Effect of Panretinal Photocoagulation on Corneal Sensation and the Corneal Subbasal Nerve Plexus in Diabetes Mellitus

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Purpose. To assess the effects of panretinal photocoagulation (PRP) for diabetic retinopathy (DR) on the human corneal subbasal nerve plexus (SBNP) and to investigate correlations between corneal subbasal nerve (SBN) density, corneal sensitivity, and diabetic peripheral neuropathy.

Methods. Thirty-eight subjects with at least a 10-year history of diabetes mellitus (DM) or DR were included. Subjects were assigned to a PRP group ($n = 19$), having undergone a treatment of retinopathy in at least one eye or a non-PRP group ($n = 19$), with no history of PRP. The Michigan Neuropathy Screening Instrument (MNSI) was administered to enable quantification of neuropathic symptoms. Laser scanning in vivo confocal microscopy was performed to capture images of the corneal SBNP to allow determination of SBNP density. Central corneal sensitivity (CST) was evaluated by noncontact aesthesiometry and peripheral vibration perception threshold was measured with a biothesiometer.

Results. Mean SBNP densities were 12.27 ± 4.28 mm/mm^2 in the PRP group and 12.75 ± 3.59 mm/mm^2 in the non-PRP group. There were no significant differences in SBNP density ($P = 0.71$), CST ($P = 0.84$), MNSI score ($P = 0.19$), and biothesiometry ($P = 0.77$) between the PRP and non-PRP groups. When data from both groups ($n = 38$) were combined, corneal sensitivity was modestly correlated with SBNP density ($r = 0.30$, $P = 0.06$), and peripheral biothesiometry ($r = 0.26$, $P = 0.11$).

Conclusions. In DM correlation of corneal sensitivity, SBNP density, and peripheral biothesiometry may have a potential role in estimating the severity of peripheral neuropathy. Corneal SBNP density and sensitivity appear to be unaffected by PRP laser treatment compared with non-PRP diabetic eyes.

Keywords: panretinal photocoagulation, diabetes, cornea, in vivo confocal microscopy, subbasal nerves

The prevalence of diabetes mellitus (DM) is expected to increase from the 2010 global estimate of 220 million, to approximately 366 million by the year 2030, as projected by the World Health Organization (WHO) Global Burden of Disease Study.[1,2] In the United States alone, DM costs the government approximately $174 billion annually and, thus, diabetes will increasingly burden health care systems throughout the world.[3] Poorly controlled diabetes may lead to a range of systemic complications including nephropathy, cardiomyopathy, and peripheral neuropathy.[4] The ocular complications of DM are numerous and range from the minor to the visually debilitating, including: dry eye, diabetic keratopathy, orbital cellulitis, early onset cataract, rubeosis iridis, and diabetic retinopathy (DR).[5–10]

DR is one of the leading causes of blindness worldwide.[11] Important therapeutic measures in the management of DR include optimizing glycaemic, lipid, and hypertensive control. However, in cases of proliferative or severe preproliferative DR, laser panretinal photocoagulation (PRP) is indicated with the aim of preventing progression of DR and vision loss.[12] Although "sight-saving" and effective in the treatment of advanced diabetic retinal disease, PRP is fundamentally associated with extensive tissue destruction and underlying anatomical changes.[13,14]

Diabetic keratopathy can be associated with significant ocular complications[9] and decreased corneal sensitivity has been reported following PRP in diabetes. This has been attributed to direct thermal injury to ciliary nerves running between the choroid and sclera.[15,16] However, only one study has directly investigated the effects of PRP on corneal nerve microstructure.[17] The aim of this current study was to utilize in vivo confocal microscopy (IVCM) to investigate the effect of PRP on the subbasal nerve plexus (SBNP) in patients with DM, and to evaluate the relationship between corneal subbasal (SBN) nerve density, corneal sensitivity, and peripheral neuropathy.

