Relationship Between Retinal and Glomerular Lesions in IDDM Patients

BLANCHE M. CHAVERS, S. MICHAEL MAUER, ROBERT C. RAMSAY, AND MICHAEL W. STEFFES

Current knowledge regarding the concordance and discordance of the eye and kidney complications of diabetes is based on observations by ophthalmoscopy of retinal structural changes, which may be present at early stages of the disorder, and renal functional changes, which only become apparent at the later stages of the disease. For this reason we investigated the relationship between retinal structural lesions and quantitative measures of glomerular structure in patients with insulin-dependent diabetes mellitus (IDDM). Renal biopsies were evaluated using morphometric techniques, and retinopathy classification was determined by retinal fundus photography in 86 patients with IDDM: age 30.4 ± 7.3 years and duration of IDDM 18.9 ± 6.3 years (mean ± SD). Retinopathy score correlated with glomerular basement membrane width ($r = 0.39$, $P = 0.0002$), mesangial volume fraction (VvMes/Glom) ($r = 0.35$, $P = 0.0009$), surface density of the peripheral capillary wall (SvPGBM/Glom) ($r = 0.34$, $P = 0.0013$), and index of arteriolar hyalinosis ($r = 0.36$, $P = 0.0008$). Abnormalities in VvMes/Glom and SvPGBM/Glom were more pronounced in patients with both retinopathy and hypertension. Four of the 15 patients (27%) with either normal urinary albumin excretion (UAE) or low-level microalbuminuria had advanced retinopathy but normal VvMes/Glom. In conclusion, the presence of advanced retinal disease with or without hypertension in patients with IDDM indicates a greater likelihood of advanced nephropathy as evidenced by increased VvMes/Glom and decreased SvPGBM/Glom. However, marked discordance between retinopathy and nephropathy occurs, as illustrated by patients with normal UAE or low-level microalbuminuria, normal glomerular structural measures, and advanced retinopathy. *Diabetes* 43:441–46, 1994

From the Department of Pediatrics (B.M.C., S.M.M.), the Department of Ophthalmology (R.C.R.), and the Department of Laboratory Medicine and Pathology (M.W.S.), University of Minnesota, Minneapolis, Minnesota.

Address correspondence and reprint requests to Dr. Blanche M. Chavers, Department of Pediatrics/Nephrology, University of Minnesota, 13–250 Moos Tower, Box 491 UMHC, 420 Delaware Street SE, Minneapolis, MN 55455.

Received for publication 28 December 1992 and accepted in revised form 21 October 1993.

IDDM, insulin-dependent diabetes mellitus; CRC, Clinical Research Center; BP, blood pressure; UAE, urinary albumin excretion; GBM, glomerular basement membrane; VvMes/Glom, volume density of the mesangium per glomerulus; SvPGBM/Glom, surface density of the peripheral glomerular basement membrane per glomerulus; GFR, glomerular filtration rate; ANOVA, analysis of variance.

Retinopathy, nephropathy, and neuropathy have been characterized as parts of the triad of common microvascular complications that can occur in patients with diabetes (1). Both concordance and discordance have been reported for retinopathy and nephropathy in patients with long-standing insulin-dependent diabetes mellitus (IDDM) (2–5). The development of recognizable retinopathy occurs in 90–97% of patients with IDDM after 15–20 years of disease (6), whereas proliferative retinopathy ultimately develops in only 40–60% of patients (7). Overt nephropathy is less common, developing in ~25–40% of patients with a peak incidence occurring after 15 years of IDDM (8,9). One possible source of error in comparing the natural history of these two microvascular problems is that one, retinopathy, is largely defined by structural alterations as visualized by direct examination of the retina, while the other, nephropathy, is typically defined by functional criteria alone. Because kidney lesions may be far advanced before functional abnormalities become detectable (10–12), a reexamination of these interrelationships using morphological criteria for both microvascular structures seemed reasonable.

