Relationship Between Retinal Blood Flow and Renal Function In Patients With Type 2 Diabetes and Chronic Kidney Disease

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Relationship between Retinal Blood Flow and Renal Function in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease

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Short title: *Retinal circulation and renal dysfunction in type 2 DM*

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ABSTRACT

OBJECTIVE--To study the relationship between retinal microcirculation and renal function in patients with type 2 diabetes mellitus.

RESEARCH DESIGN AND METHODS--Using a laser Doppler velocimetry system, we obtained the retinal blood flow (RBF) values by simultaneously measuring the retinal vessel diameter and blood velocity. To determine if the RBF is affected in the presence of renal dysfunction, we also evaluated the renal function using the estimated glomerular filtration rate calculated by age and serum creatinine level.

RESULTS—We recruited 169 eyes of 169 consecutive Japanese patients with type 2 diabetes mellitus, no or minimal diabetic retinopathy and normo/microalbuminuria (mean age ± standard deviation, 59.0±11.1 years). We divided the patients into four groups based on the stage of chronic kidney disease (CKD) (non-CKD group, n=99; CKD stage: 1, n=22; 2, n=27; 3, n=21). We found significant (P=0.035) decreases in RBF with decreased vessel diameter (P=0.017) but no difference in blood velocity (P=0.54) in stage 3 CKD compared with the non-CKD group. Multiple regression analysis showed that the CKD stage was significantly (P=0.02) and independently associated with decreased RBF.

CONCLUSIONS--Our results indicated that the vessel diameter and RBF in the retinal arterioles decrease in patients with type 2 diabetes with stage 3 CKD, suggesting that impaired renal function might be associated with decreased RBF, probably via constriction of the retinal arterioles, in early-phase diabetic retinopathy.

Although both retinopathy and nephropathy are major diabetic microvascular complications, few studies have examined the relation between retinal structural changes and renal functions in patients with diabetes. Thickening of the basement membrane in the retinal and glomerular capillary vessels has been reported in late-stage diabetic retinopathy and nephropathy (1), suggesting that retinopathy and nephropathy share similar microvascular pathologic pathways related to abnormal glucose metabolism and other processes, e.g., inflammation and endothelial dysfunction. Those findings support the clinical recommendation to monitor renal function in patients with diabetes who have signs of retinopathy. A growing body of evidence suggests that several cardiovascular risk factors are associated with the stage of chronic kidney disease (CKD) (2), which accelerates progression of atherosclerosis and increases the propensity to oxidative stress (3). Moreover, diabetes mellitus per se is the leading cause of the incidence and prevalence of CKD (4). Although we recently reported that retinal blood flow (RBF) decreases in patients with type 2 diabetes without retinopathy and with mild retinopathy using a retinal laser Doppler velocimetry (LDV) system (5), it is unclear whether renal dysfunction is associated with impaired retinal microcirculation in patients with diabetes.

Recent ophthalmic epidemiologic studies also have reported that narrowing of the retinal arterioles is associated with CKD independent of diabetes and hypertension (6) and that patients with moderate CKD were three times more likely to develop early age-related macular degeneration compared with subjects with no or mild CKD (7), suggesting that renal dysfunction might have some effect on ocular disorders. However,

no study has examined the relation between renal dysfunction and retinal microcirculation in type 2 diabetes mellitus. The current study examined the effect of renal dysfunction, evaluated by grading the CKD, on the retinal microcirculation in patients with type 2 diabetes mellitus, especially the early stages of retinopathy and nephropathy.

RESEARCH DESIGN AND METHODS--The study adhered to the tenets of the Declaration of Helsinki and followed the guidelines, which the ethics committee of our institution approved. All subjects provided written informed consent. This study included 169 consecutive patients who were native Japanese (73 men, 96 women; age, mean ± standard deviation [SD], 59.0 ± 11.1 years) and had type 2 diabetes mellitus between April 2001 and March 2005. Diabetes mellitus was diagnosed based on the criteria of the American Diabetes Association (8). If the patients were treated with insulin or oral hypoglycemic agents or if the fasting blood glucose value exceeded 126 mg/dl, they were considered to have diabetes. If the blood pressure (BP) exceeded 140/90 mmHg or if they used antihypertensive drugs, the patients were considered to have hypertension (9). Dyslipidemia was diagnosed in patients with serum low-density lipoprotein (LDL) cholesterol levels ≥140 mg/dl and/or high-density lipoprotein cholesterol levels <40 mg/dl and/or triglyceride values ≥150 mg/dl in subjects with a history of cholesterol-lowering therapy (10).

