Relationship Between Retinal Capillary Nonperfusion Area and Renal Function in Patients With Type 2 Diabetes

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Received: September 1, 2020
Accepted: November 16, 2020
Published: December 14, 2020

Citation: Min JW, Kim HD, Park SY, et al. Relationship between retinal capillary nonperfusion area and renal function in patients with type 2 diabetes. *Invest Ophthalmol Vis Sci.* 2020;61(14):14.
https://doi.org/10.1167/iovs.61.14.14

Purpose. We sought to assess the relationship between retinal nonperfusion area (NPA) on ultra-widefield fluorescein angiography (UWFA) and renal function in type 2 diabetes mellitus (DM) patients with diabetic retinopathy (DR) and nephropathy.

Methods. UWFA was performed in 248 eyes (124 patients) with DR, comprising 94 eyes from patients with chronic kidney disease (CKD) caused by diabetes and 154 eyes without CKD (non-CKD). Serum creatinine level (Cr), estimated glomerular filtration rate (eGFR), urine albumin/creatinine ratio (UACR), and urine protein/creatinine ratio (UPCR) were collected. On UWFA, retinal NPA was measured in an automated manner. The correlation between NPA and renal function was analyzed.

Results. The mean NPA value of the total eye was 33.11 ± 45.77-disc diameter (DA) in non-CKD and 100.57 ± 69.52 in CKD ($P < 0.001$). NPA of posterior pole was 1.21 ± 3.28 DA in non-CKD and 7.99 ± 6.75 in CKD group ($P < 0.001$). The NPA values of both the total eye and posterior pole were significantly correlated with Cr (r = 0.585 and 0.483), eGFR (r = −0.572 and −0.524), UACR (r = 0.541 and 0.482), and UPCR (r = 0.509 and 0.529, respectively) (all $P \leq 0.001$). Linear modeling encompassing all clinical factors and relative clinical factors suggested eGFR as the most important predictor for NPAs of the total eye and posterior pole.

Conclusions. Larger retinal NPA on UWFA is associated with worse renal function in DM patients. Renal function can be used to predict retinal NPA in type 2 DM patients with nephropathy and DR.

Keywords: diabetic retinopathy, nonperfusion, non-perfusion, diabetic nephropathy, renal function, ischemia

Diabetes mellitus (DM) is one of the most important and common chronic diseases worldwide and is expected to increase in prevalence alongside continued population growth, aging, and escalating rates of obesity.[1,2] Dealing with complications of DM is important because the morbidity and mortality rates and health care costs for diabetic patients are critical socioeconomic issues that must be brought under control.[1] Diabetic retinopathy (DR) and diabetic nephropathy are among the most significant complications of DM.

Both DR and diabetic nephropathy are typical microvascular complications of DM. DR is the most common cause of visual disability in people of working age.[3] Meanwhile, diabetic nephropathy, the primary cause of chronic kidney disease (CKD), accounts for 40% of all new cases of end-stage renal disease recorded annually.[4]

The association between DR and diabetic nephropathy has been investigated previously under the hypothesis that they share a common pathophysiology because both result from diabetic microangiopathy. The prevalence of DR was increased in DM patients with CKD.[5,6] The severity of DR has been linked to a high urine albumin to creatinine ratio (UACR), lower estimated glomerular filtration rate (eGFR), and deterioration of renal function.[7–10] Other studies have suggested that CKD and DR share common risk factors such as smoking, poor glycemic control, systolic hypertension, and dyslipidemia.[6,8,11,12] In a recent meta-analysis, DR was proved to be helpful in diagnosing nephropathy in patients with type 2 DM.[13]

Most of the previous studies used ophthalmoscopic or conventional fundus photographic findings to define DR, whereas angiographic findings can provide more valuable information on angiopathy in DR eyes. Capillary nonperfusion (NP) is an important sign of microvascular injury seen during fundus angiography of DR eyes. It represents the extent of ischemia, thus signifying impairment of the capillary perfusion status of retinal vessels. The NP area (NPA) is directly correlated with DR severity and neovascularization (NV), which is a pathognomonic finding of proliferative DR (PDR).[14,15] Classically, NPA is easily detected by

fluorescein angiography. The recent introduction of ultra-widefield imaging has enabled more detailed analyses of NPA by covering most of the retinal area.[15,16]

Microvascular pathology also plays an important role in development of renal insufficiency in diabetic nephropathy.[17] Considering the proven relationship between DR and nephropathy, microangiopathy associated with DM in the kidney, hence, renal function and proteinuria, can be correlated with microangiopathy in the retina, presented as retinal capillary NPA, proving that this can have clinical advantages. In the viewpoint of nephrologists, maintaining good vision is important for the patients' quality of life, especially those undergoing dialysis. Quantified correlation between renal function and angiopathy in DR may assist proper referral to DR care. For ophthalmologists, the parameters of renal function can give clues to estimate vascular changes in DM patients who are not suitable for fluorescein angiography because of decreased renal function.

Because most of the NPAs are located in the peripheral area, NPA measurement ultra-widefield fluorescein angiography (UWFA) can represent global vascular changes in DR eyes.[14,18] To the best of our knowledge, there have been no previous studies on the association of DR and nephropathy using quantitative angiographic parameters or ultra-wide field imaging. In this study, we sought to assess the relationship between retinal NPA on UWFA and renal function and the performance of renal function in the prediction of NPA in patients with type 2 DM and DR.

