

Longitudinal Changes in Corneal Cell and Nerve Fiber Morphology in Young Patients with Type 1 Diabetes with and without Diabetic Retinopathy: A 2-Year Follow-up Study

Eszter A. Deák,^{1,2} Eszter Szalai,² Noémi Tóth,² Rayaz A. Malik,³ András Berta,² and Adrienne Csutak²

¹Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

²Department of Ophthalmology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

³Weill Cornell Medicine and Division of Cardiovascular Sciences, University of Manchester, Manchester, United Kingdom

Correspondence: Adrienne Csutak, Department of Ophthalmology, Faculty of Medicine, University of Debrecen, Nagyerdei körút 98, Debrecen 4032, Hungary; acsutak@med.unideb.hu.

Submitted: April 6, 2018

Accepted: January 14, 2019

Citation: Deák EA, Szalai E, Tóth N, et al. Longitudinal changes in corneal cell and nerve fiber morphology in young patients with type 1 diabetes with and without diabetic retinopathy: a 2-year follow-up study. *Invest Ophthalmol Vis Sci.* 2019;60:830-837. <https://doi.org/10.1167/iops.18-24516>

PURPOSE. We have previously used in vivo corneal confocal microscopy (IVCCM) to demonstrate significant alterations in the corneal epithelial cells, stromal keratocytes, and subbasal nerves in young patients with type 1 diabetes mellitus (T1DM), especially those with diabetic retinopathy (DR). We have evaluated the change in corneal cellular and subbasal nerve morphology over 2 years in young patients with T1DM with or without DR.

METHODS. A total of 19 patients with T1DM, without ($n = 12$) and with ($n = 7$) DR and 19 age- and sex-matched healthy control subjects underwent quantification of corneal cellular and subbasal nerve plexus morphology by using IVCCM at baseline and after 2 years.

RESULTS. There was no significant change in corneal basal epithelial, posterior stromal keratocyte, or endothelial cell densities over 2 years. However, there was a significant reduction in corneal nerve branch ($P = 0.03$) and total nerve branch density ($P = 0.04$) in patients without DR and a significant reduction in corneal nerve fibre density ($P = 0.004$) in those with DR.

CONCLUSIONS. IVCCM can detect a progressive loss of corneal nerve fibers in young patients with T1DM and may allow the identification of individuals at risk of neuropathy progression for more active risk factor reduction.

Keywords: corneal confocal microscopy, type 1 diabetes mellitus, follow-up

Type 1 diabetes mellitus (T1DM) occurs in around 10% of all people with diabetes, affecting 20 million people worldwide, and the prevalence of newly diagnosed cases is predicted to increase by 0.24% to 0.30% over the next 5 to 15 years.¹ Microvascular complications can occur in young people with T1DM,²⁻⁴ and diabetic retinopathy (DR) is considered to be the earliest and most common complication.^{5,6}

Optical coherence tomography (OCT) has shown reduced thickness of the retinal nerve fiber and ganglion cell layers in children with T1DM without retinopathy, which is suggestive of early neuronal damage.⁶⁻⁸ Furthermore, in vivo corneal confocal microscopy (IVCCM), a rapid noninvasive ophthalmic imaging technique has also been shown to identify early corneal cellular and nerve fiber pathology in children and adolescents with T1DM,⁹ adults with T1DM without neuropathy,¹⁰ or retinopathy or microalbuminuria.¹¹ Furthermore, reduced corneal nerve fiber length predicts the development of clinical diabetic neuropathy¹² and the development or worsening of retinopathy.¹³ This early corneal nerve fiber damage has been attributed to elevated hemoglobin A_{1c} (HbA_{1c}) and triglycerides and a lower high-density lipoprotein (HDL).¹⁴ Corneal nerve fiber regeneration has been observed after combined pancreas and kidney transplantation¹⁵ and after treatment with the novel nonerythropoietic peptide ARA

290.¹⁶ Based on our previous study showing reduced corneal nerve fiber parameters in young adolescents with T1DM with and without DR,⁹ the present case-control follow-up study assessed the progression of corneal cellular and nerve fiber abnormalities in these young T1DM patients.

METHODS

Study Subjects

All subjects were attending the Ophthalmology Department, Faculty of Medicine, University of Debrecen. Ethical approval was obtained from the University of Debrecen Ethics Committee (number 4701A-2016), and all subjects provided written informed consent in accordance with the Declaration of Helsinki. In case of children under 18 years of age, written informed consent was obtained from their parents or legal guardians.