The urinary albumin excretion level is presented as the albumin-to-creatinine ratio (ACR) (mg/g creatinine). Diabetic nephropathy (DN) was staged based on analyses of

spot urine samples, i.e., stage I DN (normoalbuminuria), ACR <30 mg/g creatinine; stage II DN (microalbuminuria), 30 < ACR < 300 mg/g creatinine; stage III DN (macroalbuminuria), ACR >300 mg/g creatinine (or dipstick urinalysis showing 2+, 3+, 4+) (11). The serum creatinine was measured within 4 hours of fasting venous blood collection using a Hitachi 747 biochemistry analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Renal function also was evaluated based on the estimated glomerular filtration rate (eGFR), which was calculated using a previously reported formula (12). The following equation originated from the Modification of Diet in Renal Disease study group compiled for Japanese individuals and was recommended by the Japanese Society of Nephrology: eGFR (mL/min/1.73m^2) = 194 x scr$^{-1.094}$ x age$^{-0.287}$ x 0.739 (if female). The CKD stages were based on the National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines (13). The absence of CKD was defined as with nomicroalbuminuria and an eGFR exceeding 90 ml/min/1.73m^2.

In the current study, we recruited patients with type 2 diabetes, no or minimal diabetic retinopathy and no or microalbuminuria. Patients with macroalbuminuria, proteinuria, and those undergoing hemodialysis were excluded. In addition, patients with poorly controlled diabetes, i.e., with hemoglobin (Hb) A1c over 10.0%, uncontrolled hypertension, i.e., with BP over 160/100 mmHg, and severe anemia, i.e., Hb <10.0 g/dL, were excluded. The individuals who had other kidney diseases, such as acute renal failure, chronic glomerulonephritis, and interstitial nephritis, were excluded as were those with atherosclerotic cardiovascular diseases, such as coronary artery

disease, congestive heart failure, peripheral vascular disease, and ischemic stroke. The specialists at our institution diagnosed those diseases and were masked to the information from the ocular examination.

All patients underwent a baseline ophthalmologic evaluation before the RBF was measured. All patients had good visual acuity (VA) levels (>20/20) and intraocular pressure (IOP) levels that were within the normal range (<20 mmHg). After the pupils were dilated with a 0.5% tropicamide eye drop, an ophthalmologist, who was masked to the RBF measurements, assessed the retinopathy. For each eye, photographs were taken with the Topcon TRC-NW6S (Topcon, Tokyo, Japan), and the maximal grade in any of the seven standard photographic fields was determined for each lesion and used to define the retinopathy levels (14). The severity of retinopathy was categorized as none (level 10), mild nonproliferative (levels 21-37), moderate-to-severe nonproliferative (levels 43-53), or proliferative (levels 60-65) (15). Patients with moderate-to-severe nonproliferative diabetic retinopathy (NPDR) and PDR and clinically relevant macular edema were excluded. One eye of a patient was included in the study if it had the worse retinopathy that met the inclusion criteria. If both eyes of a patient had retinopathy of equal severity, one eye was assigned randomly to the study. The ophthalmologic exclusion criteria included a previous intraocular surgery, a history of laser photocoagulation, moderate-to-severe cataract, vitreous hemorrhage, tractional retinal detachment, and moderate-to-high refractive error (> ± 3.0 diopters).

RBF measurements

The ocular examination was performed first followed by measurement of the RBF. The participants had abstained from drinking coffee for at least 12 hours before the RBF was measured. We used a retinal LDV system (Canon Laser Blood Flowmeter, model CLBF 100, Canon, Tokyo, Japan) to estimate the blood flow in the superior branch of the first-order major temporal retinal artery. The details of the LDV methodology were reported previously (16). The system noninvasively measures the absolute values of the red blood cells flowing in the centerline of the vessel, based on the bi-directional LDV (16). The mean retinal blood velocity (V_{mean}) was defined as the V of the averaged maximal speed during one cardiac cycle. The diameter of the retinal artery also was determined automatically by computer analysis of the signal produced by the arterial image on the array sensor using the half height of the transmittance profile to define the vessel edge (16).

Calculations

The RBF was calculated as RBF = V_{mean} x area, where the V_{mean} was calculated as V_{mean} = V of the averaged maximal speed/2, and the area was the cross-sectional area of the retinal artery at the laser Doppler measurement site (16). The mean arterial BP was determined by the formula: diastolic BP + (systolic BP – diastolic BP)/3 (16).

