < 0.001$ ). There were no significant changes in HbA<sub>1c</sub> ( $60.5 \pm 12.5$  to  $61.6 \pm 13.7$  mmol/mol) or in modified total neuropathy scores ( $2.4 \pm 3.2$  to  $3.4 \pm 3.8$ ;  $P = 0.2$ ).

**Conclusions:** The corneal nerve damage and poorer corneal sensitivity reported in the patients with T1D did not change and displayed improvement with good glycemic control.

**Translational Relevance:** The corneal nerve changes may be of more value in those with a shorter duration of diabetes for the timely prediction of at-risk individuals likely to develop peripheral neuropathy, particularly in type 1 diabetes.

## Introduction

Much literature exists on diabetes-related complications, particularly diabetic peripheral neuropathy. It is now broadly accepted that changes in corneal nerve microstructure precede the development of diabetic peripheral neuropathy in people with type 1 diabetes mellitus (T1D) and those with type 2 diabetes mellitus (DM).<sup>1–4</sup> However, only a limited number of longitudinal studies have explored the effect of diabetes on corneal and peripheral neuropathy over a 2- to 4-year period.<sup>5–7</sup>

A number of research groups worldwide, including ours, have previously reported significant associa-

tions among the corneal nerve microstructure, corneal sensitivity, retinopathy, and peripheral neuropathy in people with diabetes.<sup>2,3,8</sup> Our previous study highlighted a prominent role for in vivo confocal microscopy (IVCM) in monitoring the effect of T1D on not just the eyes but also peripheral neuropathy.<sup>2</sup> Changes in the corneal microstructure and the development of retinopathy and peripheral neuropathy are related to metabolic control of glucose in a positive curvilinear manner.<sup>7</sup> Visual impairment in diabetic retinopathy is due to retinal vascular abnormalities that cause anatomical and functional changes in retinal neurons.<sup>9</sup> Despite utmost importance being given to sight-threatening diabetic retinopathy, diabetic changes in the cornea can lead to reduced corneal

sensitivity, compromised ocular surface, and recurrent corneal erosions.<sup>10</sup> The current longitudinal follow-up study examined corneal nerve structure and function over a period of 6 years in participants with T1D compared with controls based on changes in the corneal nerve microstructure, glycated hemoglobin, diabetic retinopathy, and clinical signs of peripheral neuropathy.

## Methods

### Study Design and Participants

This study was a prospective, observational, longitudinal study conducted at the University of Auckland Ophthalmology Clinic and the University of Auckland Cornea and Anterior Segment Unit based in the Department of Ophthalmology, Greenlane Clinical Centre, Auckland District Health Board, New Zealand (Aotearoa). The study adhered to the tenets of the Declaration of Helsinki, and the Regional Health and Disability Ethics Committee granted ethical approval (16/STH/76) in June 2016. Fifty-three participants with T1D and 40 age-matched controls were initially studied in 2011.<sup>2</sup> The participants were contacted again in 2017 by phone, email, or letter. All of the participants were sent a detailed patient information sheet, and informed consent was obtained. Exclusion criteria included bilateral cataract surgery, ocular trauma, or the development of DM in controls. The contralateral eye was only assessed if the originally examined eye underwent cataract surgery or ocular trauma that might influence the sub-basal nerve (SBN) density. Sixteen patients with T1D and 15 controls were not reviewed in 2017 due to relocation outside of Auckland or overseas, the development of other medical illness, or refusal to participate.

Past medical, current social, and family history and the duration of diabetes were recorded for all participants. The patients' up-to-date glycated hemoglobin (HbA1c) and the results from the previous 6 years were reviewed, and a time-weighted average was derived. Height, weight, and body mass index (BMI) were recorded at both visits. Each participant's best-corrected visual acuity was recorded, and a slit-lamp examination was undertaken that focused on the anterior segment and adnexae to rule out any ocular surface abnormality.

