BRIEF REPORTS

Decreased Density of Corneal Basal Epithelium and Subbasal Corneal Nerve Bundle Changes in Patients with Diabetic Retinopathy

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PURPOSE: To define alterations in the density of corneal basal epithelium in relation to diabetic retinopathic severity and the alteration of corneal innervation using confocal microscopy.

DESIGN: Prospective case-control study.

METHODS: Forty-two type II diabetic patients stratified into nondiabetic (NDR), nonproliferative diabetic (NPDR), and proliferative diabetic (PDR) retinopathy and 14 age-matched healthy control subjects were studied. Epithelial and stromal cell densities and subbasal nerves were investigated by confocal microscopy.

RESULTS: Corneal basal epithelial cell density ($P = .0001$), nerve fiber density (NFD) ($P < .0001$), nerve branch density (NBD) ($P = .0003$), and tortuosity coefficient (Tc) ($P < .0001$) were significantly different among the four groups. Basal epithelial density was significantly correlated with NFD ($r = 0.43$, $P = .0009$), NBD ($r = 0.36$, $P = .007$), and TC ($r = -0.58$, $P < .0001$).

CONCLUSIONS: Patients with diabetes show alterations in corneal innervations and basal epithelial cell density in different retinopathic stages. We demonstrate that reduced density in basal epithelial cell is correlated with changes in innervation. (Am J Ophthalmol 2006;142:488–490. © 2006 by Elsevier Inc. All rights reserved.)

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CONFOCAL MICROSCOPY, A NONINVASIVE IN VIVO method for corneal examination, has been used to quantify corneal nerve density and morphology. These measurements can be correlated with the severity of neuropathy in diabetic patients.[1,2,3] However, few studies evaluated corneal basal epithelial cell density with its possible relation to corneal problems. Our study compares basal epithelium density between diabetic retinopathic patients and controls by in vivo confocal microscopy to determine whether corneal basal epithelium density change is associated with an alteration of corneal innervation.

Forty-two type II diabetic patients, including 14 nondiabetic retinopathic (NDR), 14 nonproliferative diabetic retinopathic (NPDR), and 14 proliferative diabetic retinopathic (PDR) patients, and 14 age-matched control subjects underwent corneal confocal microscopic examination (Nidek-ConfoScan 3, Tokyo, Japan). Age, gender, duration of diabetes, fasting blood sugar, and HbA_{1c} were not significantly different among NDR, NPDR, and PDR groups (Table 1). No marked differences in the morphology of corneal basal epithelium, stroma, and

TABLE 1. Clinical Data of Study Patients with Diabetes and Non-diabetic Controls*

Parameter	Control (n = 14)	Nondiabetic Retinopathy (n = 14)	Nonproliferative Diabetic Retinopathy (n = 14)	Proliferative Diabetic Retinopathy (n = 14)
Male (%)	28.6	42.9	35.7	50
Age (yrs)	64.6 ± 9.6	63.4 ± 4.8	64.6 ± 7.3	63.4 ± 9.5
Diabetic duration (yrs)	0	14.3 ± 6.3	19.3 ± 8.2	17.6 ± 9.7
Fasting blood sugar (mg/dl)	<110	128.1 ± 24.3	120.4 ± 34.8	135.0 ± 36.7
HbA1c (%)	<6.6	7.2 ± 0.7	7.3 ± 0.8	8.26 ± 2.2

*data expressed as mean ± standard deviation.