At baseline, DR status was graded in T1DM patients according to the International Clinical Diabetic Retinopathy Disease Severity Scale by using fundus photography. Patients were classified into no DR (NDR) and DR groups and compared with age- and sex-matched healthy volunteers (CNDR and CDR) without diabetes or history of systemic inflammatory or ocular

TABLE 1. Demographics of Control Subjects and Patients with T1DM

	Groups					
	CNDR, <i>n</i> = 12	Baseline NDR, <i>n</i> = 12	Follow-up NDR, <i>n</i> = 12	CDR, <i>n</i> = 7	Baseline DR, <i>n</i> = 7	Follow-up DR, <i>n</i> = 7
Age, y						
Mean	17	14	16	37	34	36
±SD	4	3	3	6	6	6
Sex						
Male	4	4	4	6	6	6
Female	8	8	8	1	1	1

CNDR, controls for T1DM patients without DR (NDR); CDR, controls for T1DM patients with DR expressed as mean ± SD.

diseases. Exclusion criteria included contact lens wear and previous intraocular surgery.

Clinical and Ophthalmologic Investigations

Participants with T1DM underwent a comprehensive medical examination that included measurements of HbA_{1c}, serum lipids, estimated glomerular filtration rate (eGFR), blood pressure, and microalbuminuria. Ophthalmologic examination included slit-lamp examination, dilated fundus photography, intraocular pressure measurement, and IVCCM. Additional neuropathy tests and tear layer assessments were not performed.

In Vivo Corneal Confocal Microscopy

In 2014, we undertook IVCCM in 28 young patients with T1DM, with (*n* = 10) and without (*n* = 18) DR.⁹ Nineteen of these T1DM patients with (*n* = 7) and without (*n* = 12) retinopathy who had continued to be seen by their diabetologist under standard clinical care, agreed to be reassessed in 2016. IVCCM analysis was performed using the Heidelberg Retina Tomograph III Rostock Cornea Module (HRT III RCM; Heidelberg Engineering GmbH, Heidelberg, Berlin, Germany). Local anesthetic (tetracaine hydrochloride 0.5%) was applied, and the subject was asked to focus on a distant target before scanning the central cornea. The right eye of normal controls and NDR patients was used for analysis, and in patients with DR, only the eligible eye (no previous intraocular surgery) was used for analysis. Section and volume scans were recorded from the basal epithelium anterior to Bowman's layer, subbasal nerve plexus (SBP), posterior stroma anterior to Descemet's membrane and endothelial cells (volume scans from the epithelium to the endothelial cells, and good quality section scans at the missing depths).

Image Selection

Three representative images of good quality were chosen from the basal epithelium, SBP, posterior stromal (keratocytes), and endothelial cell layers. A region of interest (ROI) was chosen from each cell layer containing at least 50 cells.

Image Analysis

The ROI area was $0.003 \pm 0.007 \text{ mm}^2$ for the epithelium, $0.100 \pm 0.009 \text{ mm}^2$ for the stromal keratocyte layer, and $0.028 \pm 0.009 \text{ mm}^2$ for the endothelium. Focus position was $7 \pm 3 \mu\text{m}$ for the epithelium, $65 \pm 11 \mu\text{m}$ for the keratocytes, and $564 \pm 17 \mu\text{m}$ for the endothelium. The cells were marked manually and the instrument-based software (Heidelberg Eye Explorer software; Heidelberg Engineering GmbH, Heidelberg,

Berlin, Germany) automatically calculated cell densities (cells/mm²). SBP morphology was quantified using automated software (ACCMetrics version 2.0; University of Manchester, Manchester, UK).¹⁷ The following parameters were quantified: corneal nerve fiber density (CNFD), the number of nerve fibers/mm²; corneal nerve branch density (CNBD), the number of primary branch points on the main nerve fibers/mm²; corneal nerve fiber length (CNFL), the total length of nerves mm/mm²; corneal nerve fiber total branch density (CNTBD), the total number of branch points/mm²; corneal nerve fibre area (CNFA), the total nerve fibre area mm²/mm²; and corneal nerve fiber width (CNFW), the average nerve fiber width mm/mm².

Statistical Analysis

The statistical analysis was performed using SPSS (version 22.0) and MedCalc (version 18.2.1) statistical programs for Windows. Descriptive data are shown as mean ± standard deviation (SD) and 95% confidence interval (CI). Student's *t*-test was used to determine the differences between patients with T1DM and controls. A paired *t*-test was used to compare corneal cellular and nerve morphology between baseline and follow-up. For univariate analysis, the χ^2 test and for bivariate datasets Pearson correlation test was used. Case-control analysis was performed with statistical significance criterion set at *P* < 0.05 in all cases.

RESULTS

Demographic and Clinical Data

The demographic data are summarized in Table 1, and the clinical metabolic data of T1DM patients with and without DR are shown in Table 2. Total cholesterol decreased (*P* = 0.04) in the DR group, while triglycerides, HDL cholesterol, eGFR, blood pressure, and microalbuminuria showed no significant change over 2 years.

Corneal Cell Densities

There was no significant change in epithelial, keratocyte and endothelial cell densities, and central corneal thickness in patients with T1DM with and without DR compared to control subjects (Table 3; Fig. 1, 2).