### Corneal Esthesiometry

Corneal sensation threshold was measured with a non-contact corneal esthesiometer.<sup>11</sup> The esthesiome-

ter utilizes a controlled pulse of air to stimulate corneal nerves using a 0.5-mm-diameter aperture for a variable and user-selectable puff intensity stimulus. The sensation is measured using a yes/no response, double staircase procedure at the center of the cornea.

### Retinal Imaging

Images of the central and peripheral retina were taken using a non-mydratic retinal camera (CR-DGi; Canon, Inc., Melville, NY). The images were graded using the Early Treatment Diabetes Research Study (ETDRS) research group scale by an experienced retinal specialist (MP) masked to the patient's diabetes status.<sup>12</sup>

### Corneal Confocal Microscopy and Image Analysis

Following anesthesia (oxybuprocaine 0.4%), laser scanning IVCM was performed on the central cornea using the Heidelberg Retina Tomograph III with Rostock Corneal Module (Heidelberg Engineering, Heidelberg, Germany). The right eye of each participant was examined, unless the left eye was examined previously. This clinical technique uses a confocal arrangement to eliminate out-of-focus light, thus enabling non-invasive optical sectioning of the living human cornea. Each IVCM image represents  $400 \times 400 \mu\text{m}$  with a lateral resolution of  $2 \mu\text{m}$  and optical section thickness of  $4 \mu\text{m}$ . Three high-quality, focused individual images of SBNs were selected and then randomized for subsequent analysis. The SBN density was evaluated by tracing visible nerves with an electronic pen (Wacom Technology Group, Vancouver, BC, Canada) and measuring the total length of nerves per frame with a digital caliper tool (ImageJ with NeuronJ plugin; National Institutes of Health, Bethesda, MD).<sup>13-15</sup> It should be noted that the terms sub-basal nerve density and sub-basal nerve length, measured as  $\text{mm}/\text{mm}^2$ , are used interchangeably in the literature, as there is no consensus regarding the terminology.<sup>1-4,16</sup> Although a number of parameters are used to quantify corneal nerve microstructure, the only repeatable measure is SBN density or nerve length, measured as  $\text{mm}/\text{mm}^2$ .<sup>17</sup>

### Assessment of Neuropathy

#### Total Neuropathy Score Questionnaire

A Total Neuropathy Score questionnaire was completed at both visits by the participants with T1D before the consult or during the history taking. The

questionnaire is divided into three sections, with a maximum normal score of 13. The sections are sensory (four points), motor (four points), and autonomic neuropathy symptoms (five points). The minimum score is 0.<sup>18</sup> Neuropathy worsens with increasing score.

### Quantitative Sensory Testing

Vibration perception threshold of the feet was assessed using a biothesiometer (Bio-Medical Instruments, Newbury, OH). The voltage is analyzed against a nomogram<sup>19</sup> for the patients gender and age and then given a score based on the centile of the result.<sup>20</sup>

### Semmes–Weinstein Fine Touch Sensation

The fine touch sensation of each T1D participant was recorded and scored using a 10-g Semmes–Weinstein monofilament. A score of 0 was given for normal sensation, 1 for reduction of sensation at fingers and toes, 2 if reduction was to the wrist and ankle, 3 if it was to the elbow or knee, and 4 if it was above the elbow and knee.<sup>20,21</sup>

### Deep Tendon Reflexes

T1D participants underwent lower limb reflex testing. A score of 0 was given if knee and ankle reflexes were normal. A maximum score of 4 was given if all lower limb reflexes were absent.<sup>20</sup>

### Modified Total Neuropathy Score

The sum of the above four scores was tallied to obtain a modified total neuropathy score.