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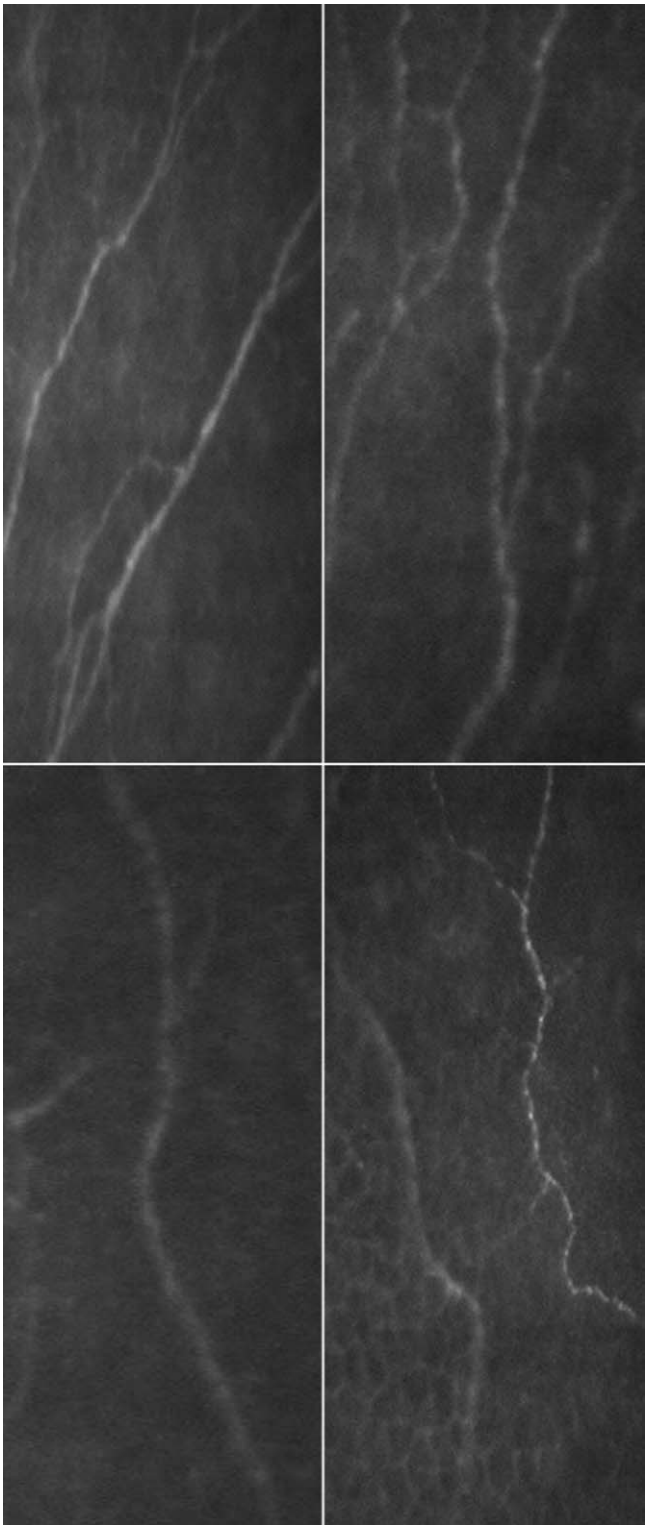

FIGURE. More tortuous and less branching of the corneal subbasal nerve plexus pattern with increasing severity of diabetic retinopathy. (Top left) This view shows the corneal subbasal nerve plexus of the study control. (Top right) This image shows the changes seen in a patient with nondiabetic retinopathy (NDR). (Bottom left) The extent of nerve changes in a patient with nonproliferative diabetic retinopathy (NPDR). (Bottom right) The nerves are shown in a patient with proliferative diabetic retinopathy (PDR).

endothelium between nondiabetic and diabetic patients were observed.

Qualitative assessment of the subbasal nerve plexus layer of the control showed nerve fibers with typical beaded appearance, normal tortuosity, and adequate branching. Comparatively, a patient with proliferative diabetic retinopathy had fewer branches from the main nerve trunk and increased tortuosity (Figure). Descriptive statistics for the cell density of each corneal layer and morphometric variables of the subbasal nerve plexus are given in Table 2. The basal epithelium cell density was significantly different among the four clinical groups ($P = .0001$)(U-ANOVA) (see Supplementary Figure 1 at AJO.com). Post hoc analysis by Dunnett test demonstrated that the basal epithelium cell density was significantly decreased in the NPDR ($P = .002$) and PDR groups ($P = .0001$) compared with control.

Nerve fiber density (NFD), the total number of major nerves observed in one image, showed a statistically significant difference among the four groups ($P < .0001$). Post hoc analysis demonstrated that NFD was significantly decreased in NDR ($P = .0011$), NPDR ($P = .0005$), and PDR groups ($P < .0001$) compared with control. Nerve branch density (NBD), the total number of branches emanating from major nerves observed in one image, showed a statistically significant difference among the four groups ($P = .0003$). Post hoc analysis demonstrated that NBD was significantly decreased in NDR ($P = .04$), NPDR ($P = .005$), and PDR groups compared with control ($P < .0001$). Additionally, tortuosity coefficient (TC), as previously described,[3] defined as the difference in slope and curvature between actual nerve trajectory and the line connecting its endpoints, revealed a statistically significant difference among the four groups ($P < .0001$). Post hoc analysis demonstrated that TC was significantly increased in the PDR group ($P < .0001$) compared with control (see Supplementary Figure 2 at AJO.com).