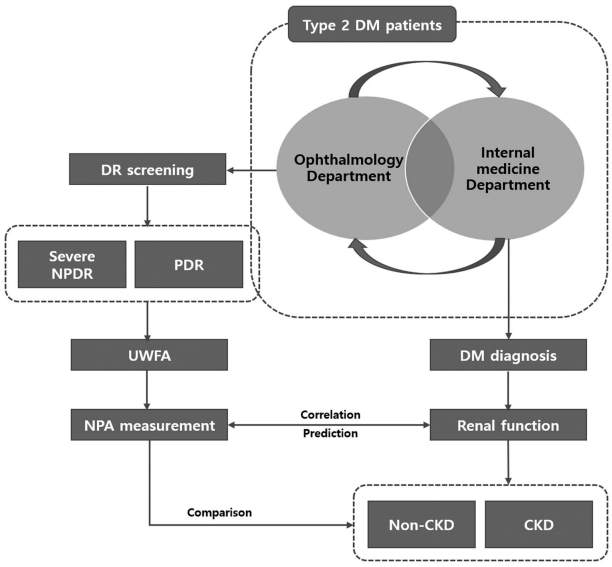

Figure 1. Simplified flow chart of the study. Consecutive type 2 DM patients who visited the outpatient departments of both ophthalmology and endocrinology and/or nephrology were identified. Of those, severe non-proliferative DR and proliferative DR patients that had ultra-widefield fluorescein (UWFA) angiography were selected. On UWFA, nonperfusion area (NPA) measurement was performed. The NPA was compared between patients with and without chronic kidney disease and correlated with parameters of renal function. Results from correlation was applied to prediction of NPA using renal function.

Methods and Materials

Study Subjects

This was a retrospective cross-sectional study performed by retrospective chart review at Bucheon St. Mary's Hospital, The Catholic University of Korea, Gyeonggi-do, Korea. The institutional review board and ethics committee of the Bucheon St. Mary's Hospital approved this study (HC20RISI0070), which adhered to the tenets of the Declaration of Helsinki. The need for informed consent was waived due to the retrospective nature of the study.

The study group consisted of all consecutive type 2 DM patients who visited the outpatient departments of ophthalmology and endocrinology and/or nephrology and underwent UWFA, between August 2016 and December 2019. Because of the invasive nature of UWFA imaging, patients with advanced stages of DR (i.e., severe nonproliferative DR [NPDR] or PDR) were included. Patients with decreased renal function were consulted to the internist for medical care before and after UWFA. Type 2 DM was diagnosed if the patient exhibited a fasting plasma glucose level of $\geq$126 mg/dL or a two-hour post-glucose level of $\geq$200 mg/dL after a 75-g oral glucose tolerance test.[1] Severity of DR was evaluated in accordance with the Early Treatment Diabetic Retinopathy Study (ETDRS) standard grading protocols.[19] PDR was manifested by posterior segment NV, which was defined as presence of NV at the disc or elsewhere, with visible angiographic evidence.[19] Diagnoses of diabetic nephropathy and CKD were made by a nephrologist according to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative classification.[20] Subjects with eGFR less than 60 ml/min/m^2 for at least three months were categorized into the CKD group. A simplified scheme for this study is presented in Figure 1.

Exclusion criteria were low-quality images (e.g., significant vitreous hemorrhage, cataract, or other media opacity); any prior treatment for DR including anti-vascular endothelial growth factor therapy, intraocular or periocular steroids, laser photocoagulation, and vitrectomy; presence of any significant retinal pathology other than PDR; renal replacement therapy including dialysis and renal transplant; and CKD caused by an etiology other than DM such as other glomerular disease, vascular disease, tubulointerstitial disease, or cystic disease.

Measurement of Retinal Capillary NPA on UWFA

Mydriatic UWFA (Optos California P200DTx icg; Optos, Dunfermline, UK) was performed using a standard image view of 200° in a single capture. Early- to mid-phase angiograms (between 40 seconds and one minute after the dye injection) with minimal eyelid or eyelash artifacts, were chosen for analysis of NPA. An NPA was defined as any area of the retina that lacked fluorescein-filled capillaries.[21] Because of variation in the overall size, shape, location, and intensity of the lesions, measurement of NPA can be a difficult job. However, NPA is characterized by change in texture and intensity compared to adjacent retinal tissue, and automated measurement is available using these properties. In this study, we used an automated method for the measurement of NPA, using MATLAB R2020a (The MathWorks, Inc., Natick, MA, USA) as described previously.[14] The method used four different properties of the images: local brightness was used to compare intensity of a region or an object to the intensity of residual background, and contextual information of the region of interest such as variance, uniformity, and entropy were used. Briefly, preprocessed images using homomorphic filtering were automatically segmented, and NPAs were selected using a threshold of properties including mean image intensity,

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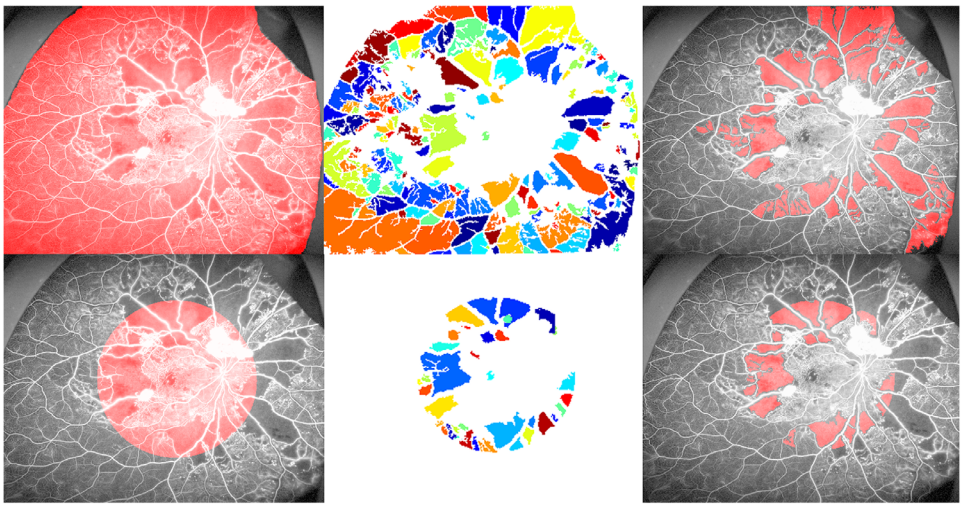

