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Effect of a Single Intravitreal Bevacizumab Injection on Proteinuria in Patients With Diabetes

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Purpose: Proteinuria is the second most common complication after hypertension after systemic administration of bevacizumab. Therefore we aimed to analyze the effect of intravitreal bevacizumab (IVB) injection on proteinuria in patients with diabetes.

Methods: Patients scheduled to receive IVB injection from May 1, 2018, to December 31, 2018, were prospectively enrolled. In total, 53 patients with diabetes (26 with nonproliferative diabetic retinopathy and 27 with proliferative diabetic retinopathy) and 37 patients without diabetes were included. Urine tests were performed within 1 month of and 7 ± 1 days after IVB injection. Urinary protein, creatinine, and albumin concentrations were quantitatively measured, and urinary protein-to-creatinine ratio and urinary albumin-to-creatinine ratio (UACR) were calculated from these data before and after IVB injection.

Results: The mean urinary microalbumin concentrations and urinary protein-to-creatinine ratio were significantly higher in patients with diabetes, both before and after IVB injection. There were no differences between patients with nonproliferative diabetic retinopathy and proliferative diabetic retinopathy. About 80% of patients with diabetes showed improved albuminuria or at least no harmful effect in terms of albuminuria. Patients with deteriorated baseline UACR showed more residual increase in UACR after IVB injection ($P < 0.05$ in all groups).

Conclusions: Close monitoring of renal function after IVB might be needed in patients with diabetes according to the severity of nephropathy.

Translational Relevance: Our results may provide information regarding the renal function of IVB-treated patients with diabetes.

Introduction

Intravitreal injections of antivascular endothelial growth factor (VEGF) agents have been widely used as a standard treatment for various retinal diseases such as age-related macular degeneration, diabetic retinopathy (DR), and retinal vascular disease.[1] Intravitreal injections typically deliver a small amount of anti-VEGF agents into the vitreous body, which can suppress the plasma-free VEGF level and may have systemic effects in addition to the local ocular effects.[2–4] Among the various types of anti-VEGF agents for intravitreal injections, higher systemic exposure was observed with bevacizumab compared with ranibizumab or aflibercept.[5] The suppression of plasma VEGF level after intravitreal injections was prominent after bevacizumab and aflibercept injections for at least 1 month.[5–7] Multiple large-scale randomized clinical trials concerning the safety of intravitreal anti-VEGF injections revealed no significant increase in systemic risk focused on serious

adverse events (cerebrovascular accident, ischemic heart disease, systemic thromboembolism, etc.).[8–11] However, there are few studies on proteinuria after intravitreal bevacizumab (IVB) injection in patients with diabetes.[12,13]

Recently, there was a case series reporting worsened proteinuria after multiple IVB injections in patients with diabetes.[14] Proteinuria is the second-most common complication after hypertension following systemic administration of bevacizumab, presenting as increased risk of all-grade proteinuria, high-grade proteinuria (grade 3 or 4), and nephrotic syndrome.[15,16] Moreover, diabetes is a significant risk factor for the development of proteinuria after systemic administration of bevacizumab.[17] Proteinuria is an independent risk factor for morbidity and death in patients with chronic kidney diseases, including diabetic nephropathy (DN).[18,19] Investigation of proteinuria including albuminuria was inconvenient in clinical settings because patients had to collect their urine sample for 24 hours; however, urinary protein-to-creatinine ratio (UPCR) or urinary albumin-to-creatinine ratio (UACR) calculated from untimed urine sample has been introduced as a marker that showed good correlation with absolute urinary protein or albumin excretion collected for 24 hours.[18,20] UPCR and UACR can be measured from untimed spot urine samples, which makes them easy to investigate on an outpatient basis in clinics, and they are currently recommended for initial evaluation of proteinuria.[19]

DN, one of the microvascular complications of diabetes, shares common pathogenesis with DR.[21] Hence, we believe that it is important to analyze the effect of systemic exposure of bevacizumab after intravitreal injection on proteinuria in patients with diabetes. There could be 3 possible courses for this effect; the systemic exposure of bevacizumab (1) is either too weak to have any effect on proteinuria, (2) would increase proteinuria by suppressing the physiological VEGF from podocytes, which is required for the maintenance of endothelial cells, or (3) would decrease proteinuria by suppressing the elevated pathologic VEGF and neovascularization, similar to that observed in proliferative DR (PDR). Accordingly, we investigated the effect of IVB injection on proteinuria using UACR in patients with diabetes.