Corneal basal epithelium density was not significantly correlated with duration of diabetes ($r = -0.41$, $P = .13$), AC sugar ($r = 0.09$, $P = .65$), or HbA_{1c} ($r = -0.31$, $P = .2$). Corneal basal epithelium density was significantly correlated with NFD ($r = 0.43$, $P = .0009$), NBD ($r = 0.36$, $P = .007$), and TC ($r = -0.58$, $P < .0001$).

These results imply corneal nerve change and basal cell density of corneal epithelium are correlated with severity of diabetic retinopathy, and possibly may explain clinical manifestations of corneal complications in the severe form of diabetic retinopathy before and after retinal surgery. Basal epithelial cells secrete a 50 nm thick basement membrane composed of type IV collagen, laminin, and other proteins. Major epithelial basement membrane components in diabetic cornea, including laminin-1, laminin-10, and type IV collagen isoforms, were markedly diminished as revealed by immunofluorescence in a previous study.[4] We hypothesize the decreased density of basal epithelial cells might have less adhesive macromolecules and poor epithelial adherence ability, which results in a fragile epithelium in diabetic patients.

TABLE 2. Confocal Data of Cell Density and Morphometric Variables of Subbasal Nerve Plexus in Study Patients and Control

	Control (n = 14)	NDR (n = 14)	NPDR (n = 14)	PDR (n = 14)	*P*
Density of basal epithelial cell	5894.1 ± 414.9	5690.1 ± 414.7	5375.7 ± 380.3	5253.6 ± 301.5	.0001*
Density of anterior stroma cell	818.3 ± 113.6	821.8 ± 113.0	773.0 ± 81.8	822.1 ± 165.4	.66
Density of posterior stroma cell	564.3 ± 60.7	606.7 ± 60.5	616.4 ± 103.6	587.9 ± 117.1	.49
Density of endothelial cell	2771.1 ± 427.7	2861.5 ± 289.7	2743.1 ± 433.2	2802.6 ± 552.7	.89
NFD (#/mm^2)	26.5 ± 7.5	17.5 ± 5.5	16.9 ± 6.1	13.8 ± 5.7	<.0001†
NBD (#/mm^2)	34.9 ± 6.8	27.5 ± 9.4	25.4 ± 4.8	21.7 ± 9.0	.0003‡
TC	19.0 ± 7.5	15.2 ± 7.9	27.3 ± 19.2	75.1 ± 41.0	<.0001§

NDR = nondiabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; NFD = nerve fiber density; NBD = nerve branch density; TC = tortuosity coefficient.

The first four rows are expressed in cells per square millimeters. Variance homogeneity among groups was determined by Bartlett test. *P* is determined by ANOVA (analysis of variance).

*NPDR vs control, $P = .002$; PDR vs control, $P < .0001$.

†NDR vs control, $P = .001$; NPDR vs control, $P = .0005$; PDR vs control; $P < .0001$.

‡NDR vs control, $P = .037$; NPDR vs control; $P < .005$, PDR vs control; $P < .0001$.

§PDR vs control; $P < .0001$.

More studies need to be conducted to support our hypothesis. Moreover, our observations further support that diabetes has a significant impact on corneal nerve integrity.[5] Corneal epithelial metabolism, cell adhesion, and wound healing depend on adequate corneal innervation.[4] By virtue of our study, we cannot prove directly that the basal epithelial changes are primarily attributable to the nerve alteration. However, this study provides evidence that corneal innervation changes are possible predictors for decreased basal epithelial cell density in diabetes.