Corneal SBP

There was no significant difference in any corneal nerve parameter between T1DM patients without DR and control subjects. CNFL was significantly lower in T1DM patients with DR compared to control subjects (*P* = 0.002). Over 2 years,

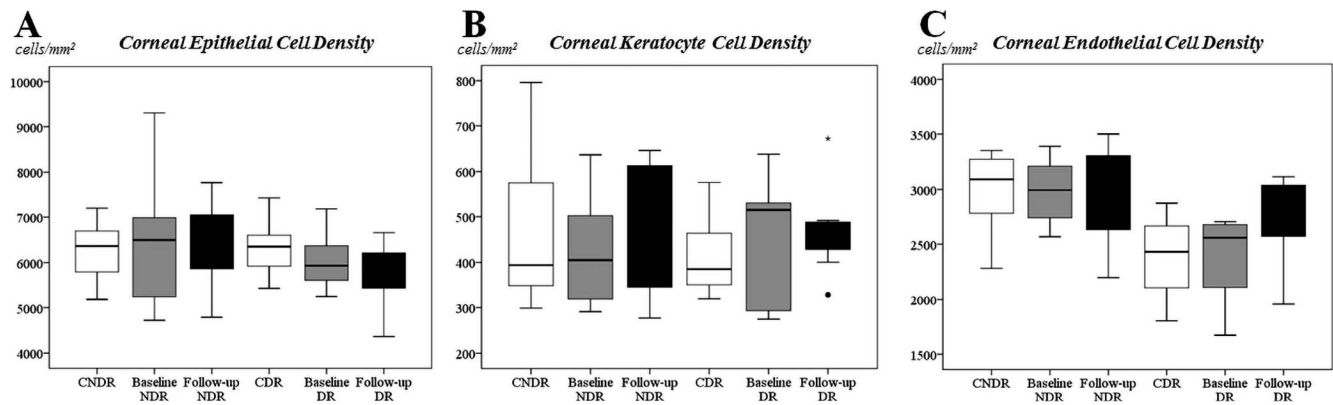


FIGURE 1. Corneal cell densities in controls compared to T1DM over 2 years. Patients with T1DM without (NDR) and with (DR) retinopathy compared to controls (CNDR and CDR) at baseline and follow-up. The basal epithelial cell (A), keratocyte (B), and endothelial cell (C) densities measured with in vivo corneal confocal microscopy (IVCCM). Controls (white bars), T1DM baseline (gray bars), and follow up (black bars). Box and whiskers show the mean values with interquartile range.

there was a significant decrease in CNBD ($P = 0.03$) and CNTBD ($P = 0.04$), with a trend for reduction in CNFA ($P = 0.08$) and CNFW ($P = 0.07$) in the NDR group (Fig. 3). There was a significant reduction in CNFD ($P = 0.04$) in the DR group (Table 4).

Correlation Analysis

In patients with T1DM, serum triglycerides correlated inversely with CNFW ($r = -0.339$, $P = 0.04$), but there was no significant correlation between the duration of diabetes, HbA_{1c}, serum triglycerides, HDL, and eGFR with any other corneal nerve parameters ($P > 0.05$).

DISCUSSION

IVCCM is a rapid, noninvasive ophthalmic imaging technique that detects subclinical nerve damage¹⁸ and predicts the development of neuropathy in adults with diabetes.^{12,19} In two large pooled analyses, a normative age range²⁰ and a good diagnostic ability for diabetic peripheral neuropathy have been established.²¹ A reduction in basal epithelial and intermediate cell density was related to diabetes duration and diastolic blood pressure, while reduced CNFD and CNFL were related to HbA_{1c} in adults with T1DM and type 2 diabetes mellitus (T2DM).²² Additionally, CNFD, CNDB, and CNFL were reduced in adults with T2DM with and without DR, while epithelial,

TABLE 2. Clinical and Metabolic Data of T1DM Patients With (DR) and Without (NDR) Retinopathy, at Baseline and Follow-up

	Baseline NDR, n = 12	Follow-up NDR, n = 12	P*	Baseline DR, n = 7	Follow-up DR, n = 7	P*
Duration of DM, y						
Mean	6	9		22	24	
±SD	3	3		7	7	
HbA _{1c} , %			0.278			0.894
Mean	7.82	8.14		8.29	8.43	
±SD	1.09	1.22		0.93	2.05	
95% CI	7.1-8.5	7.4-8.9		7.4-9.2	6.5-10.3	
Cholesterol, mmol/L			0.123			0.042
Mean	4.03	4.37		5.23	5.10	
±SD	0.51	0.73		0.59	1.35	
95% CI	3.7-4.4	3.9-4.9		4.3-6.2	1.7-8.5	
Triglycerides, mmol/L			0.067			0.317
Mean	0.83	0.73		2.43	1.45	
±SD	0.27	0.18		1.80	0.78	
95% CI	0.7 to 1.0	0.6 to 0.9		-2.0 to 6.9	-5.5 to 8.4	
HDL cholesterol, mmol/L			0.320			0.578
Mean	1.73	1.84		1.93	1.85	
±SD	0.25	0.28		1.21	0.07	
95% CI	1.6 to 1.9	1.7 to 2.0		-1.1 to 4.9	1.2 to 2.5	
eGFR, mL/min/1.73m ²			0.801			0.943
Mean	>90 in all patients	>90 in all patients		81.71	80.71	
±SD				21.92	24.57	
95% CI				61.4 to 101.9	57.9 to 103.4	
Hypertension	0	0		2	2	
Insulin pump users	5	5		0	0	
Microalbuminuria	0	0		2	2	

* P values, paired t-test to determine the differences between baseline and follow-up.