Figure 2. Assessment of nonperfusion area (NPA) on ultra-widefield fluorescein angiography (UWSA). (Left) Two regions of interest were selected (total retina and posterior pole) on UWFA and saved as Bitmap files, which were preprocessed with a homomorphic filter. (Middle) The images were then segmented automatically. (Right) NPAs were chosen using a threshold of area properties, and the sum of the selected areas was calculated.

uniformity, and entropy. The NPA of the total gradable retinal area was calculated following the final modification (total NPA). Separately, the NPA of the posterior pole (circular area of three-disc diameters centered at the fovea) was also measured (pp NPA) (Fig. 2). Measured NPAs were divided by disc area of each eye and presented as disc area (DA). The measurement of NPA was performed individually by two trained ophthalmologists (J. B. and A. L.), and the average of their two measurements in each case was used for analysis.

Table 1. Baseline Demographic and Clinical Characteristics of the Enrolled Patients

Clinical Features	Total	Non-CKD	CKD	*P* Value
Number of patients (number of eyes)	124 (248)	83 (166)	41 (82)	
Age (y)	57.90 ± 11.21	56.92 ± 11.59	59.88 ± 10.18	0.041[*,†]
Sex (male %)	54	54	54	0.934[†]
DM duration (y)	12.18 ± 9.08	11.28 ± 9.02	13.98 ± 8.98	0.028[*,‡]
HbA1c (%)	8.31 ± 2.02	8.4 ± 1.97	8.14 ± 2.12	0.370[‡]
DR grade (PDR%)	46	35	68	<0.001[*,†]
HTN (%)	45	31	73	<0.001[*,†]
HTN duration (y)	5.31 ± 8.49	3.61 ± 7.25	8.71 ± 9.73	<0.001[*,‡]
Systolic BP (mm Hg)	131.52 ± 18.57	130.28 ± 17.68	134.05 ± 20.15	0.151[‡]
Diastolic BP (mm Hg)	74.92 ± 10.54	74.59 ± 10.8	75.59 ± 10.02	0.474[‡]
MAP (mm Hg)	93.79 ± 11.88	93.15 ± 11.95	95.07 ± 11.71	0.229[‡]
Heart Rate (beats/min)	84.48 ± 13.15	85.24 ± 11.84	82.93 ± 15.43	0.234[‡]
BMI (kg/m^2)	24.83 ± 3.59	24.78 ± 3.41	24.94 ± 3.95	0.757[b]
CVD disease (%)	15	11	24	0.013[*,†]
Smoking (%)	30	31	29	0.798[†]
Serum Creatinine (mg/dL)	1.43 ± 1.44	0.84 ± 0.27	2.74 ± 1.93	<0.001[*,‡]
eGFR (mL/min/1.73 m^2)	74.38 ± 36.23	91.27 ± 26.94	34.44 ± 19.66	<0.001[*,‡]
Urine A/C ratio (mg/g)	1044.8 ± 2048.69	211.23 ± 398.53	2757.01 ± 2861.67	<0.001[*,‡]
Urine P/C ratio (mg/g)	2.01 ± 3.1	0.54 ± 1.04	4.07 ± 3.78	<0.001[*,‡]
Hemoglobin (g/dL)	13.39 ± 8.27	13.07 ± 1.88	14.03 ± 14.11	0.541[‡]
Albumin (g/dL)	4.23 ± 0.53	4.37 ± 0.38	3.97 ± 0.67	<0.001[*,‡]
Total cholesterol (mg/dL)	164.22 ± 59.97	158.03 ± 46.29	176.96 ± 80.08	0.066[‡]
Triglyceride (mg/dL)	158.7 ± 140.95	137.64 ± 106.55	200.8 ± 186.03	0.010[*,‡]
HDL cholesterol (mg/dL)	48.71 ± 16.39	50.96 ± 18.21	44.09 ± 10.49	0.001[*,‡]
LDL cholesterol (mg/dL)	102.34 ± 55.28	95.19 ± 42.78	118.78 ± 74.6	0.033[*,‡]
Calcium (mg/dL)	9.37 ± 0.65	9.58 ± 0.53	8.98 ± 0.68	<0.001[*,‡]
Phosphate (mg/dL)	3.79 ± 0.79	3.67 ± 0.63	4.03 ± 0.98	0.004[*,‡]

HTN, hypertension; BP, blood pressure; MAP, mean arterial pressure; BMI, body mass index; A/C, albumin/creatinine; P/C, protein/creatinine; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[*] Statistically significant *P* value.

[†] The χ^2 test between non-CKD and CKD group.

[‡] Independent *t*-test between non-CKD and CKD group.