RESEARCH DESIGN AND METHODS

We studied 86 patients (55 women) with IDDM at the University of Minnesota Clinical Research Center (CRC) after obtaining informed consent. Patients were referred by their physicians to be considered for pancreas transplantation and were evaluated with protocols designed to

TABLE 1
Clinical characteristics of patients

n	86
Sex (F/M)	55/31
Age at onset (years)	11 ± 5 (2–27)
Age at biopsy (years)	30 ± 7 (17–56)
Duration of diabetes (years)	19 ± 6 (5–40)
HbA_1 (%)	10.6 ± 2.7 (7.2–25.9)
BP (mmHg)	
Systolic	122 ± 12 (97–160)
Diastolic	78 ± 8 (60–94)
Creatinine clearance $ml \cdot s^{-1} \cdot 1.73\ m^{-2}$	1.7 ± 0.5 (0.6–3.0)
UAE (mg/24 h)	587 ± 892 (4–4162)

Data are means ± SD (range).

assess their suitability as pancreas transplant candidates as well as the baseline status of key diabetic complications including nephropathy, retinopathy, neuropathy, and macrovascular disease. Most came because of extreme difficulty with control of diabetes, and some were concerned about complications of diabetes. Patients <17 years of age or those with a creatinine value >176.8 μM (2 mg/dl) were not evaluated. Thus, we do not claim that this group of IDDM patients is cross-sectionally representative of all patients with IDDM of similar duration. Nonetheless, we believe that the interrelationships between kidney and eye complications within a given patient, as presented here, are representative of other IDDM patients with similar manifestations.

The patients' ages ranged from 17 to 56 years (30.4 ± 7.3) and duration of IDDM ranged from 5 to 40 years (18.9 ± 6.3) (mean ± SD). All evaluations were performed during the same CRC hospitalization and included eye examination, blood pressure (BP) measurement, total glycosylated hemoglobin level (HbA_1) measured using the Bio-Rad (Richmond, CA) column assay (normal values 5.5–8.5%) (13,14), kidney function studies, and percutaneous kidney biopsy. Table 1 summarizes the gender, age at onset and duration of IDDM, HbA_1, BP, creatinine clearance, and urinary albumin excretion (UAE) rate at the time of biopsy for the study group.

Ophthalmological evaluation. Ophthalmological evaluation included a complete clinical examination, determination of corrected visual acuity in each eye according to the method of the Diabetic Retinopathy Study, and documentation of retinopathy by seven-field color stereophotography and macular fluorescein angiography (15). The photographs were masked and graded by a single ophthalmologist (R.C.R.) using a modification of the Early Treatment Diabetic Retinopathy Study system (16). The grading ranged from no diabetic retinopathy to retinopathy ungradable because of opacity of ocular media. Patients with microaneurysms, dot-blot hemorrhages, and cotton-wool spots or less(grade ≤P2) were classified as having mild or nonproliferative retinopathy. Patients with more significant lesions such as extensive neovascularization of the retina and the intrusion of vessels into the vitreous cavity (grade ≥P3) were classified as having advanced retinopathy (Table 2). For the purpose of analysis each grade was assigned a numerical value in an attempt to establish a scale for eye measurements that represented increasing pathological severity. The worst eye measurement was used. Because it is not known whether the relationship between eye grades is linear, the eye data were log-transformed.

Kidney function studies. Kidney function studies were performed at CRC. The mean of at least 10 BP determinations per patient obtained by the trained CRC nursing staff was calculated. Hypertension was diagnosed when the systolic BP was ≥160 mmHg, when the diastolic BP was ≥90 mmHg, or when antihypertensive therapy was used (17,18). Borderline hypertension was defined as systolic BP of 140–159 mmHg or diastolic BP of 85–89 mmHg (17). Two patients with borderline hypertension were included in the no hypertension group. Hypertension was present in 36 patients (42%). Creatinine clearance represents the mean value obtained from at least two and usually three studies from carefully supervised 24-h urine samples obtained in the CRC. Serum and urine creatinine concentrations were measured with an automated kinetic method that used the Jaffe reaction (19). Creatinine clearances $<1.50\ ml \cdot s^{-1} \cdot (90\ ml/min)^{-1}$ of body surface area were considered reduced. Previously we reported the validation of this method with inulin clearances in IDDM patients (11).