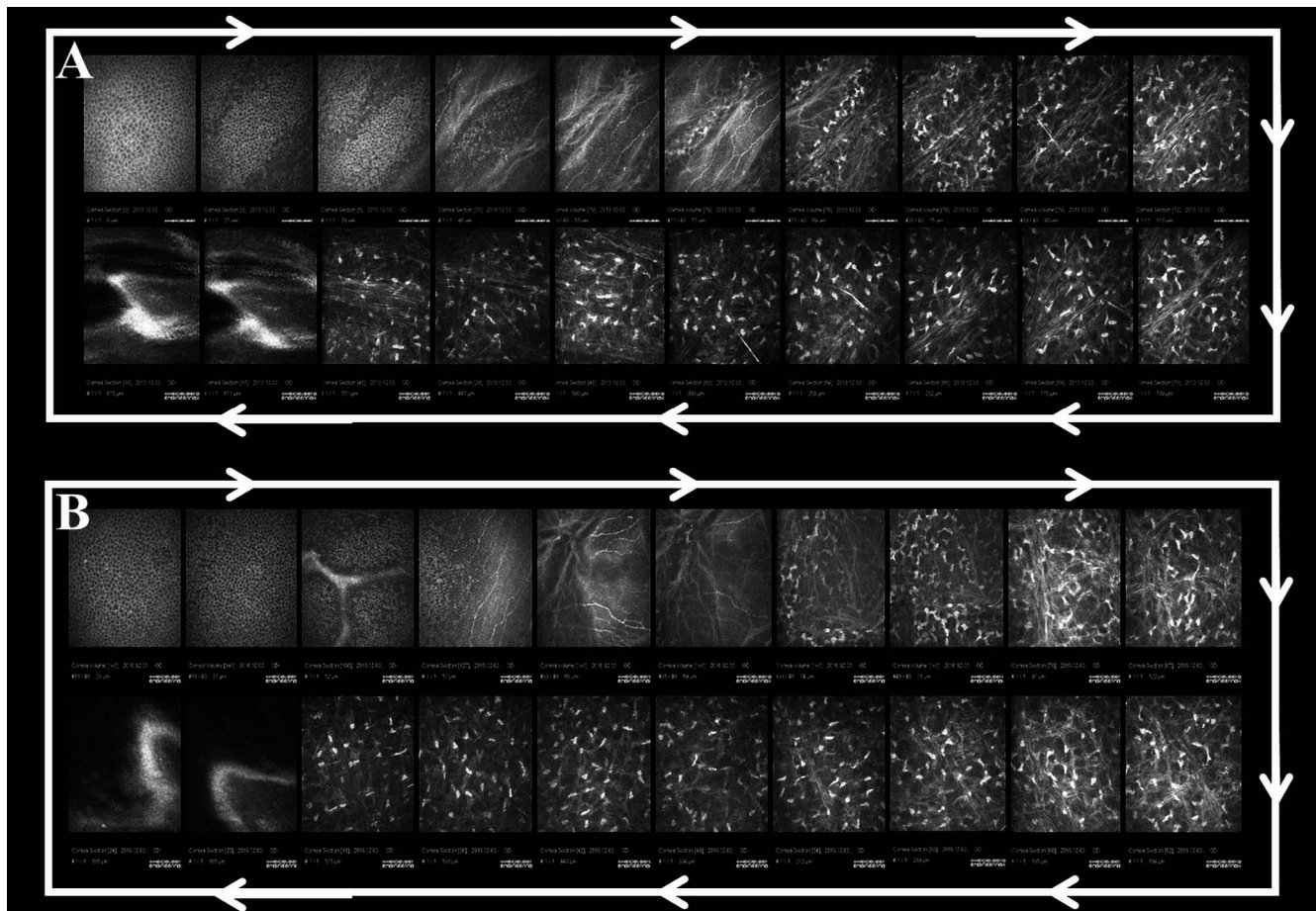


FIGURE 2. Corneal cell identification using Heidelberg Retina Tomograph instrument-based software. Section and volume scans from the corneal cell layers in the same patient's same eye with T1DM with DR at follow-up, at baseline (A) and after 2 years (B).