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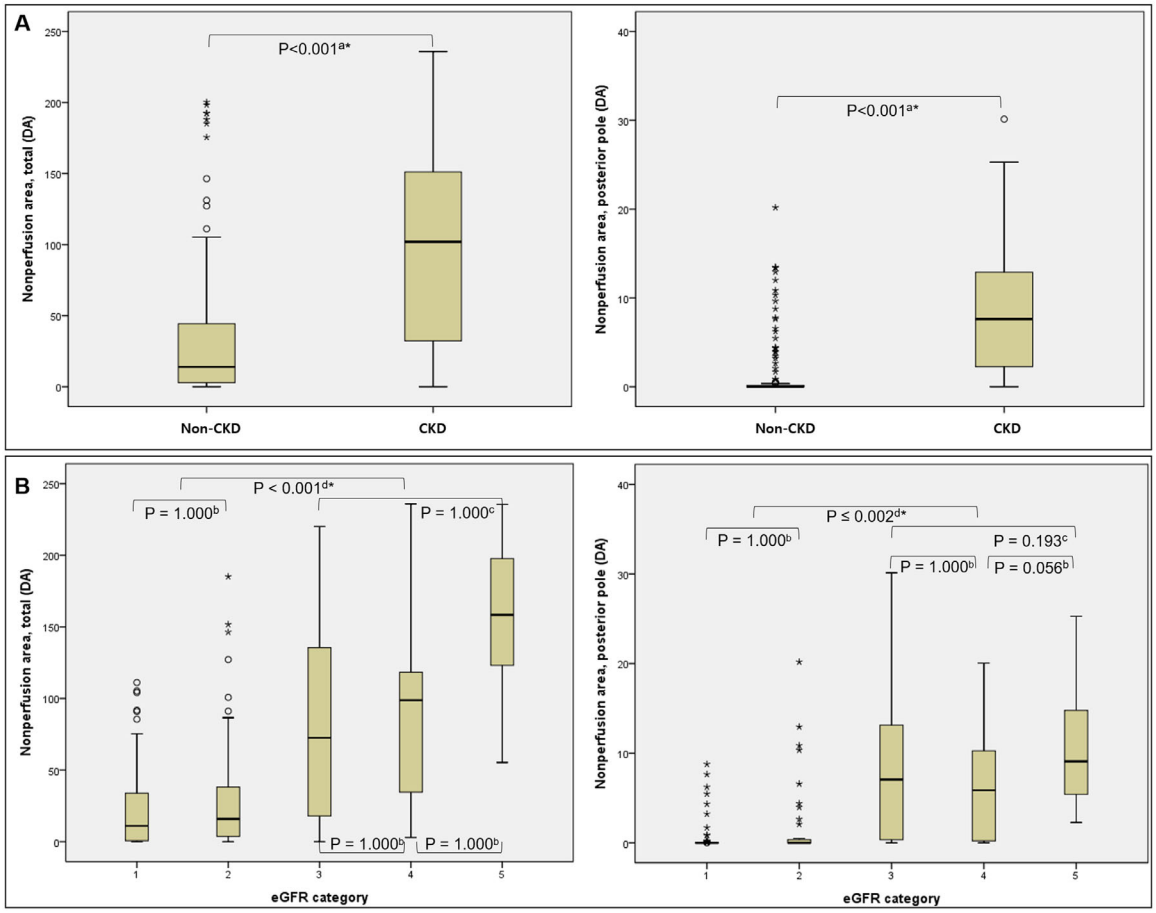

Figure 3. Comparisons of total retina and posterior pole nonperfusion according to CKD group and eGFR category. (**A**) Comparison of NPA according to CKD status shows larger NPAs in the CKD group compared to the non-CKD group. (**B**) Comparison of NPA according to eGFR category shows that the NPAs of eGFR grades 3, 4, and 5 were significantly larger than those of eGFR grades 1 and 2. o indicates outlier and * indicates extreme outlier. a. Independent t-test between non-CKD and CKD group. b. Independent t-test between two eGFR categories. c. One-way analysis of covariance between eGFR categories 3, 4, and 5. d. Independent t-test between eGFR category 1, 2 and eGFR category 3,4,5.

Clinical Information and Laboratory Analysis

Clinical information of the patient was collected and comprised age, sex, DM duration, hemoglobin A1c, comorbid hypertension, hypertension duration, systolic blood pressure, diastolic blood pressure, heart rate, body mass index, and comorbid cardio- or cerebrovascular disease (CVD). Serum creatinine level (Cr), eGFR, urine albumin–creatinine ratio (UACR), and urine protein–creatinine ratio (UPCR) were chosen as parameters for renal function. Additionally, serum levels of hemoglobin, albumin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, calcium, and phosphate were recorded. All data were obtained within one month from the date of UWFA examination.

Statistical Analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences version 22.0.1 for Windows (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation. Independent *t*-test was used to compare continuous variables between groups. The Mann-Whitney U test was used when normal distribution could not be confirmed. The χ^2 test was used for comparison of categorical variables. Intraclass correlation coefficient (ICC) between two graders for NPA measurements were calculated. Analysis of variance (ANOVA) was performed considering NPAs of the eGFR categories, and the Bonferroni test was applied as the post-hoc test. Pearson's correlation analysis was used to determine the correlations between NPA and clinical parameters. Multiple linear regression analysis was performed for covariate adjustment. A P value <0.05 was considered statistically significant.

Results

Baseline Demographic and Clinical Features

In total, 248 eyes of 124 type 2 DM patients with treatment-naïve DR were enrolled. The mean age was 57.90 ± 11.21 years, and 54% of the study population was male. One-hundred sixty-six eyes were classified as non-CKD, and 82 eyes were classified as CKD. For non-CKD and CKD eyes, the mean Cr, eGFR, UACR, and UPCR values were 0.84 ± 0.27 versus 2.74 ± 1.93 mg/dL, 91.27 ± 26.94 versus 34.44 ± 19.66 mL/min/1.73 m^2, 211.23 ± 398.53 versus 2757.01 ± 2861.67 mg/g, and 0.54 ± 1.04 versus 4.07 ± 3.78 mg/g,

Table 2. Correlation Between Retinal Capillary Nonperfusion Area and Clinical Parameters in Total Subjects