### Glycated Hemoglobin

Glycated hemoglobin was not always measured at the same interval in each patient over the 6 years of follow-up. To avoid potential bias, we used a time-weighted average of HbA1c (TW-HbA1c) instead of simple mean HbA1c.<sup>22</sup> TW-HbA1c was calculated using the formula  $TW-HbA1c = \frac{\sum_2^n (T \times HbA1c) + (\text{initial HbA1c} \times mHbA1c)}{\sum_1^n HbA1c}$ , where mHbA1c is the mean HbA1c level between the last and the first measurement, after the start of the time period. Inter-year and intra-year measurements were also calculated and found to show no statistically significant difference over time. This method has previously been used for analyzing large numbers of thyroid-stimulating hormone measurements.<sup>23</sup> HbA1c is reported in both mmol/mol and percentage.

### Statistical Analysis

The Shapiro–Wilk test was performed to confirm normality of the data distributions. Dependent *t*-tests

or independent *t*-tests were performed to determine the statistical difference between the groups, as needed. A Pearson correlation (two-tailed) analysis was applied to determine the relationship between numeric variables, and a Spearman analysis was done for the ordinal data.  $P < 0.05$  was considered significant. Data are presented as mean  $\pm$  SD. SPSS Statistics 23 (IBM Corporation, Armonk, NY) was used to conduct the data analysis.

## Results

The study re-assessed 62 participants that were seen after a gap of 6 years. Of the initial 53 participants with T1D studied at baseline, 37 were available for re-examination (69.8%) and 25 of 40 controls (62.5%) accepted the invitation for the follow-up visit. Only the patients who were assessed at the follow-up visit were included in data processing and the comparative statistical analysis. Participants with diabetes followed the treatment regimens as advised by their endocrinologists throughout the 6-year period. The main reason for participants not being able to attend a follow-up appointment was geographic relocation (77.4%). At the follow-up visit, the mean age of the T1D subjects was  $56.4 \pm 12.6$  years and that of the controls was  $55 \pm 14.8$  years ( $P = 0.33$ ). The mean duration of diabetes mellitus was  $26.2 \pm 12.0$  years at baseline and  $32.1 \pm 12.0$  years at follow-up. The Table shows the clinical characteristics and demographic data of the participants with diabetes and the controls at their follow-up visit. There was no significant change in HbA1c in the control (from  $35.0 \pm 2.5$  mmol/mol to  $34.9 \pm 3.3$  mmol/mol;  $P = 0.80$ ) or T1D participants (from  $60.5 \pm 12.5$  mmol/mol to  $61.6 \pm 13.7$  mmol/mol;  $P = 0.12$ ).

The SBN density was lower in the participants with T1D than in the controls at baseline ( $P < 0.001$ )<sup>2</sup> and remained unchanged at the 6-year time point for the participants with T1D (from  $11.07 \pm 4.03$  mm/mm<sup>2</sup> to  $11.41 \pm 4.1$  mm/mm<sup>2</sup>;  $P = 0.71$ ), but it improved marginally, without reaching statistical significance, in the controls (from  $19.5 \pm 3.7$  mm/mm<sup>2</sup> to  $21.63 \pm 4.03$  mm/mm<sup>2</sup>;  $P = 0.06$ ). Significant differences were noted in SBN density between the controls and the T1D participants ( $P < 0.001$ ) at the follow-up visit. Corneal sensitivity in the T1D participants was poorer at baseline compared with controls ( $P < 0.001$ )<sup>2</sup> and did not change over time (from  $1.3 \pm 1.0$  mbar to  $1.4 \pm 1.0$  mbar;  $P = 0.80$ ), but it showed a reduction in the control group (from  $0.2 \pm 0.3$  mbar to  $0.6 \pm 0.3$  mbar;  $P < 0.001$ ). A reduction in the corneal sensitivity threshold value indicates an increase in the corneal sensitivity. Modified total neuropathy scores did not