TABLE 3. Corneal Cell Densities and Central Corneal Thickness in Patients with T1DM Compared to Control Subjects at Baseline and Follow-up

	Groups					
	CNDR, n = 12	Baseline NDR, n = 12	Follow-up NDR, n = 12	CDR, n = 7	Baseline DR, n = 7	Follow-up DR, n = 7
Epithelial cell density, cells/mm ²						
Mean	6,248.33	6,333.72	6,514.74	6,327.58	6,048.71	5,743.95
±SD	639.62	1,290.25	877.19	650.76	671.17	772.87
95% CI	5,818.6–6,678.0	5,513.9–7,153.5	5,925.4–7,104.1	5,783.5–6,871.6	5,428.0–6,669.5	5,029.2–6,458.7
P†	0.845*		0.701†	0.894*		0.446†
Keratocyte cell density, cells/mm ²						
Mean	475.73	414.62	466.40	385.00	439.38	472.40
±SD	168.97	115.21	140.48	70.14	148.27	105.18
95% CI	362.2–5,889.2	337.2–492.0	372.0–560.8	311.4–458.6	302.3–576.5	375.1–569.7
P	0.334*		0.356†	0.668*		0.639†
Endothelial cell density, cells/mm ²						
Mean	2,977.95	2,980.48	2,980.70	2,384.50	2,343.53	2,652.37
±SD	406.53	292.12	490.10	442.18	445.26	464.62
95% CI	2,551.3–3,404.6	2,736.3–3,224.7	2,604.0–3,357.4	1,680.9–3,088.1	1,790.6–2,896.4	2,075.5–3,229.3
P	0.989*		0.999†	0.429*		0.315†
Central corneal thickness, μm						
Mean	551.54	562.067	560.00	545.06	560.46	576.86
±SD	28.21	25.48	34.89	20.21	23.55	33.28
95% CI	532.6–570.5	545.8–578.3	538.4–582.7	528.1–562.0	538.7–582.2	546.1–607.6
P	0.358*		0.907†	0.196*		0.308†

CNDR, controls for NDR group; CDR, controls for DR group.

* P value determined using Student's *t*-test to compare the baseline T1DM groups with controls.

† P value determined using paired *t*-test to determine the difference between baseline and follow-up.

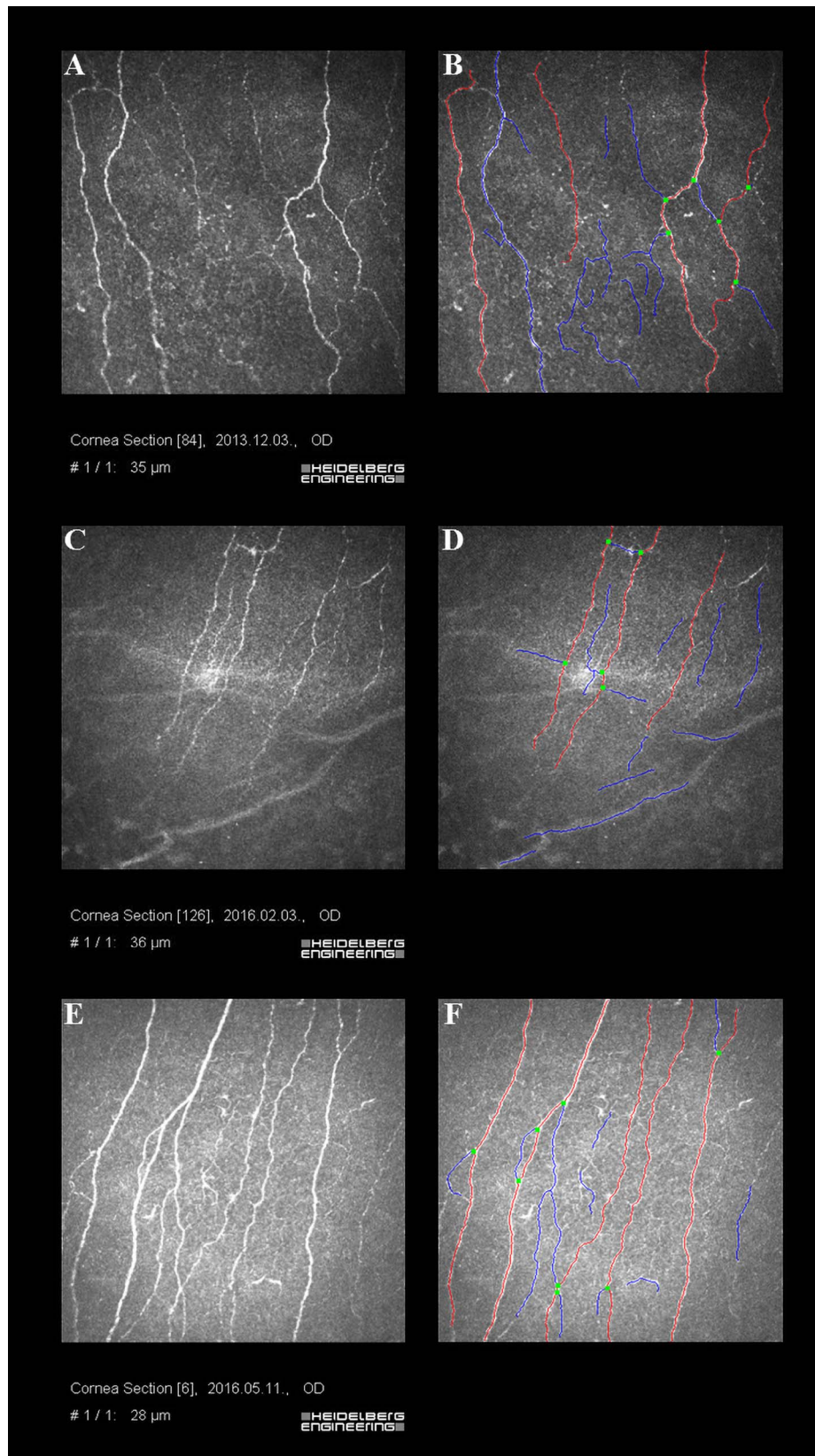


FIGURE 3. Original and annotated representative images of the SBP by using ACCMetrics software. SBP morphology of a patient with T1DM with DR at baseline (**A, B**) and after 2 years (**C, D**) compared with a healthy age- and sex-matched subject (**E, F**). *Red lines* (main fibers), *blue lines* (branches), and *green points* (branch points) showing reduced corneal nerves at baseline with a further reduction at follow up.

TABLE 4. SBP Morphology in Control Subjects and Patients with T1DM at Baseline and Follow-up

	Groups					
	CNDR, n = 12	Baseline NDR, n = 12	Follow-up NDR, n = 12	CDR, n = 7	Baseline DR, n = 7	Follow-up DR, n = 7
CNFD, no./mm ²						
Mean	13.97	10.81	10.76	16.93	14.58	6.60
±SD	7.36	11.09	9.26	4.45	7.36	3.59
95% CI	9.0-18.9	0.5-21.1	1.0-20.5	13.2-20.7	5.4-23.7	2.8-10.4
P	0.530*		0.173†	0.485*		0.043†
CNBD, no./mm ²						
Mean	12.59	12.36	6.25	16.66	16.25	6.25
±SD	7.48	9.50	5.43	5.59	12.79	5.43
95% CI	7.6-17.6	3.6-21.2	0.5-11.9	11.9-21.3	0.4-32.1	0.5-11.9
P	0.196*		0.029†	0.936*		0.114†
CNFL, mm/mm ²						
Mean	10.33	10.67	8.58	12.52	8.43	7.71
±SD	3.19	4.98	3.49	0.85	2.96	1.44
95% CI	8.2-12.5	6.1-15.3	6.2-10.9	11.8-13.2	5.7-11.2	6.4-9.1
P	0.494*		0.153†	0.002*		0.573†
CNTBD, no./mm ²						
Mean	28.50	28.82	20.36	31.44	21.73	20.83
±SD	12.93	14.44	10.67	10.64	13.28	12.15
95% CI	19.8-37.2	15.5-42.2	13.2-7.5	22.5-40.3	9.5-34.0	9.6-32.1
P	0.214*		0.035†	0.140*		0.898†
CNFA, mm ² /mm ²						
Mean	0.0047	0.0057	0.0047	0.0053	0.0046	0.0051
±SD	0.0020	0.0013	0.0012	0.0001	0.0017	0.0019
95% CI	0.003-0.006	0.005-0.007	0.004-0.006	0.005-0.006	0.003-0.006	0.003-0.007
P	0.160*		0.086†	0.404*		0.694†
CNFW, mm/mm ²						
Mean	0.0235	0.0240	0.0260	0.0220	0.0240	0.0250
±SD	0.0028	0.0031	0.0038	0.0019	0.0031	0.0044
95% CI	0.022-0.025	0.021-0.027	0.023-0.028	0.021-0.024	0.021-0.028	0.021-0.028
P	0.839*		0.074†	0.239*		0.760†

CNDR, controls for T1DM without NDR; CDR, controls for T1DM with DR.

* P values determined with Student's *t*-test to compare baseline T1DM to controls.

† P values determined with paired *t*-test to determine the difference between baseline and follow-up.

stromal, and endothelial cell densities were only reduced in patients with DR.²³ Furthermore, in T1DM we have shown an early reduction in corneal nerve fibers in patients without DR or microalbuminuria.²⁴

IVCCM can be undertaken reliably and with good reproducibility in children with T1DM.²⁵ An initial study in children and adolescents with T1DM showed no difference in corneal nerve morphology.²⁶ Although, more recently we have shown a reduction in epithelial and endothelial cell densities and a reduction in CNDB and CNFL in adolescent T1DM patients without DR and a further reduction in CNFD and CNBD in those with DR.⁹

There are no longitudinal studies assessing change in corneal cellular morphology, and only one previous study has assessed nerve fiber morphology over time in adults with T1DM, showing a reduction in CNFD and CNBD, which was related to HbA_{1c}.¹⁴ This is the first longitudinal study in young adolescents with T1DM and it shows no significant change in epithelial, stromal, and endothelial cell densities over 2 years. With regard to early nerve fiber alterations, previous studies have shown a reduction in corneal and retinal nerve fiber parameters prior to the development of retinopathy^{13,27} and a relatively greater reduction in CNFL in patients with T1DM compared to T2DM.²⁸ In the present study over 2 years, there was a significant decrease in the distal branches (CNBD and CNTBD) in young patients with T1DM without DR and a reduction in more proximal nerves (CNFD) in patients with