Decreased creatinine clearance was present in 34 subjects (39%). UAE in two and usually three 24-h urine collections was measured using a solid-phase fluoroimmunoassay (20). Mean UAE values were used for patients with more than one urine collection ($n = 63$). The normal UAE for our laboratory based on measurements in 100 normal adults is 8.3 ± 4.8 (range 1.1–21.0) mg/24 h. Normal UAE (≤22 mg/24 h) was present in 23 patients (27%), microalbuminuria (>22 and <250 mg/24 h) was present in 22 patients (25%), and 8 of these patients had low-level microalbuminuria (>22 but <45 mg/24 h). Overt proteinuria (UAE >250 mg/24 h) was present in 41 subjects (48%).

Kidney structure studies. Kidney structure studies were performed on percutaneous needle biopsy specimens processed for morphometric examination by light and electron microscopy (10). Tissues were coded before evaluation. Glomeruli per specimen (30 ± 19; range 8–99) were evaluated for the light microscopic measurement of mean glomerular volume (21).

The percentage of sclerosed glomeruli was determined on Zenker-fixed, paraffin-embedded tissues in 45 patients with ≥20 glomeruli/specimen (22). Glomeruli were considered to have global sclerosis if all of the glomerular tufts were totally replaced by dense scarring or were collapsed and involuted as evidenced by wrinkling of the glomerular basement membrane (GBM) and marked irregularity of the capillary luminal space (22).

The index of arteriolar hyalinosis is the estimate of the degree of replacement of afferent and efferent glomerular arteriolar smooth muscle by waxy homogeneous periodic acid Schiff–positive material. Incomplete replacement of a few vessels was graded as 0.5, incomplete replacement of most vessels as 1.5, complete replacement of about one-half of the arterioles as 2.5, and complete replacement of most vessels as 3.5 (22).

TABLE 2
Modified Early Treatment Diabetic Retinopathy Study grading system

Grade	Findings
Mild	
No retinopathy/no therapy	Normal retina; no photocoagulation
No classifiable retinopathy	Microaneurysms only
P0	Microaneurysms plus edema/exudate
P1	Nonproliferative disease > standard photo 2A in 1 field
P2	Nonproliferative disease with cotton-wool spots, venous beading, and intraretinal microvascular abnormalities in more than 1 field
Advanced	
P3	Flat retinal neovascularization
P4	Flat optic nerve neovascularization
P5	Flat retinal and optic nerve neovascularization
P6–8	Elevated neovascularization 1/4–1 disc diameter from retina
P9–11	Elevated neovascularization greater than 1 disc diameter from retina
P12	Involutional proliferative retinopathy
P13	Involuted retinopathy after pan retinal photocoagulation
P14	Unable to grade because of opacity of ocular media

Quantitative electron microscopic morphometric analyses were performed as described previously (10). Briefly, GBM width was determined using a minimum of 100 measurements/biopsy by the orthogonal intercept method at ×11,000 (23). Normal GBM width in our laboratory is 349 ± 49 nm (24). The volume density of the mesangium per glomerulus (VvMes/Glom) was measured by the point counting technique on low-magnification montages (25,26). The normal value for VvMes/Glom is 0.14 ± 0.04 $\mu m^3/\mu m^3$ (24). Surface density of the peripheral GBM per glomerulus (SvPGBM/Glom) was measured on montages using a line intercept counting technique (11,25,26). The normal value for SvPGBM/Glom is 0.13 ± 0.02 $\mu m^2/\mu m^3$ (24).

Statistical analysis. Statistical analyses included analysis of variance (ANOVA), Student's *t* test, χ^2 test, log transformation, and linear regression. Calculations were performed on an Apple Macintosh SE computer (Cupertino, CA) with the programs Statview 512+ (BrainPower, Calabasas, CA) and Microsoft Excel (Bellevue, WA). Two-tailed $P \leq 0.05$ was considered significant. All values are expressed as means ± SD.