Methods

Patients

Patients scheduled to receive IVB injection owing to various retinal diseases from May 1, 2018, to December 31, 2018, were prospectively enrolled in this study. This study was approved by the Institutional Review Board of Ajou University Hospital, Suwon, Korea (IRB No. AJIRB-BMR-OBS-18-035), and it complied with the Declaration of Helsinki. Written informed consent was obtained from all patients. The exclusion criteria were as follow: (1) age <20 years; (2) a history of vitrectomy; (3) prior intravitreal anti-VEGF injections within 6 months from the time of inclusion; (4) a pre-existing kidney disease or current dialysis; (5) withdrawn consent; and (6) loss of follow-up.

The medical history, clinical demographics, and information regarding current medications were obtained from the patients at the time of inclusion. Systolic and diastolic blood pressure were also measured at each visit. HbA1c data within 3 months were collected, and the grade of diabetic retinopathy was assessed using fundus photographs and fluorescein angiography findings in patients with diabetes. The underlying retinal diseases that required IVB were diagnosed by fundus examinations, and additional examinations such as fluorescein angiography and optical coherence tomography. IVB was administered as 1.25 mg/0.05 mL by 1 of the 3 participating retinal specialists (Y.H.K, Y.R.C., or K.L.).

Proteinuria and Albuminuria

Untimed urine sample was collected before and after 7 ± 1 days of IVB injection, based on the fact that bevacizumab shows prominent suppression of plasma-free VEGF concentration at 1 week after injection.[6] Urinary protein, albumin, and creatinine concentrations were measured, and UPCR and UACR were calculated from these measurements using the following formula:

$$\text{UPCR (mg/g)} = \text{urine protein (mg/dL)/creatinine (g/dL)}$$

$$\text{UACR (mg/g)} = \text{urine albumin (mg/dL)/creatinine (g/dL)}$$

UACR was categorized into 3 groups as follows: A1 (<30 mg/g), A2 (≥30 mg/g and ≤300 mg/g), and A3 (>300 mg/g) groups, according to KDIGO 2012 clinical practice guidelines (Supplementary Fig. S1).[19] Because patients with higher value at baseline tend to have a lower change on a subsequent measurement and vice versa, i.e., regression-to-the-mean, we used the categorical analyses from the study by Jun et al.[22] The categorical changes to account for regression-to-the-mean are described in Online Resource 1.

Statistical Analysis and Sample Size

The sample size was based on primary outcomes, and it was calculated using the G*power 3.1.9.2 software[23] assuming a moderate effect, because there were no prior studies. We used an α error of 0.05 and power of 80%, and a sample size of 35 patients was required.

All statistical analyses were performed using SPSS (version 25.0; IBM Corp., Armonk, NY). Categorical variables were compared by use of the χ^2 test, and continuous variables were compared using the independent *t*-test or Mann–Whitney *U* test. Paired *t*-test or Wilcoxon signed rank test was used to compare pre-IVB and post-IVB values. Because UPCR and UACR showed extremely skewed distribution, log10-transformed values of the original values were created and used for statistical analysis. For expression of the log10-transformed data, the values were back-transformed to geometric means and presented as the geometric mean with a 95% confidence interval. A *P* value <0.05 was considered significant.

Results

A total of 108 patients were initially enrolled, but 17 patients refused post-IVB urine tests or were lost to follow-up at 7 ± 1 days after IVB. One patient who showed over 6000 mg of urinary protein level at baseline assessment was diagnosed as having nephrotic syndrome immediately after IVB injection. Accordingly, 90 patients were finally included in this study. Among these, 37 patients without diabetes were assigned to the control group, and 53 patients with diabetes were assigned to the diabetic group (26 with nonproliferative DR [NPDR] and 27 with PDR). This fulfilled the required sample size of 35 patients in each group. The baseline characteristics of the included patients are summarized in Table 1. The