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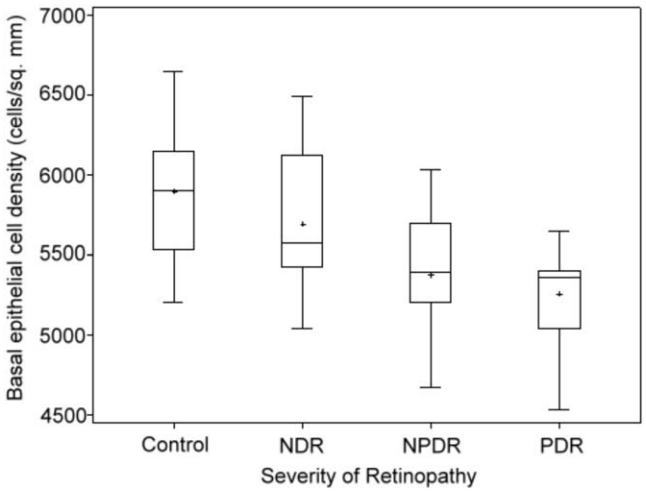
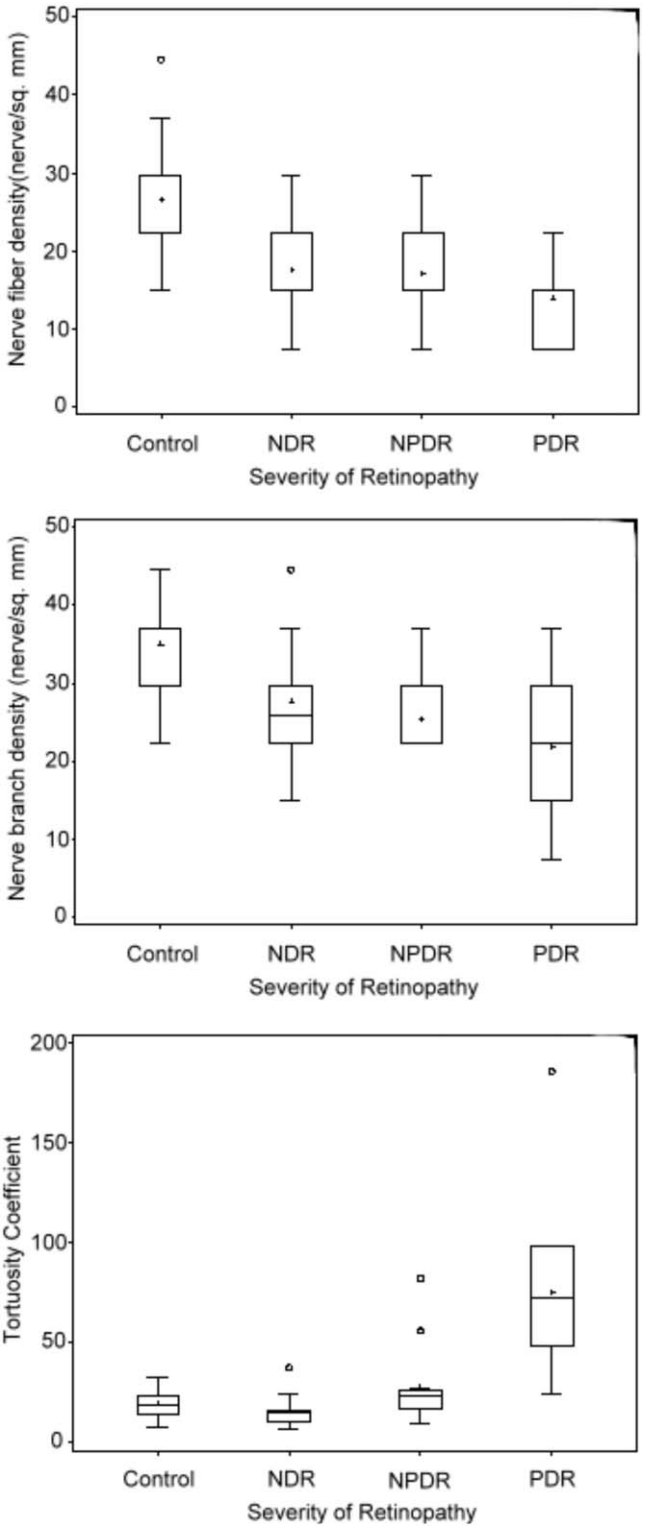


SUPPLEMENTARY FIGURE 1. Basal epithelial densities in control and diabetic subjects. The "*" is the mean of the data set. The horizontal line within the box represents the median of the data set. The upper and lower limits of the box indicate the 75th and 25th centiles, respectively. The upper and lower limits of the whiskers indicate the 90th and 10th centiles, respectively. (NDR = nondiabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy).



SUPPLEMENTARY FIGURE 2. (Top). Nerve fiber density (NFD) in control and diabetic subjects. (Middle) Nerve branch density (NBD) in control and diabetic subjects. (Bottom) Tortuosity coefficient (TC) in control and diabetic subjects. Description of the statistics shown in the box and whisker plots. (NDR = nondiabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy).