Proliferative Retinopathy in Type 1 Diabetes Is Associated With Cerebral Microbleeds, Which Is Part of Generalized Microangiopathy

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OBJECTIVE

We investigated whether proliferative diabetic retinopathy in type 1 diabetic patients can be generalized to cerebral small vessel disease and whether it is associated with impaired peripheral microvascular function.

RESEARCH DESIGN AND METHODS

Thirty-three patients with proliferative diabetic retinopathy (PDR+), 34 patients without proliferative diabetic retinopathy, and 33 controls underwent magnetic resonance imaging to assess cerebral microangiopathy (cerebral microbleeds) and ischemic damage (white matter hyperintensities and lacunes). Peripheral microvascular function, i.e., skin capillary density and capillary recruitment, was assessed by capillary microscopy.

RESULTS

Cerebral microbleeds, but not ischemic damage, were more prevalent in PDR+ patients versus the other groups ($P < 0.05$). A trend was found across groups for the lowest baseline capillary density in PDR+ patients (P for trend = 0.05). In individuals with microbleeds, capillary recruitment was impaired compared with those without microbleeds ($P = 0.04$).

CONCLUSIONS

In PDR+ patients, cerebral microbleed prevalence was higher and seems part of generalized microangiopathy that may affect the skin and the brain.

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Studies, such as ACCORD (Action to Control Cardiovascular Risk in Diabetes), have shown retinopathy and cerebral small vessel disease to be related to stroke in diabetes (1–3), whereas prevalence of ischemic small vessel disease (white matter hyperintensities and lacunes) does not seem to be increased in type 1 diabetes (4,5). This may suggest that only subgroups, i.e., those with (proliferative) retinopathy, are more at risk to develop small vessel disease. Retinopathy is closely linked to cumulative hyperglycemic exposure and longer disease duration (6) and has been related to brain changes (7). Cerebral microbleeds indicate hemosiderin leakage mainly at the capillary level, representing permanent cerebral vascular damage (8,9). It seems a more direct measure of cerebral microangiopathy than ischemic tissue damage (9,10). Cerebral microbleeds have not been studied in type 1 diabetes.

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Furthermore, it is not known whether cerebral small vessel disease is associated with peripheral microvascular functioning and thus part of generalized microangiopathy. Therefore, we assessed the presence of cerebral small vessel disease and peripheral microvascular function in type 1 diabetic patients with and without proliferative retinopathy, as a marker of cumulative hyperglycemic damage, and healthy controls.

RESEARCH DESIGN AND METHODS

This study was performed as part of a larger cross-sectional observational study (7) to which microbleed imaging and capillary microscopy were added at a later stage. In brief, 33 consecutive patients with proliferative diabetic retinopathy (PDR+), 34 patients without proliferative diabetic retinopathy (PDR−), and 33 healthy controls underwent these measurements. Proliferative or no retinopathy was ascertained by fundus photography. Microalbuminuria (albumin-to-creatinine ratio [ACR] >2.5 mg/mmol for men and >3.5 mg/mmol for women) was determined by 24-h urine sampling, and neuropathy was based on results of annual neuropathy checkup or self-report in case results were not available. PDR− patients were free of clinically manifest microangiopathy, whereas PDR+ patients could have other complications. The study was approved by the local ethics review board, and written informed consent was obtained of all participants.

Magnetic resonance imaging was performed on a 1.5 T magnetic resonance system (Siemens-Sonata, Erlangen, Germany) using an eight-channel phased-array head coil. Cerebral microbleeds were assessed using T2* susceptibility weighted imaging [repetition time 48.0 ms; echo time 40.0 ms; flip angle 150; slice thickness 2.0 mm (11)] and white matter hyperintensities and lacunes using T2-FLAIR [repetition time 6500 ms; echo time 385 ms; variable flip angle (12); slice thickness 1.3 mm]. All images were rated by a trained neuroradiologist. Cerebral small vessel disease markers were rated as absent or present, and number of cerebral microbleeds was determined.

Peripheral microvascular function was assessed by capillary microscopy as described previously (13). Nail fold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope. Baseline capillary density was defined as the number of continuously erythrocyte-perfused capillaries per square millimeter. Capillary density during peak reactive hyperemia was counted after 4 min of arterial occlusion. Capillary recruitment was calculated as the relative increase in capillary density from baseline to capillary density during peak reactive hyperemia. Only Caucasians were included since skin capillary microscopy cannot be performed on pigmented skin.

With 100 participants, $\alpha = 0.05$, power $(1 - \beta) = 0.80$, an effect size $\delta = 0.51$ could be detected. Differences in subject characteristics were analyzed using one-way ANOVA or χ^2 test. Participants were dichotomized on the presence or absence of small vessel disease. Logistic regression was performed to determine differences in prevalence of these markers, corrected for age, sex, and hypertension. Multivariate ANCOVA, corrected for age, sex, and hypertension, was used to determine differences in skin capillary density between those with and without markers of small vessels disease. $P < 0.05$ was considered statistically significant. All analyses were performed using IMB-SPSS-20.0 (IBM-SPSS, Chicago, IL).

RESULTS

Participant characteristics are shown in Table 1. PDR+ patients were significantly older and had higher systolic blood pressure than the other two groups. A1C was significantly higher in both diabetes groups compared with healthy controls. PDR+ versus PDR− patients had longer disease duration, earlier onset age, and higher hypertension rates (all $P < 0.05$). There were no statistically significant differences between included and excluded participants (Supplementary Table 1).

In 11 participants, cerebral microbleeds were detected. Six had one, four had two, and one participant had eight microbleeds. Cerebral microbleeds were more prevalent in PDR+ ($n = 7$; 21%) compared with PDR− patients ($n = 1$; 3%; $P = 0.01$) and controls ($n = 3$; 9%; $P = 0.02$). Prevalence did not differ between PDR− and controls ($P = 0.49$; uncorrected χ^2 P for trend = 0.17). The difference between patient groups remained significant after adjustment for A1C, diabetes duration, and ACR ($P = 0.05$). Due to the low absolute number of cerebral microbleeds in these participants, further statistical testing was not performed. There was no difference in white matter hyperintensity prevalence between PDR+ patients ($n = 8$; 24.2%), PDR− patients ($n = 5$; 14.7%), and controls ($n = 5$; 15.2%; all $P > 0.05$). Lacunes were not found in this cohort.

Capillary microscopy data are shown in Supplementary Fig. 1. There was a trend across groups toward lower baseline capillary density (PDR+, 45 ± 7 n/mm^2; PDR−, 46 ± 9 n/mm^2; controls, 48 ± 10 n/mm^2; P for trend = 0.05; Supplementary Fig. 1*A*) and during peak reactive hyperemia (PDR+, 62 ± 12 n/mm^2; PDR−, 65 ± 16 n/mm^2; controls, 67 ± 17 n/mm^2; P for trend = 0.06; Supplementary Fig. 1*B*) in PDR+ patients, whereas none of the between-group differences were significant. In all subjects with versus without cerebral microbleeds, irrespective of group allocation, capillary recruitment was lower (with, 29 ± 15%, density at baseline 46 ± 7 to 60 ± 13 n/mm^2 during peak reactive hyperemia; without, 40 ± 16%, density at baseline 47 ± 9 to 65 ± 15 n/mm^2 during peak reactive hyperemia; $P = 0.04$; Supplementary Fig. 1*C* and *D*). This difference was most notable in PDR+ patients (with cerebral microbleeds, 28 ± 14%; without cerebral microbleeds, 41 ± 17%; $P = 0.03$; after adjustment for A1C, diabetes duration, and ACR, $P = 0.02$).