Materials and Methods

The study was conducted in the University of Auckland Research Unit, Department of Ophthalmology, Greenlane

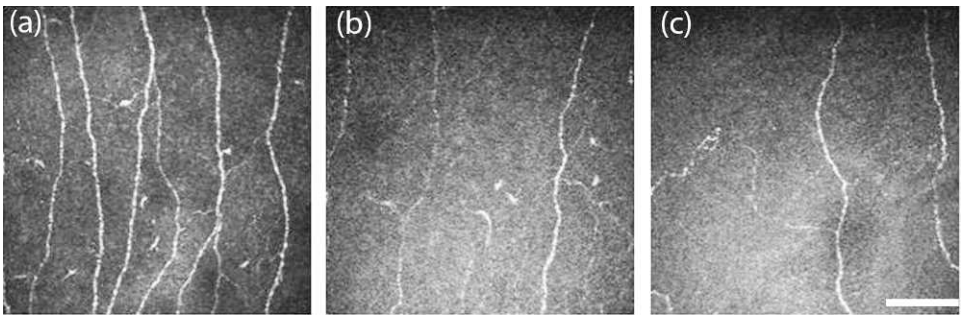

FIGURE 1. (a) A representative laser scanning in vivo confocal image of the central cornea showing SBNs in a healthy eye. (b) Visibly reduced SBN density in a subject with DM without a history of PRP. (c) SBNs in a subject with DR treated with PRP. *Scale bar*: 100 μm.

Clinical Centre, Auckland, New Zealand with ethical approval granted by the Regional Ethics Committee (NTX/09/12/122) and adhered to the tenets of the Declaration of Helsinki. Inclusion criteria were: a 10 or more year history of DM or the presence of any DR subjects with a history of ocular surgery or trauma; contact lens wear, or a history of corneal disease or systemic disease that may affect the cornea (other than DM) were excluded. The study was explained in detail and written consent to participate was obtained.

Subjects were categorized into two groups according to their treatment history; the PRP group with a history of PRP at least 6 months previously, and the non-PRP group with no history of PRP. Subjects with recent PRP (<6 months) were excluded from the study.

A detailed medical history was obtained from all subjects regarding duration of DM, known diabetic complications, comorbidities, medications, and smoking and alcohol history. A validated questionnaire (Michigan Neuropathy Screening Instrument [MNSI]) was administered to calculate the severity score of neuropathic symptoms.[18,19] The results of recent glycated hemoglobin (HbA1c) blood tests, performed within the preceding 3 months, were also recorded. Although fasting glucose remains a useful diagnostic criterion for DM, it only provides an instantaneous, single point, indication of glucose control. In contrast HbA1c gives an indication of the degree of control over the previous 3 months and is considered to be a major diagnostic criterion of DM.[20,21]

Central corneal sensitivity threshold (CST) was measured with a noncontact corneal aesthesiometer (NCCA).[22] After the stimulus was presented to the patient at a suprathreshold pressure for demonstration purposes, CST was determined by following a forced-choice double-staircase protocol.[23] Vibration perception threshold (VPT) of the feet was measured by biothesiometery (Bio-Medical Instrument Co., Newbury, OH), and VPT was also determined by forced-choice double-staircase technique.[24] The examination was performed at the medial malleolus, at the plantar and dorsal aspects of the distal interphalangeal (DIP) joint of the great toe of the foot ipsilateral to the examined eye. Digital images of the central and peripheral retina were captured with a nonmydriatic retinal camera (Non-Mydriatic Retinal Camera DR-DGi; Canon, Inc., Melville, NY) for DR grading. All retinal images were graded by a fellowship-trained medical retina specialist (MP).

In vivo confocal microscopy using the corneal module of the Heidelberg Retina Tomograph (HRT II; Heidelberg Engineering GmbH, Heidelberg, Germany) was performed in the selected eye of each patient.[17] A drop of local anesthetic (Oxybuprocaine hydrochloride 0.4%; Chauvin Pharmaceuticals Ltd., Kingston, UK) was administered to both eyes and lubricating gel (Viscotears Carbomer; Novartis Pharmaceuticals Ltd., Surrey, UK) applied to the nonexamined eye to protect the ocular surface from drying during the examination. The participant was asked to fixate on a distant target aligned to enable examination of the central cornea, while images of the corneal SBNP were captured with the aid of the device's "section mode."

The examination took approximately 5 minutes to perform in each case and none of the participants experienced any visual or corneal sequelae as a result of examination. All examinations were performed by a single experienced examiner (SM).