Data analysis

All values are expressed as the mean ± SD. The assumption of data normality was assessed using the Shapiro-Wilk test. Comparisons between groups were made using

one-way analysis of variance (ANOVA) (for continuous variables) and the χ^2 test (for

categorical variables). One way-ANOVA was followed by a post-hoc comparison with

Tukey-Kramer's procedure. Standardized regression coefficients from multiple

regression analysis of the RBF in relation to various factors were analyzed. $P < 0.05$

was considered significant. All analyses were conducted using the SPSS version 16,

statistical software (Chicago, IL).

RESULTS—We divided the patients with type 2 diabetes mellitus with

normoalbuminuria (DN stage I; n = 120) or microalbuminuria (DN stage 2; n = 49) into

four groups based on the CKD stage (non-CKD group, n=99; CKD groups stage 1,

n=22; stage 2, n=27; stage 3, n=21). No patient had stage 4 or 5 CKD. Although the

group-averaged values of age and duration of diabetes were significantly higher in stage

2 CKD compared with the other groups, there were no significant differences in the

HbA1c, systemic BP, heart rate, IOP, Hemoglobin, total cholesterol, triglyceride, HDL,

or LDL among the groups. The serum creatinine and eGFR were higher in stage 1 CKD

but lower in stages 2 and 3 CKD compared with the non-CKD group (Table 1). We

found significant decreases in the vessel diameter ($P=0.017$) and RBF ($P=0.035$) in

stage 3 CKD compared with those in the non-CKD group; however, there were no

differences in the retinal arteriolar blood velocity among the groups (Table 2). In the

current study, 47 (28%) patients had mild retinopathy in at least one eye. There were no

significant differences in retinal circulatory parameters (vessel diameter, velocity, and

RBF) between the no retinopathy group and the mild NPDR group (data not shown). In

addition, there also were no significant differences in the retinal circulatory parameters between DN stages 1 and 2 (data not shown). Multiple regression analysis was performed to determine whether the CKD stage was an independent variable related to the RBF in patients with type 2 diabetes mellitus. Table 3 shows the multiple regression analysis of the RBF in relation to the renal stage of CKD, serum LDL, mean arterial BP, and HbA1c, based on our previous findings (5). This analysis confirmed that the CKD stage was a significant and independent variable associated with the RBF in these patients (P=0.02) (Table 3) in addition to serum LDL and mean arterial BP.

DISCUSSION--Although diabetic retinopathy is associated closely with the preclinical morphologic changes of diabetic nephropathy (17), about one-third of patients with diabetes have decreased kidney function without either albuminuria or retinopathy (18). These findings suggested that the relation between renal dysfunction and impaired retinal microcirculation remains unclear, especially in early-stage diabetes. We recently reported that the RBF decreases in patients with type 2 diabetes without retinopathy and with mild retinopathy (5). The current study also found that the vessel diameter and RBF in the retinal arterioles decreased in patients with type 2 diabetes with stage 3 CKD and normo/microalbuminuria, suggesting that impaired renal dysfunction might be associated with decreased retinal microcirculation in early-stage type 2 diabetes mellitus. Taken together, we hypothesized that impaired retinal and renal microcirculation might be involved with the common pathogenesis of diabetic microvascular complications, especially in early-stage type 2 diabetes mellitus.

Tonelli et al. reported that higher baseline C-reactive protein (CRP) was associated with decreased eGFR in patients with hyperlipidemia and a history of myocardial infarction (19), suggesting that chronic low-grade inflammation might be associated with renal dysfunction. Although we did not measure the serum level of CRP in patients with diabetes mellitus in the current study, we reported previously that the CRP impaired retinal endothelial function in isolated porcine retinal arterioles (20). Because chronic kidney disease is associated with low-grade inflammation, endothelial dysfunction, and platelet activation, even among patients with moderate renal impairment (21), chronic inflammation might be associated with the pathogenesis of diabetic microvascular complications including retinopathy and nephropathy in patients with type 2 diabetes mellitus.

Previous epidemiologic studies have reported that renal dysfunction might have some effect on ocular disorders in patients without diabetes (6, 7). In addition, recent clinical studies have reported that the vessel calibers of retinal arterioles and venules decreased progressively with each CKD stage of renal failure (22, 23), suggesting that progression of renal dysfunction might be associated with retinal vessel constriction. Although those studies did not include patients with diabetes, the findings seemed to support the current finding that vessel diameter and blood flow in the retinal arterioles were significantly lower in the patients with stage 3 CKD compared with those without CKD in patients with type 2 diabetes and no or minimal retinopathy.