	NPA, Total			NPA, Posterior Pole		
Clinical Features	**Correlation Coefficient***	***P* Value**	***N***	**Correlation Coefficient***	***P* Value**	***N***
NPA, total (DA)	1.000	—	248	0.737†	0.000	248
NPA, posterior pole (DA)	0.737†	0.000	248	1.000	—	248
Age (y)	0.097	0.126	248	0.006	0.925	248
Sex	−0.048	0.449	248	0.065	0.305	248
DM duration (y)	0.147†	0.021	246	0.047	0.465	246
HbA1c (%)	−0.077	0.230	244	−0.194†	0.002	244
DR grade	0.277†	0.000	248	0.243†	0.000	248
HTN	0.128†	0.044	248	0.209†	0.001	248
HTN duration (y)	0.091	0.155	246	0.102	0.110	246
Systolic BP (mm Hg)	0.033	0.601	248	0.013	0.843	248
Diastolic BP (mm Hg)	−0.095	0.137	248	−0.089	0.163	248
MAP (mm Hg)	−0.039	0.545	248	−0.046	0.472	248
Heart Rate (beats/min)	−0.090	0.159	248	−0.082	0.198	248
BMI (kg/m2)	−0.119	0.064	242	−0.061	0.347	242
CVD	0.036	0.577	248	0.056	0.381	248
Smoking	−0.043	0.501	244	0.069	0.284	244
Serum Creatinine (mg/dL)	0.585†	0.000	248	0.483†	0.000	248
eGFR (mL/min/1.73 m^2)	−0.572†	0.000	248	−0.524†	0.000	248
Urine A/C ratio (mg/g)	0.541†	0.000	226	0.482†	0.000	226
Urine P/C ratio (mg/g)	0.509†	0.000	182	0.529†	0.000	182
Hb (g/dL)	−0.037	0.563	246	0.016	0.803	246
Albumin (g/dl)	−0.334†	0.000	236	−0.327†	0.000	236
Total cholesterol (mg/dL)	0.006	0.928	220	0.048	0.475	220
Triglyceride (mg/dL)	0.169†	0.014	210	0.141†	0.041	210
HDL cholesterol (mg/dL)	−0.149†	0.031	208	−0.184†	0.008	208
LDL cholesterol (mg/dL)	0.036	0.637	178	0.076	0.311	178
Calcium (mg/dL)	−0.384†	0.000	234	−0.392†	0.000	234
Phosphate (mg/dL)	0.269†	0.000	234	0.270†	0.000	234

HTN, hypertension; BP, blood pressure; MAP, mean arterial pressure; BMI, body mass index; CVD, cardio/cerebrovascular disease; A/C, albumin/creatinine; P/C, protein/creatinine; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Two-tailed Pearson correlation between each clinical feature and NPA.

† Statistically significant value.

respectively (all $P < 0.001$). Furthermore, the non-CKD and CKD groups differed in age, DM duration, DR grade, comorbid hypertension, hypertension duration, and comorbid CVD (all $P \leq 0.041$). The demographic and clinical features of the study eyes are summarized and compared between the non-CKD and CKD groups in Table 1.

Comparison of NPAs and Correlations Between Retinal NPA and Clinical Parameters

The mean total and pp NPA values were significantly larger in the CKD group than in the non-CKD group (33.11 ± 45.77 DA and 100.57 ± 69.52 DA versus 1.21 ± 3.28 DA and 7.99 ± 6.75 DA; both $P < 0.001$) (Fig. 3A). The ICCs for total and pp NPAs were 0.956 and 0.951, respectively. ANOVA revealed that the NPA values differed according to eGFR category, whereas post-hoc analysis indicated that NPAs of eGFR grades 3, 4, and 5 were significantly larger than those of eGFR grades 1 and 2 (all $P \leq 0.002$) (Fig. 3B).

Total NPA showed positive correlations with pp NPA, DM duration, DR grade, comorbid hypertension, Cr, UACR, and UPCR ($r = 0.737$, 0.147, 0.277, 0.585, 0.541, and 0.509; all $P \leq 0.021$), but a negative correlation with eGFR ($r = -0.572$; $P < 0.001$). Meanwhile, pp NPA showed positive correlations with total NPA, DR grade, comorbid hypertension, Cr, UACR, and UPCR ($r = 0.737$, 0.243, 0.209, 0.483, 0.482, and 0.529; all $P \leq 0.001$) and negative correlations with HbA1c and Cr ($r = -0.194$ and −0.524; both $P \leq 0.002$). Correlation coefficients and P values are summarized in Table 2.

Detailed Analysis of the Association Between NPA and Renal Function

The clinical factors that differed between non-CKD and CKD eyes (i.e., DM duration, DR grade, hypertension, and CVD) did not affect the finding that NPAs in the CKD group were significantly larger than those in the non-CKD group (all $P \leq 0.007$), except with respect to the impact of CVD on total NPA ($P = 0.107$) (Fig. 4). Two-way ANOVA also revealed no significant effect of DM duration, DR grade, hypertension, and CVD on NPA differences between non-CKD and CKD eyes ($F = 1.349$, 0.99, 0.267, and 1.122 and $P = 0.247$, 0.753, 0.606, and 0.291 for total NPA; $F = 0.340$, 0.629, 1.060, and 1.720 and $P = 0.560$, 0.428, 0.304, and 0.191 for pp NPA, respectively).

Separately, correlations between NPAs and all parameters concerning renal function (i.e., Cr, eGFR, UACR, and UPCR) remained significant after adjusting for clinical factors that showed correlation with NPA (i.e. DM duration, DR grade, and hypertension) (all $P < 0.001$). Linear regression analysis including all these factors resulted in good prediction of NPA ($R^2 = 0.444$ for total NPA and $R^2 = 0.448$ for pp NPA; both $P < 0.001$). Overall, the coefficients were significant for