RESULTS

Retinopathy was present in all patients and advanced retinopathy, grades P3 and above, was present in 60 patients (70%) (Table 2). Age of onset did not differ significantly in patients with and without advanced retinopathy, but IDDM duration was shorter in patients with mild retinopathy (17 ± 5 vs. 20 ± 6 years, $P = 0.03$). For all patients, retinopathy correlated with systolic ($r = 0.41$, $P = 0.0001$), diastolic ($r = 0.43$, $P = 0.0001$), and mean BP ($r = 0.45$, $P = 0.0001$). Advanced retinopathy was present in 32 of the 36 patients with hypertension (89%).

GBM width was 648 ± 144 nm (range 310–968 nm) and was >447 nm (2 SD above the normal mean) in 77 patients (89%). VvMes/Glom was 0.32 ± 0.13 $\mu m^3/\mu m^3$ (0.08–0.84 $\mu m^3/\mu m^3$) and >0.22 mm^3/mm^3 (2 SD above the normal mean) in 66 patients (77%). SvPGBM/Glom was 0.08 ± 0.03 $\mu m^2/\mu m^3$ (0.01–0.14 $\mu m^2/\mu m^3$) and <0.09 mm^3/mm^3 (>2 SD below the normal mean) in 55 patients (64%). The percentage of sclerosed glomeruli in 45 patients was 9.7 ± 12.0% (range 0–45%) and >9.9% (2 SD above the normal mean) in 15 patients (33%). The index of arteriolar hyalinosis was 1.7 ± 0.9 (range 0–3.5) and was >0 (normal value) in 81 of 84 patients (96%).

Retinopathy and nephropathy: concordance. Overt nephropathy (UAE >250 mg/24 h) was present in 34 of 60 patients (57%) with advanced retinopathy and in 7 of 26 patients (27%) with mild retinopathy. In 28 patients with overt nephropathy and hypertension, retinopathy was advanced in 26 (93%) and mild in 2 (7%), and in 12 patients with overt nephropathy and normal BP, retinopathy was advanced in 7 (58%) and mild in 5 (42%) (χ^2 $P = 0.03$).

Retinopathy scores correlated with GBM width ($r = 0.39$, $P = 0.0002$) (Fig. 1), VvMes/Glom ($r = 0.35$, $P = 0.0009$), SvPGBM/Glom ($r = 0.34$, $P = 0.0013$), and index of arteriolar hyalinosis ($r = 0.36$, $P = 0.0008$). Log-transformed retinopathy scores also correlated with GBM width ($r = 0.44$, $P = 0.0001$), VvMes/Glom ($r = 0.41$, $P = 0.0001$), SvPGBM/Glom ($r = 0.40$, $P = 0.0001$), and index of arteriolar hyalinosis ($r = 0.40$, $P = 0.0002$).

Glomerular structure was more abnormal in patients with advanced retinopathy and hypertension compared with those with advanced retinopathy and normal BP (GBM width, $P = 0.01$; VvMes/Glom, $P = 0.0002$; SvPGBM/Glom, $P = 0.0001$) or those with mild retinopathy and normal BP (GBM width, $P = 0.0001$; VvMes/Glom, $P = 0.0001$; SvPGBM/Glom, $P = 0.0001$) (Tables 3 and 4). Matched for severity of glomerular lesions based on VvMes/Glom, retinopathy was more severe in patients with hypertension (mean retinopathy score = 12) compared with patients with normal BP (mean retinopathy score = 9, $P = 0.03$).

Retinopathy and nephropathy: discordance. Of the 60 patients with advanced retinopathy, 8 (13%) had normal VvMes/Glom (0.17 ± 0.04 $\mu m^3/\mu m^3$, range 0.12–0.22). In 4 of these 8 patients, UAE was <45 mg/24 h. Five of the 8 patients were normotensive, one had borderline hypertension, and two were hypertensive. These 8 patients did not differ from the remaining 52 patients with