From the images showing well-focused nerves from the central cornea, six were randomly selected for each subject and the SBN density calculated in terms of length of nerves per millimeters squared of image area.[23] SBN density was analyzed by an independent observer (HA) using computer software (analySIS 3.1; Soft Imaging System, Münster, Germany).[23]

Statistical analysis was performed with SPSS 19.0 for Windows (Chicago, IL). On the basis of previously reported nerve fibre density data, a power calculation for this study (Statistical Solutions, LLC, Clearwater, FL) determined a minimum sample size of 10 in each group in order to detect a 5% difference in nerve density between the PRP and non-PRP groups, with 80% power and 95% confidence. An independent samples *t*-test was applied to compare patient characteristics of the PRP and non-PRP groups, and one-way ANOVA was performed to compare ocular and peripheral neuropathy features between the two groups. Bivariate Pearson correlation (two-tailed) was used to determine the correlations between a number of different factors. A P value of less than 0.05 was considered significant. A correlation coefficient (Pearson's r) of less than ± 0.3 was considered weak; ± 0.3 to ± 0.6, was moderate; and greater than ± 0.6, was strong.[25]

RESULTS

Thirty-eight eyes of 38 participants with DM were enrolled for this study, of which 20 eyes had previously undergone PRP. Fifteen left eyes and 23 right eyes were assessed. There was no significant difference in the mean age, body mass index (BMI), current severity of retinopathy, and smoking and alcohol history between the PRP and non-PRP groups (Table 1). The mean duration of DM and HbA1c values were not significantly different between the two groups (Table 2).

The mean SBN densities were 12.27 ± 4.28 mm/mm^2 in the PRP group and 12.75 ± 3.59 mm/mm^2 in the non-PRP group. Representative subbasal nerve plexus images of the central cornea are illustrated in Figure 1. There were no significant differences between the PRP and the non-PRP groups in terms of SBN density ($P = 0.71$), CST ($P = 0.84$), MNSI score ($P = 0.19$), and biothesiometry ($P = 0.77$, Table 2).

The variables included from both groups in the correlation analysis were SBN density, HbA1c, CST, and biothesiometry

Table 1. Demographics Including Male:Female Ratio, Age, BMI, Smoking History, Alcohol Score, Ethnicity, Duration of DM (Years), and Type of DR (Subject Percentage Included) in Subjects With Retinopathy Treated by PRP and Untreated Retinopathy (Non-PRP) Group

	Groups		*t*-test, *P* Values
	PRP	Non-PRP	
Subjects, *n*	19	19	-
Male:female ratio	13:06	14:05	-
Age, y	57.0 ± 7.9	59.5 ± 14.2	0.50
BMI, kg/m^2	29.6 ± 4.0	29.2 ± 5.1	0.78
Smoking history, pack y	5.8 ± 12.1	4.4 ± 9.75	0.70
Alcohol score	2.20 ± 2.3	2.0 ± 2.6	0.75
Type 1:type 2	1:18	1:18	-
Mean DM duration, y	18.0 ± 10.4	13.6 ± 7.2	-
<10	2	4	-
10–20	11	13	-
>20	6	2	-
No active DR	0 (0%)	1 (5.3%)	-
Mild NPDR	0 (0%)	10 (52.6%)	-
Moderate NPDR	3 (15.8%)	2 (10.5%)	-
Severe NPDR	4 (21.1%)	5 (26.3%)	-
PDR	12 (63.2%)	1 (5.3%)	-

NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

(medial malleolus, great toe plantar, and great toe dorsal) and MNSI score (Table 3). HbA1c showed no significant correlations with nerve density, corneal sensitivity, biothesiometry, or neuropathy symptoms score. SBN density showed a modest correlation with CST (Pearson's correlation $r = 0.30$, $P = 0.06$), and corneal sensitivity was poorly correlated with biothesiometry (medial malleolus) ($r = 0.26$, $P = 0.11$, Fig. 2). All three biothesiometry measurements were strongly correlated with each other (Table 3). Symptomatic MNSI scores were also significantly correlated with the biothesiometry measurements ($r = 0.83$, $P < 0.001$).

Discussion

The corneal SBNP lies between the basal epithelium and Bowman's layer.[26]

The SBN density in diabetic corneas has been widely reported to be reduced, particularly in patients with associated DR and neuropathy.[27–29] Messmer et al. reported mean SBN density of 16.1 mm/mm^2 in patients with a mean of 14.8-year history of type 2 DM as compared with 12.2 and 12.7 mm/mm^2 in PRP and non-PRP patients in the current study, using the same IVCM.[30] Importantly, a recent global study showed that the patients with a less than 10-year history of DM had only a 21.0% prevalence of any degree of DR as compared with 54.2% in patients with a 10- to 20-year history of DM.[31] Additionally, only 1.2% of the former group developed proliferative retinopathy compared with 9.0%, possibly requiring treatment, in the latter group.[31] The normal SBN density has been reported to be between 21.6 and 45.9 mm/mm^2 as measured by in vivo and ex vivo studies.[32–34] The reduction in nerve density is associated with a loss of corneal sensitivity in these eyes.[35] The corneal sensitivity threshold in the healthy population is noted to be significantly lower than that found in the diabetic subjects of the current study, at 0.38 millibars (mBAR).[23] A number of previous studies have investigated the corneal nerve changes in DM patients compared with healthy controls,[30,36–38] but there has been little exploration of the possible effects of PRP on corneal nerves.[17]