The exact mechanism by which vasoconstriction of the retinal arterioles and decreased RBF without any change in blood velocity in patients with CKD stage 3

might be associated with renal dysfunction remains unclear. CKD is associated with low-grade inflammation, platelet activation, and endothelial dysfunction even among patients with moderate renal impairment (21). Indeed, the reduced endothelium-dependent dilation in the brachial artery of patients with CKD is related to the severity of the renal failure (24). Evidence indicates that oxidative stress occurs early in the course of CKD and is amplified with disease progression (3). In addition, the nitric oxide (NO) production decreases in renal disease due to impaired endothelial function and renal NO production (25). Because we previously reported that NO has an important role in vasoregulation in RBF (26), the decreased NO production in patients with diabetes with CKD might be involved with constriction of the retinal arterioles and decreased RBF seen in the current study. Moreover, the current data do not definitively explain why there were no significant differences in blood velocity among the four diabetic groups. Based on our previous findings that the blood velocity in the retinal arterioles decreased in patients with type 2 diabetes with no and/or minimal retinopathy compared with healthy subjects (5), we speculated that the blood velocity in the retinal arterioles decreased in all patients with diabetes independent of the CKD stage without a further decrease according to the CKD stage.

In the current study, the multivariable regression model included serum-LDL, mean arterial systemic BP, and HbA1c, which we selected based on previous findings (5), and suggested that the CKD stage, serum-LDL, and systemic BP are independent risk factors for RBF in our patients. Because LDLs specifically impair endothelium-dependent vasodilation by reducing NO synthesis and producing

superoxide anion in isolated coronary arterioles (27), the increased serum LDL might be involved with the decreased RBF via the reduced NO production in the retinal arterioles, resulting in constricted retinal arterioles.

At the onset of diabetes, the kidney enlarges along with glomerular hypertrophy, and the GFR becomes supranormal (28, 29). Hypertrophy also contributes to cellular oxidative stress, which might precede the reactive oxygen species perturbation (30). This eventually leads to gradual kidney deterioration with reduced GFR and subsequently results in sclerosis and kidney failure. Moreover, renal arterial resistance plays an important role in deteriorating renal function in patients with diabetes (31). In the current study, we found that the eGFR increased in the group with stage 1 CKD with microalbuminuria compared with the group with no CKD, whereas there was no difference in RBF between the non-CKD and stage 1 CKD groups, suggesting a possible discrepancy in the changes in the retinal and renal microcirculation in early-stage type 2 diabetes mellitus with microalbuminuria. Because the retinal microvasculature can be visualized directly, non-invasive measurement of the RBF using a LDV system might be a good indicator of general microvascular dysfunction in patients with type 2 diabetes mellitus.

The study limitations included its cross-sectional nature. A prospective study is needed to identify the interaction of the pathogenesis between retinopathy and nephropathy in type 2 diabetes mellitus. We also did not examine the GFR directly but estimated it from the serum creatinine. Moreover, all patients in the current study were Japanese, which might have affected our results, because Asian patients with type 2

diabetes have a high prevalence of albuminuria, reportedly 60% (32). Moreover, we included only patients with early-stage diabetic retinopathy and nephropathy, and therefore, could not comment on the retinal hemodynamics in patients with type 2 diabetes with late-stage diabetic microvascular complications. In addition to the current findings obtained from patients with early-stage renal dysfunction, we previously reported a negative linear correlation between the changes in systemic BP and the RBF induced by hemodialysis in patients with end-stage renal disease (33), suggesting that autoregulation of the RBF might be impaired in these patients. The current finding that the systemic BP is an independent risk factor for RBF in our multivariable regression model (Table 3) might be associated with impaired autoregulation of the RBF in patients with diabetes with renal dysfunction. Another clinical study is needed to elucidate the relation between renal function and retinal microcirculation in the advanced stages of diabetic microvascular complications. Finally, we used only one method to quantify the changes in RBF and had no technique to measure the renal blood flow. Although numerous and some larger scale studies have reported that the vessel diameter in the retinal arterioles is reduced when using other techniques to evaluate the vessel diameter, which seems to be in line with current results, further study is warranted to substantiate these changes in blood flow and vessel diameter in the retinal arterioles by using other techniques to evaluate the retinal vessel diameter and renal blood flow in these patients.

In conclusion, our results indicated that the RBF decreases in patients with type 2 diabetes with stage 3 CKD, suggesting that impaired renal function might be associated

with decreased RBF in early-phase diabetic retinopathy. Because the LDV system

enables noninvasive and quantitative evaluation of RBF, evaluation of the retinal

microcirculation likely is useful for detecting early changes in the microvasculature in

patients with type 2 diabetes.