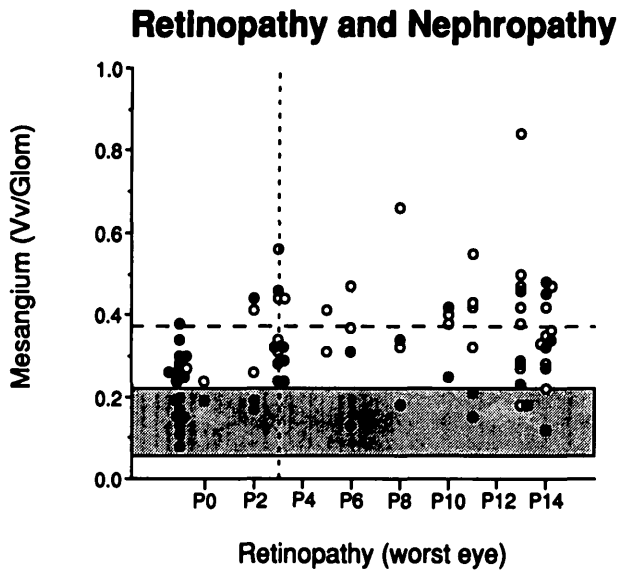

FIG. 1. Fractional volume of the mesangium in 85 patients: ○, patients with hypertension; ●, patients with normal blood pressure. The shaded area is the mean ± 2 SD in normal subjects. The dotted horizontal line represents a VvMes/Glom of 0.37, the level above which virtually all patients have overt nephropathy. The dotted vertical line separates patients with milder degrees of retinopathy (grade <P3) from those with advanced retinopathy (grade ≥P3).

advanced retinopathy and increased VvMes/Glom for gender, age at onset of IDDM, and HbA_1. However, glomerular filtration rate (GFR) was greater ($P = 0.02$), and trends toward lower UAE ($P = 0.06$) and shorter duration of IDDM ($P = 0.053$) were present in the 8 patients with normal VvMes/Glom. The mean GBM width was 736 ± 112 nm (range 591–880 nm) and did not differ from that in patients with increased VvMes/Glom. The index of arteriolar hyalinosis and the percentage of sclerosed glomeruli were similar in patients with advanced retinopathy whether the VvMes/Glom was normal or increased.

Of the 22 patients with mild retinopathy and normal BP, 11 (50%) had increased VvMes/Glom (0.30 ± 0.06, range 0.24–0.44 $\mu m^3/\mu m^3$) and/or decreased SvPGBM/Glom (0.08 ± 0.03, range 0.03–0.13 $\mu m^2/\mu m^3$). Of these 11 patients, 3 (27%) had overt proteinuria and 5 (45%) had advanced mesangial lesions (VvMes/Glom ≥0.3 $\mu m^3/\mu m^3$). These patients differed from patients with mild retinopathy, normal BP, and normal VvMes/Glom in that their duration of IDDM was longer (20 vs. 14 years, $P = 0.03$).

TABLE 3
Volume fraction of the mesangium relative to retinopathy and hypertension

	Hypertension	
	Yes	No
Mild retinopathy	0.26 ± 0.11	0.23 ± 0.09
Advanced retinopathy	0.41 ± 0.13	0.29 ± 0.10

Data are means ± SD. Two-way ANOVA: retinopathy, $P = 0.002$; hypertension, $P = 0.02$; no interaction. Student's t tests: advanced retinopathy and hypertension vs. advanced retinopathy without hypertension, $P = 0.0002$; advanced retinopathy without hypertension vs. mild retinopathy without hypertension, $P = 0.03$.

TABLE 4
Surface density of the glomerular peripheral capillary wall relative to retinopathy and hypertension

	Hypertension	
	Yes	No
Mild retinopathy	0.08 ± 0.02	0.10 ± 0.03
Advanced retinopathy	0.06 ± 0.02	0.08 ± 0.02

Data are means ± SD. Two-way ANOVA: retinopathy, $P = 0.007$; hypertension, $P = 0.005$; no interaction. Student's t tests: advanced retinopathy and hypertension vs. advanced retinopathy without hypertension, $P = 0.0001$; advanced retinopathy without hypertension vs. mild retinopathy without hypertension, $P = 0.05$.

Exceptions also were present in the 31 patients with normal UAE or low-level microalbuminuria. Of the 16 with mild retinopathy, 6 (37%) had increased VvMes/Glom and 3 (19%) had decreased SvPGBM/Glom.