CONCLUSIONS

In this study, the relationship between cerebral small vessel disease and peripheral microvascular function in type 1 diabetic patients with and without proliferative retinopathy and healthy controls was investigated. Our results show that cerebral microbleeds, in contrast to ischemic small vessel disease, are more prevalent in PDR+

Table 1—Characteristics of the participants

		Type 1 diabetes		
	Healthy controls	PDR−	PDR+	Overall *P* value
N	33	34	33	—
Sex (men/women; % male)	16/17 (48.5)	13/21 (38.2)	12/21 (36.4)	0.56
Age (years)	37.3 ± 10.7	38.8 ± 9.1	44.5 ± 6.8*†	<0.01
Disease duration (years)	—	21.6 ± 9.1	35.3 ± 8.2	<0.01
Disease onset age (years)	—	17.3 ± 9.7	9.2 ± 7.7	<0.01
Diabetes early onset (%)‡	—	5 (14.7)	14 (42.4)	0.01
Severe hypoglycemia§	—	5.6 ± 8.5	7.1 ± 10.7	0.51
BMI (kg/m^2)	24.2 ± 3.2	24.8 ± 3.3	25.7 ± 4.4	0.23
Systolic blood pressure (mmHg)	123.7 ± 11.5	131.0 ± 15.7	133.0 ± 18.3*†	0.04
Diastolic blood pressure (mmHg)	76.7 ± 7.8	79.2 ± 9.8	76.0 ± 9.3	0.32
Hypertension (%)‖	—	10 (29.4)	23 (69.7)	<0.01
Antihypertensive medication (%)	—	21 (63.6)	5 (14.7)	<0.01
ACR (mg/mmol)	—	0.6 ± 0.6	4.1 ± 7.1	<0.01
Microalbuminuria (%)	—	—	11 (33.3)	—
Neuropathy (%)#	—	—	19 (57.6)	—
A1C (%)	5.3 ± 0.3	7.8 ± 1.0*	8.1 ± 1.4*	<0.01
A1C (mmol/mol)	34.4 ± 2.7	62.2 ± 10.5*	63.9 ± 15*	<0.01
Updated A1C (%)**	—	7.9 ± 0.8	8.0 ± 1.2	0.83
Updated A1C (mmol/mol)**	—	63.2 ± 8.7	64.9 ± 13.6	0.83
Total cholesterol (mmol/L)	4.4 ± 0.9	4.6 ± 0.7	4.4 ± 0.7	0.31
Cholesterol medication (%)	—	11 (33.3)	8 (23.5)	0.43

Data are presented as mean (SD) unless otherwise indicated. *Statistically significantly different from healthy controls (post hoc Bonferroni-corrected $P < 0.05$). †Statistically significantly different from PDR− patients (post hoc Bonferroni-corrected $P < 0.05$). ‡Diabetes early onset was defined as an onset age below the age of 7 years. §Severe hypoglycemic events were self-reported and defined as those events for which the patient needs assistance from a third person to recuperate due to loss of consciousness or seriously deranged functioning, coma, or seizure due to low glucose levels. ‖Hypertension was defined as a systolic blood pressure of 140 mmHg or above, a diastolic blood pressure of 90 mmHg or above, or use of antihypertensive drugs. Microalbuminuria was defined as ACR >2.5 for men and >3.5 mg/mmol for women. #Neuropathy was based on the results of the annual checkup patients receive, of which the results are incorporated into the medical records, or were self-reported if not available. **Updated A1C represents the mean A1C level for the group with a median period of 7.0 years.

patients relative to the other groups. The prevalence of microbleeds in the other groups was low and most likely resembles previously described incidental findings (14). Furthermore, capillary recruitment in skin was significantly impaired in participants with cerebral microbleeds, most notably in PDR+ patients.

Our finding that cerebral microbleed prevalence is related to retinal microvascular abnormities has also been observed in type 2 diabetic patients (15). We did not observe this relationship for ischemic small vessel disease. This may be due to etiological differences between these markers. Cerebral microbleeds are a marker of vessel wall leakage (9), whereas white matter hyperintensities and lacunes represent ischemic tissue damage. Thus microbleeds may more closely resemble cerebral microangiopathy and be comparable to microangiopathy measured at the retina and in skin than the other markers. More research in larger samples is needed to confirm and expand these findings.