DR. This is consistent with a retrograde process of neurodegeneration that has been demonstrated recently with a greater reduction in the inferior whorl compared to central CNFL.²⁹ The diagnostic ability of IVCCM may be further enhanced by assessing the inferior whorl or using wide-area mosaics, which take into account both proximal and distal corneal nerve morphology.^{30,31}

Multiple pathogenetic mechanisms, including advanced glycation, increased flux through the sorbitol pathway, and oxidative stress, have been implicated in the development of diabetic neuropathy.³² Experimental studies using a combination of menhaden oil, α -lipoic acid, and enalapril have been shown to ameliorate oxidative and inflammatory stress and improve distal corneal nerve morphology.³³ Several recent studies have shown early corneal nerve fiber regeneration in T1DM patients after combined pancreas and kidney transplantation,¹⁵ T2DM patients treated with the novel nonerythropoietic peptide ARA 290,¹⁶ and T1DM patients treated with omega-3 polyunsaturated fatty acid supplementation.³⁴ Furthermore, a recent study in patients with T2DM has shown that multifactorial intervention that reduces HbA_{1c}, blood pressure, and weight results in corneal nerve fiber regeneration.³⁵ Indeed, a change in corneal nerve morphology may prove to be a more sensitive end-point to assess the benefits of risk factor reduction on microvascular complications, given that the recent Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial showed no change in albumin excretion and

retinopathy in children treated with a statin and ACE inhibitor over 4 years.³⁶

A limitation of the current study is the relatively small number of patients assessed at follow-up. However, this is the first longitudinal study of young adolescents with T1DM and it shows an early and progressive reduction in corneal nerve morphology. We believe these data support the potential utility of corneal nerve morphology quantification to assess the benefits of new therapies for diabetic neuropathy.

Acknowledgments

Supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences, TÁMOP-4.2.2.A-11/1 KONV-2012-0045 by the European Union, cofinanced by the European Social Fund.

Disclosure: **E.A. Deák**, None; **E. Szalai**, None; **N. Tóth**, None; **R.A. Malik**, None; **A. Berta**, None; **A. Csutak**, None