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TABLE 1--Characteristics of patients with type 2 diabetes mellitus with early-stage

retinopathy

CDK stage	No CKD (n=99)	Stage 1 CKD (n=22)	Stage 2 CKD (n=27)	Stage 3 CKD (n=21)	P value
Mean age (years)	58.2±11.2	52.6±10.1	64.9±9.9*	61.6±8.7	0.001
HbA1c (%)	8.0±2.1	7.7±1.8	7.7±1.8	7.5±1.9	0.65
Duration of diabetes (years)	7.6±7.1	8.4±7.7	12.9±7.9*	9.6±8.4	0.01
Serum creatinine (mg/dL)	0.60±0.14	0.49±0.10*	0.76±0.12*	1.14±0.32*	<0.001
eGFR (mL/min/1.73 m^2)	92.8±21.3	116.7±16.7*	71.3±7.3*	47.3±8.6*	<0.001
SBP (mmHg)	133.8±18.5	144.6±18.3	140.4±20.9	136.4±21.2	0.07
DBP (mmHg)	75.7±10.9	73.4±10.6	73.4±10.6	76.3±12.9	0.49
MBP (mmHg)	95.1±12.2	100.4±11.6	95.8±12.0	96.3±14.5	0.35
Heart rate (beats/min)	71.2±10.7	73.5±12.0	69.6±13.7	68.8±15.1	0.61
IOP (mmHg)	15.6±3.2	15.6±3.0	15.3±2.3	14.5±2.4	0.45
Hemoglobin (%)	14.2±1.5	13.8±1.1	13.5±1.3	13.1±1.6	0.08
Total cholesterol (mg/dl)	198.6±38.5	205.1±47.1	201.8±43.3	189.5±45.6	0.63
Triglycerides (mg/dL)	144.1±65.4	155.3±94.3	135.1±65.9	168.0±95.6	0.42
HDL (mg/dL)	54.2±14.0	57.1±17.5	54.4±13.8	45.9±11.4	0.07

LDL (mg/dL)	117.4±34.7	117.7±44.2	118.5±34.7	115.2±33.8	0.99
Insulin use, n (%)	27 (27)	6 (27)	5 (19)	9 (43)	0.31
Oral antidiabetic drugs, n (%)	52 (53)	17 (77)	21 (78)	11 (52)	0.03
Hypertension, n (%)	46 (46)	11 (50)	19 (70)	17 (81)	0.01
Dyslipidemia, n (%)	53 (54)	11 (50)	12 (44)	13 (62)	0.67
Medications					
ß-antagonist, n (%)	9 (9)	1 (5)	3 (11)	4 (19)	0.43
Angiotensin converting enzyme inhibitor, n (%)	7 (7)	0 (0)	3 (11)	6 (29)	0.007
Angiotensin II type 1 receptor blocker, n (%)	21 (21)	7 (32)	10 (37)	7 (33)	0.28
Calcium channel antagonist, n (%)	18 (18)	4 (18)	12 (44)	7 (33)	0.02
Diuretic, n (%)	8 (8)	1 (5)	4 (15)	5 (24)	0.12
Antihyperlipidemic drugs, n (%)	45 (45)	12 (55)	11 (41)	13 (62)	0.28

P values were obtained by one-way ANOVA or the χ^2 test.

*$P < 0.05$ compared with the group without CKD.

CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; SBP =

systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure;

IOP = intraocular pressure; HDL = high-density lipoprotein; LDL = low-density

lipoprotein.

TABLE 2—Retinal circulatory parameters in the study groups

CDK stage	No CKD (n=99)	Stage 1 (n=22)	Stage 2 (n=27)	Stage 3 (n=21)	P value
Vessel diameter (μm)	108.2±12.0	105.8±12.6	108.0±10.5	99.6±13.1*	0.017
Blood velocity (mm/sec)	34.9±7.7	33.6±8.1	32.9±9.2	33.0±8.2	0.54
RBF (μL/min)	9.8±3.1	9.0±3.0	9.1±2.8	7.8±2.5*	0.035

P values were obtained by one-way ANOVA. *$P < 0.05$ compared with the group without CKD.

CKD = chronic kidney disease; RBF = retinal blood flow.

TABLE 3--Multiple regression analysis of RBF in patients with type 2 diabetes

	Regression coefficient	95% CI	*P* value
CKD stage	-0.46	-0.89, -0.04	0.02
MBP	0.04	+0.003, +0.078	0.03
LDL	-0.014	-0.027, -0.002	0.04
HbA1c	-0.067	-0.307, +0.175	0.58

R=0.28, *P*=0.01.

CI = confidence interval; CKD = chronic kidney disease; MBP = mean arterial blood pressure; LDL = low-density lipoprotein; HbA1c = hemoglobin A1c.