Interestingly, De Cilla et al. reported lower SBN density in patients with proliferative diabetic retinopathy (PDR) treated by PRP when compared with untreated PDR.[17] However, it is important to note that the extent of DR, per se, has been shown to correlate with reduced corneal sensitivity irrespective of the treatment.[39] The presence of basement membrane abnormalities in the corneal epithelium and retinal blood vessels in DM has been put forward as a possible explanation for this correlation.[40,41] A recent study highlighted differences in corneal sensitivity between the diabetic participants and control subjects, but identified no differences in corneal sensitivity threshold between PRP treated and PRP untreated diabetic participants.[42]

The current study identified no significant differences between the PRP and non-PRP groups with respect to: corneal SBNP density, corneal sensitivity, biothesiometry, and subjective neuropathy score. Thus, PRP did not appear to have any adverse effect on the corneal SBNP density or corneal sensitivity in this study when compared with comparable, non-PRP treated, diabetic eyes. Therefore, these data suggest the reduction in corneal SBN density observed in the PRP group appear to be attributable to the effect of diabetes itself rather than laser treatment. The current study has confirmed that SBNP density is unaffected by PRP in the long term. However, further studies comparing preoperative and 6-month postoperative SBN density would be useful to determine if these nerves are affected in the short term following PRP.

Reflectivity and tortuosity are two other parameters that have been commonly used to quantify IVCM SBNs in previous studies. Some studies have simply used a subjective grading system for SBN tortuosity.[17,29] More complex objective mathematical methods have also been used to define a tortuosity coefficient in DM using a measure of nerve fibre curvature with the observer selecting nerve branches for calculation.[36,43,44] Unfortunately, these subjective and objective measurements of nerve tortuosity are not directly

Table 2. Comparison of SBN Density, HbA1c, Corneal Sensitivity, Michigan Neuropathy Symptomatic Score, and Beiothesiometry for Medial Malleolus (MM), Great Toe Plantar (GTP), and Great Toe Dorsal (GTD) of Patients With ($n = 19$) and Without ($n = 19$) a History of PRP

	PRP, Mean ± SD	Non-PRP, Mean ± SD	ANOVA, *P* Values
SBN density, mm/mm^2	12.27 ± 4.28	12.75 ± 3.59	0.71
HbA1c, %	9.12 ± 1.89	8.56 ± 1.64	0.33
Corneal sensitivity, mBar	2.32 ± 2.23	2.46 ± 1.76	0.84
MNSI score	3.00 ± 2.60	2.10 ± 1.37	0.19
Biothesiometry - MM, V	27.15 ± 10.04	26.670 ± 8.89	0.77
Biothesiometry - GTP, V	20.21 ± 10.50	20.94 ± 8.62	0.81
Biothesiometry - GTD, V	19.630 ± 10.11	20.63 ± 8.59	0.71

SD, standard deviation.

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Table 3. Correlations Between SBN Density (mm/mm²), HbA1c (%), Corneal Sensitivity (mBAR), Michigan Neuropathy Symptomatic Score, and Biothesiometry (V) for MM, GTP, and GTD of All Subjects With DM ($N = 38$)

	SBN Density, µm/mm²	HbA1c, %	CST, mBar	VPT - MM, V	VPT - GTP, V	VPT - GTD, V	MNSI Score
SBN density, µm/mm²							
Pearson correlation	1	0.098	0.302*	0.028	0.015	−0.051	0.000
Significance, two-tailed	-	0.557	0.065	0.907	0.929	0.763	0.998
HbA1c, %							
Pearson correlation	-	1	0.084	−0.154	−0.094	−0.041	−0.043
Significance, two-tailed	-	-	0.617	0.356	0.574	0.807	0.797
CST, mBar							
Pearson correlation	-	-	1	0.263	0.155	0.285	0.148
Significance, two-tailed	-	-	-	0.110	0.353	0.082	0.374
VPT - MM, V							
Pearson correlation	-	-	-	1	0.811†	0.753†	0.240
Significance, two-tailed	-	-	-	-	0.000	0.000	0.147
VPT - GTP, V							
Pearson correlation	-	-	-	-	1	0.836†	0.528†
Significance, two-tailed	-	-	-	-	-	0.000	0.001
VPT - GTD, V							
Pearson correlation	-	-	-	-	-	1	0.586†
Significance, two-tailed	-	-	-	-	-	-	0.000