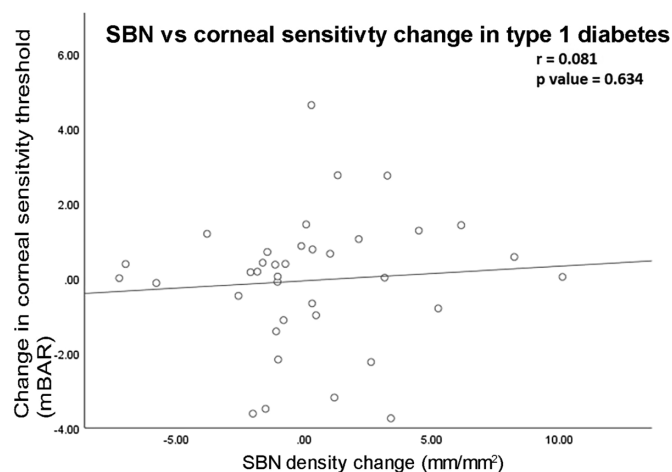
**Table.** Demographic and Clinical Characteristics of the Same 37 Participants With T1D and 25 Controls at Baseline and at Follow-Up Visits After a Period of 6 Years

Characteristic	Baseline		6-Year Follow-Up	
	Controls	T1D	Controls	T1D
Ratio of males to females	11:14	17:20	11:14	17:20
Age (y), mean $\pm$ SD	46.6 $\pm$ 15.0	50.2 $\pm$ 12.4	52.8 $\pm$ 14.8	56.4 $\pm$ 12.6
BMI, mean $\pm$ SD	25.0 $\pm$ 3.8	26.9 $\pm$ 4.6	26.3 $\pm$ 4.3	28 $\pm$ 5.5
Smoking history (packs/y), mean $\pm$ SD	0.2 $\pm$ 1.0	3.5 $\pm$ 10.2	0.2 $\pm$ 0.7	4 $\pm$ 8.3
Alcohol (units/wk), mean $\pm$ SD	5.4 $\pm$ 0.2	3.5 $\pm$ 2.1	2.0 $\pm$ 1.7	3.2 $\pm$ 2.5
HbA1c (mmol/mol), mean $\pm$ SD	35.0 $\pm$ 2.5	60.5 $\pm$ 12.5	34.9 $\pm$ 3.3	61.6 $\pm$ 13.7
HbA1c (%), mean $\pm$ SD	5.4 $\pm$ 0.2	7.6 $\pm$ 1.1	5.8 $\pm$ 1.4	7.8 $\pm$ 3.4
DM duration (y), mean $\pm$ SD		26.2 $\pm$ 12.0		32.1 $\pm$ 12.0
DM duration (y), <i>n</i>				
<10	—	4	—	0
10–20	—	6	—	5
21–30	—	12	—	11
>30	—	15	—	21
Corneal SBN density (mm/mm <sup>2</sup> ), mean $\pm$ SD	19.57 $\pm$ 3.7	11.07 $\pm$ 4.0	21.63 $\pm$ 4.04	11.41 $\pm$ 4.1
Corneal sensitivity threshold (mbar), mean $\pm$ SD	0.2 $\pm$ 0.3	1.3 $\pm$ 1.0	0.6 $\pm$ 0.3	1.4 $\pm$ 1.5
Neuropathy score (modified TNS), mean $\pm$ SD	—	2.4 $\pm$ 3.2	—	3.4 $\pm$ 3.8