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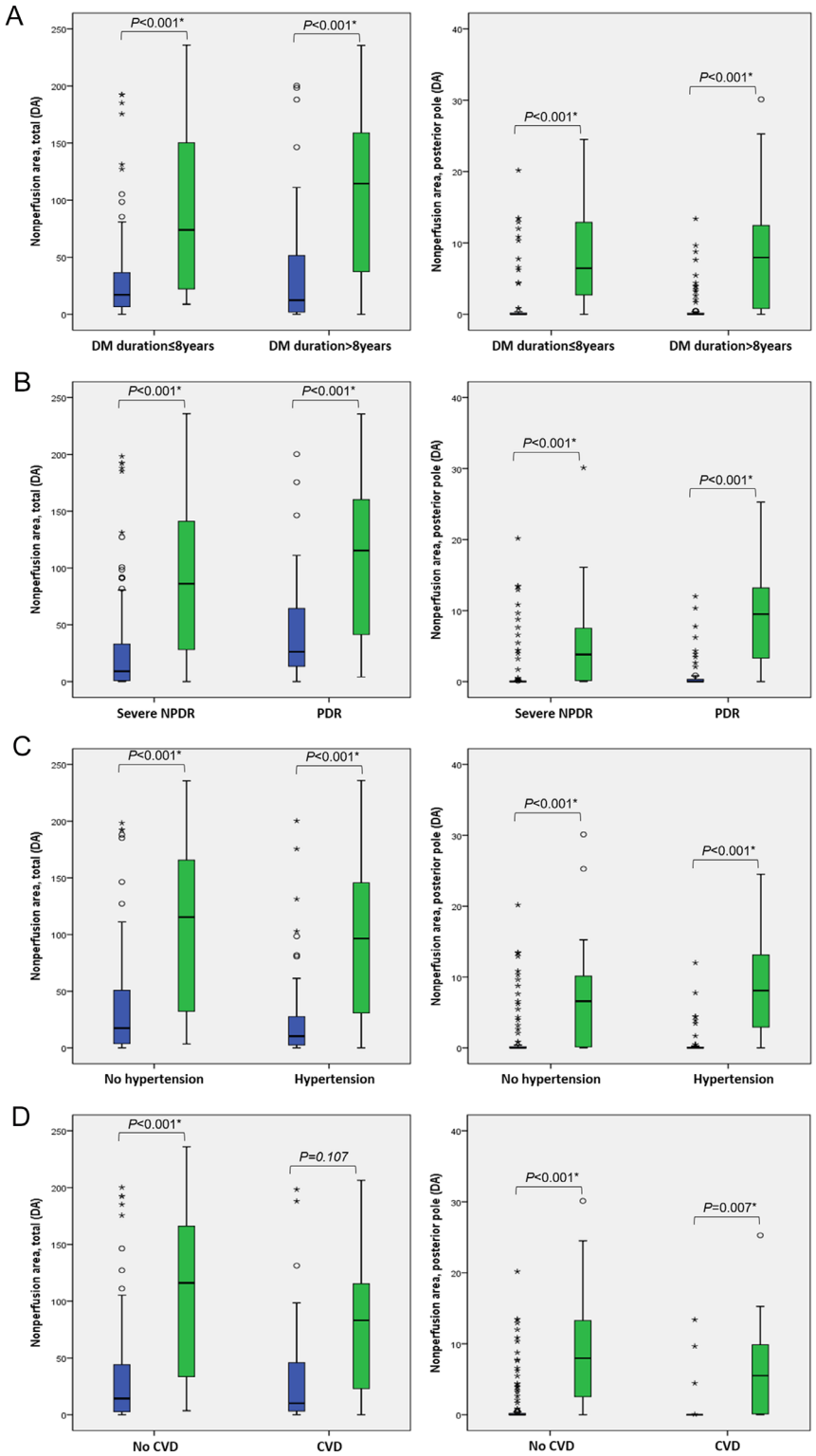

Figure 4. Comparisons of NPAs between the CKD and non-CKD groups with regard to factors that differed between the groups. (**A**) NPAs were larger in the CKD group than in the non-CKD group when the subjects were divided according to disease duration. (**B**) NPAs were larger in the CKD group than in the non-CKD group when the subjects were divided according to DR grade. (**C**) NPAs were larger in the CKD group than in the non-CKD group when the subjects were divided according to presence of hypertension. (**D**) Posterior polar NPAs were larger in the CKD group than in the non-CKD group when the subjects were divided according to presence of comorbid cardio-/cerebrovascular disease. In the presence of CVD, total NPA was larger in the CKD group, but it failed to reach statistical significance. Blue bar indicates non-CKD and green bar indicates CKD group. P-values are obtained from independent t-test between non-CKD and CKD group.

Table 3A. Correlation Between Nonperfusion Areas and Parameters for Renal Function Adjusted by Retinopathy Grade, Disease Duration, and Comorbid Hypertension: Total NPA

Parameters	R^2	Constant	Cr	eGFR	UACR	UPCR	DR Grade	DM Duration	HTN
Cr									
Coefficient	0.370*	−41.685	24.556*,†	—	—	—	16.635*	0.511	−6.971
P value	<0.001	0.088	<0.001	—	—	—	0.016	0.167	0.311
eGFR									
Coefficient	0.361*	83.974*	—	−1.015*,†	—	—	13.777	0.219	−13.112
P value	<0.001	0.006	—	<0.001	—	—	0.051	0.564	0.068
UACR									
Coefficient	0.344*	−40.293	—	—	0.016*,†	—	18.440*	1.236*	3.151
P value	<0.001	0.127	—	—	<0.001	—	0.013	0.002	0.664
UPCR									
Coefficient	0.307*	−14.000	—	—	—	11.030*,†	11.597	1.421*	1.473
P value	<0.001	0.665	—	—	—	<0.001	0.190	0.003	0.864
All									
Coefficient	0.444*	88.290*	10.240*	−0.579*	0.005	1.051	−0.361	0.229	−20.178*
P value	<0.001	0.018	0.009	0.001	0.343	0.780	0.965	0.623	0.018

Cr, serum creatinine level; HTN, hypertension; All: multiple linear regression model including Cr, eGFR, UACR, UPCR, DR grade, DM duration, and HTN.

* Statistically significant value.

† Correlation coefficient of each parameter for renal function after adjusted by DR grade, DM duration, and HTN using multiple linear regression.