DISCUSSION

The stages of diabetic retinopathy can be documented with the use of noninvasive ophthalmological techniques. However, unless overt proteinuria or high-level microalbuminuria with elevated BP and/or decreased GFR is present, the severity of the underlying lesions of diabetic nephropathy can only be ascertained by kidney biopsy (12). Patients without these renal functional abnormalities may have normal or near normal glomerular structure or lesions of a severity bordering on that regularly associated with overt nephropathy (12).

This study compares retinal lesions determined by fundus photography and glomerular lesions determined by renal biopsy in a large cohort of IDDM patients. These patients were not selected to represent the general diabetic population in that children and patients with advanced renal failure were excluded, and complication rates may be higher in patients evaluated for pancreas transplantation. For example, hypertension may have been present in many patients as a result of advanced diabetic nephropathy. However, we have no reason to believe that the interrelationships of eye and kidney abnormalities presented here are not broadly valid.

These studies show significant concordance between retinal lesions and glomerular structure in patients with IDDM and advanced retinopathy and in this sense are consistent with previous reports in IDDM patients with proteinuria or microalbuminuria. However, the r values were low, and this was best explained by the patients whose symptoms are discordant. Of the patients with advanced retinopathy, 13% had VvMes/Glom within the normal range, whereas 54% of patients with mild retinopathy had increased VvMes/Glom. Discordance between retinal and glomerular lesions was more common in patients with mild retinopathy and normal BP. Discordance was more pronounced in patients with normal or low-level microalbuminuria in that 27% of the patients

with advanced retinopathy had normal VvMes/Glom and 40% had normal SvPGBM/Glom. These exceptions are consistent with the idea that end-organ responses can be markedly different within a given patient with IDDM. These results are in keeping with studies of Krolewski et al. (7), which show that the risk of developing proliferative retinopathy persists over time, but the risk of developing nephropathy decreases after 20–30 years of IDDM. Our data shed light on these epidemiological studies by identifying a significant subset of patients who have advanced diabetic retinal vascular injury, although important glomerular structural parameters were within the normal range. This finding is consistent with the hypothesis that there are important differences in at least some aspects of the pathogenesis of eye and renal lesions of diabetes.

Glomerular structure (VvMes/Glom and SvPGBM/Glom) correlated with both retinopathy and hypertension. Hypertension has been reported to be a risk factor for both retinopathy and nephropathy (27,28). Hypertension in IDDM patients most frequently occurs in association with advanced glomerular lesions and appears to represent the rise in systemic BP commonly seen in patients with progressive glomerular injury from a number of causes (29). This finding suggests that, at least in part, the greater concordance between the eye and kidney complications in patients with overt nephropathy may occur because the development of clinical renal disease with concomitant hypertension might then accelerate retinopathy. Nevertheless, advanced retinopathy also occurred without the presence of advanced nephropathy.

In summary, our measurements of glomerular structure and retinopathy in patients with IDDM show that significant microvascular disease of the kidney is more common in patients with advanced retinopathy and hypertension. In this study, 92% of patients with advanced retinopathy also had elevated VvMes/Glom and/or decreased SvPGBM/Glom. However, severe microvascular disease of the eye and kidney may occur in isolation, suggesting specific organ-related pathogenetic factors (7). Ophthalmologists and endocrinologists treating patients with advanced retinopathy alone or advanced retinopathy and hypertension should have these patients evaluated for diabetic nephropathy, including measures of UAE if a dipstick test for proteinuria is negative. Patients with UAE >45 mg/24 h or persistent dipstick-positive proteinuria will probably have serious renal lesions. In the absence of these findings, advanced retinopathy is an imprecise indicator of renal structure.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (AM-22206B, AM-13083, AM-43605, and M01-RR400), the Minnesota Medical Foundation, and the Children's Kidney Disease Society.

We are indebted to John Basgen for technical assistance in performing the electron microscopic studies, to Sandra Spier for measurement of urinary albumin, to the nursing staff of the University of Minnesota Hospital Clinical Research Center, to Marshall Hoff for medical illustration, and to Sandy Cragg for help in preparing the manuscript.

Parts of this paper were presented in abstract form at the annual meeting of the American Society of Nephrology in Baltimore, Maryland, 17–20 November 1991.

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