* Correlation is significant at the 0.05 level (two-tailed).
† Correlation is significant at the 0.01 level (two-tailed).

comparable. Furthermore, in the absence of a repeatable method for analyzing tortuosity,[44] this technique was deemed unsuitable for evaluation of subjects in this study. In the context of reflectivity, the laser scanning in vivo confocal microscope used in the current study automatically adjusts the illuminating brightness to maximize image quality, whilst generally producing higher quality images than white light IVCM, this automatic adjustment leads to inconsistent reflectivity of highly reflective structures, such as corneal SBNs.[45] Another means of measurement is nerve branching analysis, however, this has been reported to be unreliable due to the potential for a single branching fibre to be interpreted as two fibers without branches.[44] Additionally, beading frequency comparison of SBN was not evaluated, as, in order to be comparable, all the images should be captured using fixed illumination intensity.[46] For these reasons, corneal nerve density was chosen as the most reliable parameter for comparison between the groups in this study.

The current study suggests that PRP does not compromise the integrity of the corneal SBNP and purely from the corneal

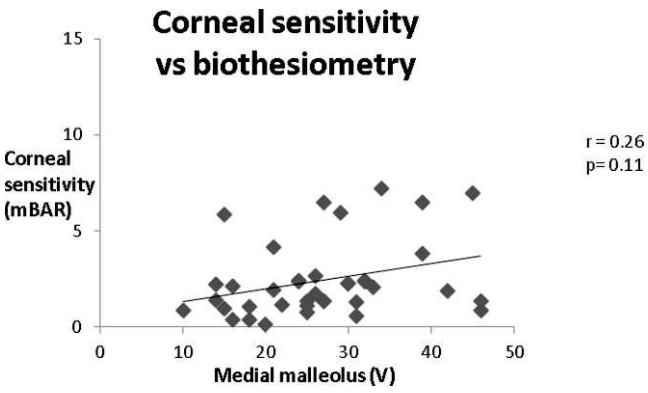

Figure 2. Scatter plot graph showing a modest positive correlation between corneal sensitivity and biothesiometry of medial malleolus.

perspective remains a relatively safe treatment modality in the treatment of DR. The contradictory results to the De Cilla et al.[17] study could relate to our strict exclusion criteria. We only included patients who had undergone PRP more than 6 months previously, but no other previous ocular surgery.[17] De Cilla et al.[17] included subjects with a history of cataract surgery, an intraocular procedure known to cause reduction in corneal sensitivity and innervation.[47] Another possible explanation could be a difference in the severity of thermal injury to underlying nerves related to the number of laser burns applied to treated subjects within in the two studies. These data were not available for comparison.

Peripheral neuropathy causes early damage to Aδ and unmyelinated C-class small nerve fibers leading to hyperesthesia, paraesthesia, and loss of pain and temperature.[48] Arguably, small corneal nerve fibers are also affected at this early stage. Previous studies have demonstrated that SBN density and corneal sensitivity correlate significantly with the severity of diabetic peripheral neuropathy.[36,37] Biothesiometry (to measure VPT) is a noninvasive and practical technique that can be easily performed in a clinical setting and is recognized as a reliable predictor of diabetic peripheral neuropathy.[49,50] Therefore, a trend in correlation between SBN density and corneal sensitivity; between nerve density and biothesiometry; and between MNSI score and biothesiometry might be anticipated in this study. Indeed, both of the correlation pairs: CST and, MNSI score and VPT, showed a positive correlation with each other. The correlation between MNSI score and VPT suggests that severe peripheral neuropathy is associated with increased symptoms and vice versa. Therefore, in agreement with other studies, these data suggest that noninvasive clinical tools, such as corneal aesthesiometry and the MNSI questionnaire, have the potential to rapidly predict peripheral neuropathy severity in lower limbs. Other studies have also shown positive correlation of peripheral neuropathy with corneal sensitivity[51] and the MNSI questionnaire.[28]

In conclusion, the current study has shown that PRP treatment does not have any significant effect on corneal SBN

density or corneal sensitivity in patients with DM compared with subjects with diabetes who have not undergone PRP.

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