Table 3B. Correlation Between Nonperfusion Areas and Parameters for Renal Function Adjusted by Retinopathy Grade, Disease Duration, and Comorbid Hypertension: Posterior Polar NPA

Parameters	R^2	Constant	Cr	eGFR	UACR	UPCR	DR Grade	HTN	HbA1c
Cr									
Coefficient	0.370*	−41.685	24.556*,†	—	—	—	16.635*	0.511	−6.971
P value	<0.001	0.088	<0.001	—	—	—	0.016	0.167	0.311
eGFR									
Coefficient	0.361*	83.974*	—	−1.015*,†	—	—	13.777	0.219	−13.112
P value	<0.001	0.006	—	<0.001	—	—	0.051	0.564	0.068
UACR									
Coefficient	0.344*	−40.293	—	—	0.016*,†	—	18.440*	1.236*	3.151
P value	<0.001	0.127	—	—	<0.001	—	0.013	0.002	0.664
UPCR									
Coefficient	0.307*	−14.000	—	—	—	11.030*,†	11.597	1.421*	1.473
P value	<0.001	0.665	—	—	—	<0.001	0.190	0.003	0.864
All									
Coefficient	0.444*	88.290*	10.240*	−0.579*	0.005	1.051	−0.361	0.229	−20.178*
P value	<0.001	0.018	0.009	0.001	0.343	0.780	0.965	0.623	0.018

Cr, serum creatinine level; HTN, hypertension; All, multiple linear regression model including Cr, eGFR, UACR, UPCR, DR grade, DM duration, and HTN.

* Statistically significant value.

† Correlation coefficient of each parameter for renal function after adjusted by DR grade, DM duration, and HTN using multiple linear regression.

hypertension, Cr, and eGFR ($P = 0.009$, 0.001, and 0.018 for total NPA, respectively, and $P = 0.006$, < 0.001, and 0.002 for pp NPA) (Tables 3A, 3B).

Automatic linear modeling with all clinical factors revealed accuracy of 51.8% for total NPA and 48.0% for pp NPA. The eGFR category was the most important predictor for both total and pp NPAs (Fig. 5A). Meanwhile, automatic linear modeling including only parameters showing correlation with NPA displayed accuracy of 48.7% for total NPA and 46.7% for pp NPA. Again, the eGFR category was the most important predictor for both total and pp NPAs (Fig. 5B).

Discussion

The association between DR and diabetic nephropathy, two important microvascular complications of DM, has been actively investigated, and the results have implied an intimate relationship between the manifestations and severity of DR and renal function and its progression in patients with DM.[5,6,8,9,22–26] Retinal capillary nonperfusion is a major sign of microvascular insufficiency of DR, reflecting the extent of ischemia and is directly involved in development of PDR, the end stage of DR.[14,16,18,27] Ultra-widefield imaging has enabled more thorough assessments of retinal vascularity

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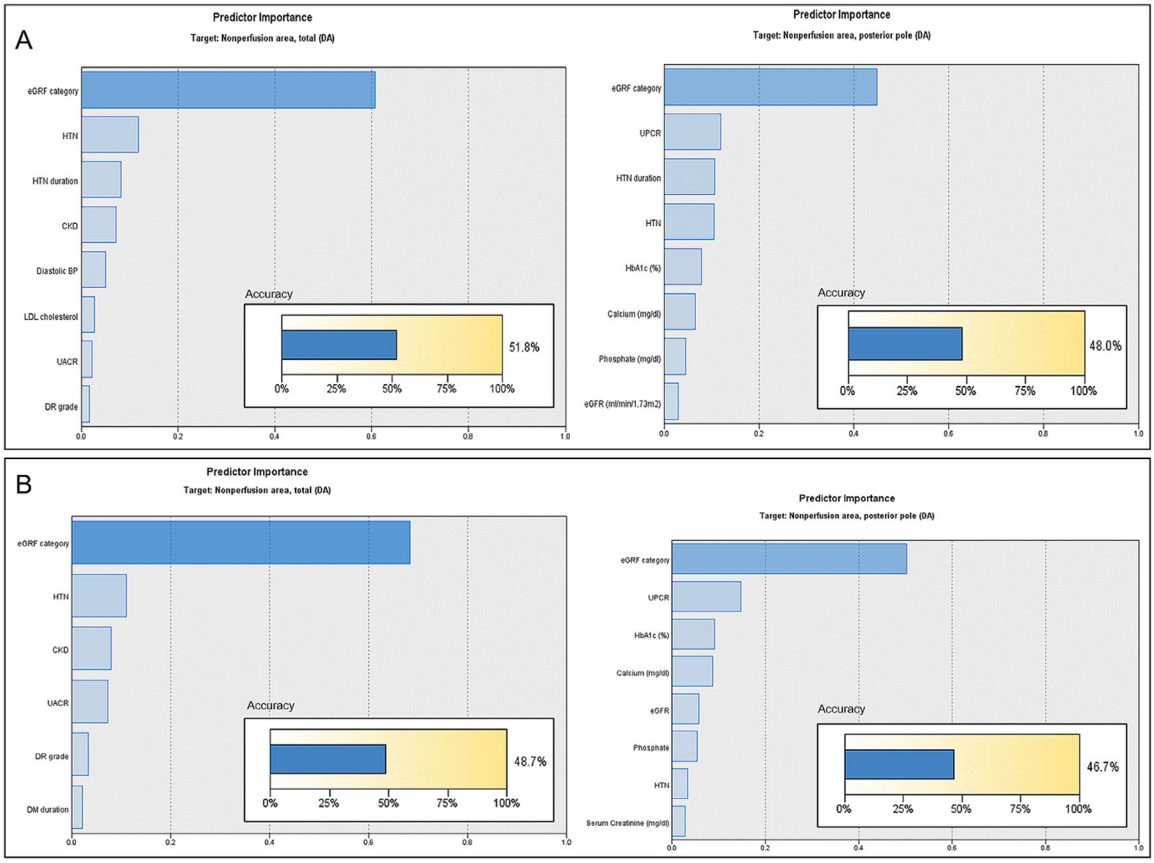