The associations between cerebral microbleeds and peripheral microvascular function in skin has not been reported before and supports the hypothesis that cerebral microbleeds are part of a generalized microvascular disorder, not limited to peripheral organs, such as the eyes and renal system.

Limitations of this study are differences in age and hypertension rates between the groups, for which all analyses were corrected. Additionally correcting for diabetes duration, A1C and ACR did not alter the results.

In conclusion, our findings show that cerebral microbleeds, but not ischemic small vessel disease, are most prevalent in type 1 diabetic patients with proliferative retinopathy (who were oldest and had longest disease duration) relative to the other groups, which seems part of generalized microangiopathy that may affect the skin and the brain.

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Author Contributions. J.W. analyzed all the skin capillary data, performed the statistical analysis, and wrote the manuscript. E.v.D. participated in the design of the study, collected all magnetic resonance imaging data and part of skin capillary data, performed the statistical analysis, and wrote the manuscript. M.P.W. rated the magnetic resonance imaging scans. F.B., F.J.S., and M.K. participated in the design of

the study. A.C.M. rated the fundus photographs to ascertain proliferative or no retinopathy in diabetic patients. M.P.d.B. collected part of the skin capillary data. R.G.I., E.H.S., and M.D. participated in the design of the study and supervised the statistical analyses. All authors were involved in drafting the manuscript and making revisions to the manuscript. M.D. and E.v.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Gerstein HC, Ambrosius WT, Danis R, et al.; ACCORD Study Group. Diabetic retinopathy, its progression, and incident cardiovascular events in the ACCORD trial. Diabetes Care 2013;36:1266–1271
2. Sundquist K, Li X. Type 1 diabetes as a risk factor for stroke in men and women aged 15-49: a nationwide study from Sweden. Diabet Med 2006;23:1261–1267
3. Klein BEK, Klein R, McBride PE, et al. Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy. Arch Intern Med 2004;164:1917–1924
4. Weinger K, Jacobson AM, Musen G, et al. The effects of type 1 diabetes on cerebral white matter. Diabetologia 2008;51:417–425
5. Brands AMA, Kessels RPC, Hoogma RPLM, et al. Cognitive performance, psychological well-being, and brain magnetic resonance imaging in older patients with type 1 diabetes. Diabetes 2006;55:1800–1806
6. Wessels AM, Scheltens P, Barkhof F, Heine RJ. Hyperglycaemia as a determinant of cognitive decline in patients with type 1 diabetes. Eur J Pharmacol 2008;585:88–96
7. van Duinkerken E, Schoonheim MM, Sanz-Arigita EJ, et al. Resting-state brain networks in type 1 diabetic patients with and without microangiopathy and their relation to cognitive functions and disease variables. Diabetes 2012;61:1814–1821
8. Goos JD, Henneman WJ, Sluimer JD, et al. Incidence of cerebral microbleeds: a longitudinal study in a memory clinic population. Neurology 2010;74:1954–1960
9. Fisher M, French S, Ji P, Kim RC. Cerebral microbleeds in the elderly: a pathological analysis. Stroke 2010;41:2782–2785
10. Vernooij MW, van der Lugt A, Ikram MA, et al. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. Neurology 2008;70:1208–1214
11. Haacke EM, Mittal S, Wu Z, Neelavalli J, Cheng Y-CN. Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. AJNR Am J Neuroradiol 2009;30:19–30
12. Mugler JP 3rd, Bao S, Mulkern RV, et al. Optimized single-slab three-dimensional spin-echo MR imaging of the brain. Radiology 2000;216:891–899
13. Serné EH, Stehouwer CD, ter Maaten JC, et al. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. Circulation 1999;99:896–902
14. Vernooij MW, Ikram MA, Tanghe HL, et al. Incidental findings on brain MRI in the general population. N Engl J Med 2007;357:1821–1828
15. Qiu C, Cotch MF, Sigurdsson S, et al. Retinal and cerebral microvascular signs and diabetes: the age, gene/environment susceptibility-Reykjavik study. Diabetes 2008;57:1645–1650