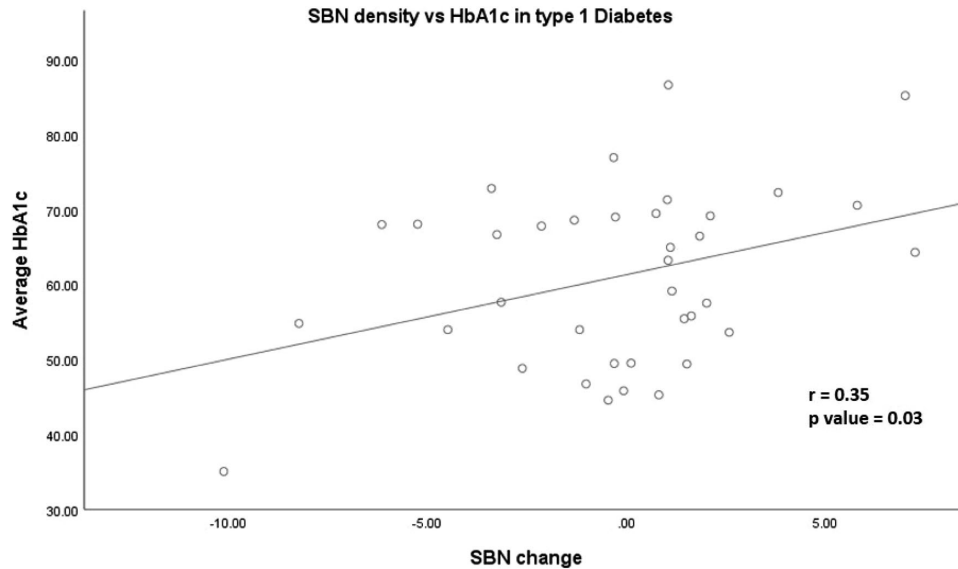
change over time in the patients with T1D (from  $2.4 \pm 3.2$  to  $3.4 \pm 3.8$ ;  $P = 0.2$ ). Of the 37 people with T1D, only 12 had some level of non-proliferative diabetic retinopathy and eight had minimal maculopathy when assessed at the initial assessment 6 years previous.<sup>2</sup> In total, 23 participants with T1D had varying degrees of non-proliferative diabetic retinopathy at the follow-up visit: 10 mild, seven moderate, and six severe. Four of these patients also had severe maculopathy, and overall 11 had mild to moderate maculopathy. There was no statistically significant correlation between the retinopathy grading and corneal SBN microstructure changes over the two time points. Spearman correlation analysis did not show any association between retinopathy grading and HbA1c ( $\rho = 0.07$ ;  $P = 0.68$ ). SBN density and corneal sensitivity did not show any association ( $r = 0.08$ ;  $P = 0.63$ ) (Figs. 1, 2). A negative change in the corneal sensitivity threshold indicates an increase in the corneal sensitivity.

Interestingly, T1D participants with poor glycemic control over time developed a marginal decrease in SBN density ( $r = 0.35$ ;  $P = 0.03$ ) (Fig. 2). If the average HbA1c was higher, T1D participants had a greater reduction in SBN density ( $P = 0.03$ ). The time-weighted HbA1c calculations are described in the Methods section.

SBN density changes were examined in participants divided by time-weighted tertiles of glycemic control. Those with the highest HbA1c (68.1–

**Figure 1.** Pearson correlation analysis of the change in SBN density versus the change in corneal sensitivity in 37 people with T1D assessed 6 years apart ( $r = 0.08$ ;  $P = 0.63$ ).

86.7 mmol/mol) displayed a greater reduction SBN density ( $0.377 \text{ mm/mm}^2$ ). In the middle tertile (HbA1c, 54.8–67.8 mmol/mol), there was a smaller reduction of  $0.064 \text{ mm/mm}^2$ . Those in the best controlled tertile (HbA1c, 35.0–54.0 mmol/mol) displayed an increase in SBN density of  $1.263 \text{ mm/mm}^2$ . The correlation analysis of change in SBN density and change in modified TNS showed no relationship (Spearman  $\rho = 0.16$ ;  $P = 0.33$ ).



**Figure 2.** Pearson correlation analysis of the change in SBN versus time-weighted average HbA1c in 37 people with T1D assessed 6 years apart ( $r = 0.35$ ;  $P = 0.03$ ).