References

- Rogers MAM, Kim C, Banerjee T, Lee JM. Fluctuations in the incidence of type 1 diabetes in the United States from 2001 to 2015: a longitudinal study. *BMC Med.* 2017;15:199.
- Amutha A, Anjana RM, Venkatesan U, et al. Incidence of complications in young-onset diabetes: comparing type 2 with type 1 (the young diab study). *Diabetes Res Clin Pract.* 2017;123:1-8.
- Costacou T, Crandell J, Kahkoska AR, et al. Dietary patterns over time and microalbuminuria in youth and young adults with type 1 diabetes: the SEARCH nutrition ancillary study. *Diabetes Care.* 2018;41:1615-1622.
- Metwalley KA, Hamed SA, Farghaly HS. Cardiac autonomic function in children with type 1 diabetes. *Int J Endocrinol.* 2018;177:805-813.
- Tönnies T, Stahl-Peche A, Baechle C, et al. Risk of microvascular complications and macrovascular risk factors in early-onset type 1 diabetes after at least 10 years duration: an analysis of three population-based cross-sectional surveys in Germany between 2009 and 2016. *Cardiovasc Diabetol.* 2018;2018:7806980.
- Karti O, Nalbantoglu O, Abali S, et al. Retinal ganglion cell loss in children with type 1 diabetes mellitus without diabetic retinopathy. *Ophthalmic Surg Lasers Imaging Retina.* 2017;48:473-477.
- El-Fayoumi D, Badr Eldine NM, Esmael AF, Ghalwash D, Soliman HM. Retinal nerve fiber layer and ganglion cell complex thicknesses are reduced in children with type 1 diabetes with no evidence of vascular retinopathy. *Invest Ophthalmol Vis Sci.* 2016;57:5355-5360.
- Tekin K, Inanc M, Kurnaz E, et al. Quantitative evaluation of early retinal changes in children with type 1 diabetes mellitus without retinopathy. *Clin Exp Optom.* 2018;101:680-685.
- Szalai E, Deák E, Modis L Jr, et al. Early corneal cellular and nerve fiber pathology in young patients with type 1 diabetes mellitus identified using corneal confocal microscopy. *Invest Ophthalmol Vis Sci.* 2016;57:853-858.
- Petropoulos IN, Alam U, Fadavi H, et al. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care.* 2013;36:3646-3651.
- Petropoulos IN, Green P, Chan AW, et al. Corneal confocal microscopy detects neuropathy in patients with type 1 diabetes without retinopathy or microalbuminuria. *PLoS One.* 2015;10:e0123517.
- Pritchard N, Edwards K, Russell AW, Perkins BA, Malik RA, Efron N. Corneal confocal microscopy predicts 4-Year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care.* 2015;38:671-675.
- Srinivasan S, Dehghani C, Pritchard N, et al. Ophthalmic and clinical factors that predict four-year development and worsening of diabetic retinopathy in type 1 diabetes. *J Diabetes Complications.* 2018;32:67-74.
- Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Risk factors associated with corneal nerve alteration in type 1 diabetes in the absence of neuropathy: a longitudinal in vivo corneal confocal microscopy study. *Cornea.* 2016;35:847-852.
- Tavakoli M, Mitu-Pretorian M, Petropoulos IN, et al. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes.* 2013;62:254-260.
- Brines M, Dunne AN, van Velzen M, et al. ARA 290, a nonerythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes. *Mol Med.* 2015;20:658-666.
- Dabbah MA, Graham J, Petropoulos IN, Tavakoli M, Malik RA. Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging. *Med Image Anal.* 2011;15:738-747.
- Chen X, Graham J, Dabbah MA, et al. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intracutaneous nerve fiber density. *Diabetes Care.* 2015;38:1138-1144.
- Edwards K, Pritchard N, Dehghani C, et al. Corneal confocal microscopy best identifies the development and progression of neuropathy in patients with type 1 diabetes. *J Diabetes Complications.* 2017;31:1325-1327.
- Tavakoli M, Ferdousi M, Petropoulos IN, et al. Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. *Diabetes Care.* 2015;38:838-843.
- Perkins BA, Lovblom LE, Bril V, et al. Corneal confocal microscopy for identification of diabetic sensorimotor polyneuropathy: a pooled multinational consortium study. *Diabetologia.* 2018;61:1856-1861.
- Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Abnormal anterior corneal morphology in diabetes observed using in vivo laser-scanning confocal microscopy. *Ocul Surf.* 2016;14:507-514.
- Bitirgen G, Ozkagnici A, Malik RA, Kerimoglu H. Corneal nerve fibre damage precedes diabetic retinopathy in patients with type 2 diabetes mellitus. *Diabet Med.* 2014;31:431-438.
- Petropoulos IN, Green P, Chan AW, et al. Corneal confocal microscopy detects neuropathy in patients with type 1 diabetes without retinopathy or microalbuminuria. *PLoS One.* 2015;10:e0123517.
- Pacaud D, Romanchuk KG, Tavakoli M, et al. The reliability and reproducibility of corneal confocal microscopy in children. *Invest Ophthalmol Vis Sci.* 2015;56:5636-5640.
- Sellers EA, Clark I, Tavakoli M, Dean HJ, McGavock J, Malik RA. The acceptability and feasibility of corneal confocal microscopy to detect early diabetic neuropathy in children: a pilot study. *Diabet Med.* 2013;30:630-631.
- Srinivasan S, Dehghani C, Pritchard N, et al. Corneal and retinal neuronal degeneration in early stages of diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 2017;58:6365-6373.
- Stem MS, Hussain M, Lentz SI, et al. Differential reduction in corneal nerve fiber length in patients with type 1 or type 2 diabetes mellitus. *J Diabetes Complications.* 2014;28:658-661.
- Kalteniece A, Ferdousi M, Petropoulos I, et al. Greater corneal nerve loss at the inferior whorl is related to the presence of diabetic neuropathy and painful diabetic neuropathy. 2018;8:3283.

30. Lagali NS, Allgeier S, Guimarães P, et al. Reduced corneal nerve fiber density in type 2 diabetes by wide-area mosaic analysis. *Invest Ophthalmol Vis Sci.* 2017;58:6318-6327.
31. Lagali NS, Allgeier S, Guimarães P, et al. Wide-field corneal subbasal nerve plexus mosaics in age-controlled healthy and type 2 diabetes populations. *Sci Data.* 2018;5:180075.
32. Malik RA. Wherefore art thou, o treatment for diabetic neuropathy? *Int Rev Neurobiol.* 2016;127:287-317.
33. Yorek MS, Obrosova A, Shevalye H, Coppey LJ, Kardon RH, Yorek MA. Early vs. late intervention of high fat/low dose streptozotocin treated C57Bl/6J mice with enalapril, alpha-lipoic acid, menhaden oil or their combination: effect on diabetic neuropathy related endpoints. *Neuropharmacology.* 2017;116:122-131.
34. Lewis EJH, Perkins BA, Lovblom LE, Bazinet RP, Wolever TMS, Bril V. Effect of omega-3 supplementation on neuropathy in type 1 diabetes: A 12-month pilot trial. *Neurology.* 2017;88:2294-2301.
35. Ishibashi F, Taniguchi M, Kosaka A, Uetake H, Tavakoli M. Improvement in neuropathy outcomes with normalizing HbA(1c) in patients with type 2 diabetes. *Diabetes Care.* 2018;42:110-118.
36. Marcovecchio ML, Chiesa ST, Bond S, et al. ACE inhibitors and statins in adolescents with type 1 diabetes. *N Engl J Med.* 2017;377:1733-1745.