Figure 5. Automatic linear modeling using clinical factors and renal function parameters for total and posterior polar nonperfusion areas. (**A**) Linear modeling with all clinical factors revealed an accuracy of 51.8% for total NPA and 48.0% for pp NPA. eGFR category was the most important predictor for both total and pp NPAs. (**B**) Linear modeling including only parameters showing correlation with NPA revealed accuracy of 48.7% for total NPA and 46.7% for pp NPA. The eGFR category was the most important predictor for both total and pp NPA.

including the far peripheral retina, therefore enabling a more precise assessment of the NPA in DR eyes.[14–16,18,28] In this study, we measured retinal NPAs quantitatively on UWFA in an objective manner and analyzed their correlation with renal function in patients with type 2 DM with DR. The results demonstrated a significant association between retinal NPA and renal function and indicated that eGFR grade was the most potent predictor of retinal NPA.

The mean NPA values were significantly larger in the CKD group than in the non-CKD group and, when stratified according to eGFR category, the NPA values of eGFR grade 3 or more were significantly larger than those of eGFR grades 1 and 2. Retinal capillary nonperfusion is an irreversible microvascular change in DR.[29] Retinal hypoxia is a powerful stimulator for capillary abnormalities and formation of NV, while nonperfusion is the leading cause of hypoxia in DR eyes.[14,30] Therefore, assessment of nonperfusion can highlight more severe stages of microangiopathy in DM patients and this can reflect the status of renal function in these patients. Although most previous studies have revealed an association between DR and diabetic nephropathy on the basis of color fundus photography, the current study adopted angiographic imaging, which enabled assessment of NPA. In addition, in contrast to conventional color fundus photography, which can only show the posterior pole of the retina, the UWFA imaging used in this study can reveal more than 80% of the total retinal area. This is a major evolution relative to the outcomes of recent reports on values of peripheral lesions in DR.[28,31,32]

DM duration, DR grade, and comorbid hypertension and CVD are well-known risk factors for CKD in DM.[5,8,33] In this study, these characteristics were also revealed as baseline clinical factors that significantly varied between non-CKD and CKD eyes. The difference in NPA values between the non-CKD and CKD conditions was significant after adjusting for DM duration, DR grade, and comorbid hypertension, suggesting an independent association between NPA and CKD regardless of other relevant factors. This is in accordance with previous research contending an association between presence of DR and renal function deterioration independent of associated DM or hypertension.[7] When divided by comorbid CVD, NPA values between the non-CKD and CKD groups did not differ significantly. A strong association between DR and CVD was previously suggested by Grunwald et al.,[33] even following adjustments for renal dysfunction and traditional CVD risk factors. The ischemic status of the posterior pole can be affected not only by renal function, but also by other factors associated with CVD, such as atherosclerosis. Nonetheless, total NPA, which represents the global ischemic status of DR, was still correlated with presence of CKD.

Both total and pp NPA showed positive correlations with renal function. Other involved clinical factors were DR grade, DM duration, and hypertension for total NPA and

DR grade, hypertension, and HbA1c for pp NPA, respectively. The correlations between NPA and Cr, eGFR, UACR, and UPCR, respectively, remained significant after adjusting these factors, suggesting an independent correlation between NPA and renal function.

Furthermore, linear modeling with all clinical factors and relative clinical factors revealed eGFR category to be the most important predictor for both total and pp NPA. The eGFR category is influenced by all parameters of retinal function. Thus this result may be interpreted to mean that renal function, among other clinical factors, has the most intimate relationship with NPA. Prediction of NPA using renal function can improve management of DM patients with CKD by assisting proper referral of these patients and enabling prompt treatment for NPAs which deteriorates DR state and causes eventual vision loss. Preservation of vision is especially important in these patients because many CKD patients require either peritoneal dialysis or hemodialysis. For patients on peritoneal dialysis, preservation of vision is mandatory to reduce possibilities of infection that increases mortality of the patient. And patients on hemodialysis also require enough vision for three times per week ambulation to the hospital. In addition, the strong correlation between NPA and renal function may offer a rationale for estimation of renal function using NPA observed via angiography in DM patients.

There are several limitations to consider in this study. First, treatment regimens for DM and diabetic nephropathy were not controlled because of the retrospective nature of the study. This factor requires further validation. Second, the patients involved in this study had advanced stages of DR, so the results of this investigation may not be applicable to earlier stages of DR. Third, angiographic findings other than nonperfusion, such as extent of microaneurysm, leakage, and neovascularization, should be considered in DR. This also requires further research, despite inherent difficulties in quantification of these parameters. Forth, selection bias can occur by omitting patients with advanced DR and not followed-up for systemic conditions in our institution. Further, NPA at the posterior pole can be more precisely visualized using OCT angiography, so additional validation of our results regarding the posterior pole warrants further assessment by OCT angiography. And finally, the subject of this study was restricted to advanced stages of DR because of the invasive nature of UWFA imaging. Prospective studies involving patients at earlier stages of DR are warranted to encompass this correlation in the general DR population.

In conclusion, DR eyes of CKD patients showed larger retinal NPA compared with those of non-CKD patients, and larger retinal NPA was associated with worse renal function in DM patients independent of other risk factors. These results imply a strong relationship between retinal NPA and renal function in DM. Also, eGFR category was the most potent predictor of retinal NPA, suggesting that renal function can be used to predict retinal NPA. Further studies relying on other methods for angiography and other angiographic findings are warranted.

Acknowledgments

Supported by Busan Sungmo Eye Hospital Sodam Scholarship Committee.

Disclosure: **J.W. Min,** None; **H.D. Kim,** None; **S.Y. Park,** None; **J.H. Lee,** None; **J.H. Park,** None; **A. Lee,** None; **H. Ra,** None; **J. Baek,** None

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