## Discussion

Electrophysiology and skin or sural nerve biopsy are the gold standards for the diagnosis and quantification of diabetic peripheral neuropathy.<sup>24</sup> However, during the initial stages in the development of neuropathy, damage is often only seen in small nerve fibers that are assessed quantitatively using invasive tests, unsuitable for either routine diagnostic or repeated testing. In randomized controlled trials, subjective assessments that include health surveys and assessments of mood states or pain scores are used.<sup>25</sup> An objective, non-invasive, repeatable diagnostic test is necessary to monitor and preempt disease progression. During the early stages of diabetic peripheral neuropathy, the myelinated A $\delta$  and unmyelinated C-class small nerve fibers are damaged, leading to loss of pain and temperature sensation, paresthesia and hyperesthesia.<sup>26</sup> It is now well established that the small corneal nerve fibers are also affected at an early stage.<sup>1-3</sup>

The current study showed that there was no clinically significant change in corneal nerve density in those with T1D over a 6-year period of follow-up. However, the nerve density values for those with T1D were significantly lower than for the controls at each time point associated consistently with moderately severe peripheral neuropathy.<sup>27</sup> A similar result was reported in a recent study of 19 patients with T1D followed up for a period 2 years; however, the patients were not compared with a control cohort, so the study lacked a direct aging comparison.<sup>5</sup> Our study has similarities to a previous longitudinal study that

observed the progression of SBN density in healthy subjects and in those with T1D. A study of 108 patients with T1D in Australia demonstrated reductions in SBN length (as measured by mm/mm<sup>2</sup>) compared with controls, but the decrease was not significant over a 4-year period.<sup>28</sup> This could be due to the plateau in corneal nerve alteration during the initial stages of diabetic corneal disease.

SBN density was very low and corneal sensitivity poorer at baseline in the participants with T1D, probably due to the long duration of diabetes in these people. Not surprisingly, no improvement or deterioration was seen over the follow-up as a group, although small improvements in SBN were seen in the tertile of patients whose follow-up mean weighted HbA1c was on the lower end. Further studies of SBN and corneal sensitivity in those with T1D of shorter duration are needed to assess whether an early loss of SBN density, reflecting early neuropathy, is reversible with improved glucose control.

A reduction in corneal sensitivity (increase in corneal sensitivity threshold) was noted in the control participants at the 6-year follow-up visit. This was not an unexpected finding in this cohort. Age-related changes in corneal sensitivity are well established, and assessments via conventional Cochet-Bonnet or non-contact esthesiometers have been widely reported.<sup>29,30</sup>

As expected, a significant number of T1D participants developed some degree of diabetic retinopathy over the course of the 6-year follow-up.<sup>31</sup> However, the SBN densities at both time points and changes in SBN density did not correlate with the degree of diabetic retinopathy. The difference in SBN density

between controls and those with diabetes has been widely established, but the association of SBN density with retinopathy is variable.<sup>4,5</sup> This could be attributed to the different mechanisms responsible for corneal neuropathy and retinopathy, as mentioned previously.

A previous study showed an improvement in the SBN density in participants with tighter diabetic control over a 2-year period.<sup>32</sup> This was also seen in the current study in the tertile of people with the lowest mean weighted HbA1c over the follow-up period. Corneal nerve density has previously been shown to predict the 4-year incidence of distal peripheral neuropathy with 63% sensitivity and 74% specificity.<sup>8</sup> The modified TNS of participants with diabetes in the current study did indeed deteriorate; however, there was no statistically significant correlation with SBN density.

A limitation of the study is the participant drop-out rate for various personal reasons, and the limited number of participants may have had an impact on the statistical analysis. In any case, it should be emphasized that only data from the 62 participants (37 T1D and 25 controls) who attended both visits were compared.

The use of IVCM in a clinical setting to determine corneal nerve changes may be of more value in those with a shorter duration of diabetes so as to predict which people may develop peripheral neuropathy and thus provide an intervention strategy to prevent development of the complications of diabetes. Our study highlights the marked reduction in corneal SBN density in patients with long-standing T1D compared with age-matched controls. Furthermore, there was no significant change over the 6-year period in the corneal SBN density, modified total neuropathy, or non-contact corneal esthesiometry, despite many patients developing retinopathy in the intervening 6 years of follow-up.

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