Table 1. Baseline Characteristics of Included Patients

Variable	All Patients			Diabetic Patients		
	Control	DM	*P* Value	NPDR	PDR	*P* Value
No. of patients	37	53		26	27	
Age (years)	58.8 (13.6)	59.8 (14.3)	0.396	60.2 (10.9)	53.2 (9.5)	0.015
Sex, male	29 (55%)	29 (55%)	0.026*	15 (58%)	14 (52%)	0.669
HbA1c, %	5.6 (0.9)	8.5 (2.1)	0.011*	7.9 (1.4)	9.0 (2.5)	0.053
Medications						
ACEI/ARB	3 (8%)	21 (40%)	<0.001*	11 (42%)	10 (37%)	0.491
β-blocker	1 (3%)	7 (13%)	0.031*	2 (8%)	5 (19%)	0.295
CCB	4 (11%)	19 (36%)	0.002*	9 (35%)	10 (37%)	0.949
Diuretics	0 (0%)	8 (15%)	0.006*	4 (15%)	4 (15%)	0.843
NSAID	2 (5%)	6 (11%)	0.293	4 (15%)	2 (7%)	0.329
Compound analgesics	1 (3%)	6 (11%)	0.115	3 (12%)	3 (11%)	0.917
Statins	6 (16%)	26 (49%)	<0.001*	12 (46%)	14 (52%)	0.920
Vasodilator	2 (5%)	3 (6%)	0.906	1 (4%)	2 (7%)	0.600
Antidiabetic medications						
Metformin	—	—	—	15	18	0.775
Sulfonylurea	—	—	—	8	14	0.178
SGLT2 inhibitor	—	—	—	0	3	0.237
DPP4 inhibitor	—	—	—	10	19	0.032*
Thiazolidinedione	—	—	—	1	5	0.194
Insulin	—	—	—	4	7	0.428
Systolic BP (mmHg)	133.2 (16.1)	135.4 (20.9)	0.606	138.3 (19.1)	132.6 (22.6)	0.325
Diastolic BP (mmHg)	78.7 (12.0)	77.4 (12.6)	0.627	77.0 (14.4)	77.7 (10.9)	0.824

Data are expressed as mean (SD) or number (percentage).

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; β-blocker, β adrenergic receptor blocker; BP, blood pressure; CCB, calcium channel blocker; DM, diabetic mellitus; DPP4, dipeptidyl peptidase-4; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SGLT2, sodium glucose cotransporter-2.

*P value < 0.05 by χ^2 test.

Table 2. Urine Analysis of Proteinuria before and after IVB

Variable	All Patients			Diabetic Patients		
	Control	DM	*P* Value	NPDR	PDR	*P* Value
Pre-IVB lab						
Microalbumin (mg/dL)	2.9 (7.6)	23.3 (43.3)	<0.001*	20.1 (51.6)	26.3 (34.1)	0.608
Protein (mg/dL)	12.3 (14.0)	43.0 (70.0)	0.003*	32.5 (65.6)	53.1 (73.8)	0.289
Creatinine (mg/dL)	104.8 (63.0)	89.4 (67.4)	0.268	88.5 (63.6)	90.2 (72.1)	0.925
UPCR (mg/g)	96.1 (66.0–188.8)	249.2 (133.8–774.2)	<0.001†	202.4 (95.1–353.0)	346.2 (154.9–977.1)	0.122
Post-IVB lab						
Microalbumin (mg/dL)	1.4 (2.4)	21.7 (36.4)	<0.001*	17.2 (40.1)	26.0 (32.5)	0.388
Protein (mg/dL)	9.5 (5.1)	33.4 (42.1)	<0.001*	28.3 (48.2)	38.2 (35.5)	0.397
Creatinine (mg/dL)	101.3 (67.1)	76.4 (51.5)	0.062	72.2 (44.6)	80.4 (58.0)	0.569
UPCR (mg/g)	89.4 (58.3–196.5)	296.1 (141.9–769.4)	<0.001†	188.2 (138.4–358.1)	353.4 (143.1–951.7)	0.262
Delta of UPCR	1.02	0.97	0.323	0.86	1.09	0.393

Data are expressed as mean (SD), except UPCR which is expressed as median (interquartile interval).
*P value < 0.05 by independent t-test.
†P value < 0.05 by Mann-Whitney test using $\log_{10}$-transformed values of the original values.

control group comprised 13 patients with central serous chorioretinopathy, 12 with retinal vein occlusion (8 with branch retinal vein occlusion and 4 with central retinal vein occlusion), 10 with neovascular age-related macular degeneration (7 with choroidal neovascularization and 3 with polypoidal choroidal vasculopathy), and 2 with retinal macroaneurysm. Among the control subjects, there was 1 patient with suspected underlying hypertension (Supplementary Table S1). Compared with the control subjects, more patients with diabetes were on concomitant systemic medications; meanwhile, there were no significant differences between patients with NPDR and PDR.

The UPCR and the mean concentrations of urinary microalbumin were significantly higher in patients with diabetes than in control subjects, both before and after IVB injection (Table 2). Among patients with diabetes, there were no differences between those with NPDR and PDR. The mean difference of UPCR before and after IVB injection showed no statistical difference between the groups.

In terms of UACR, the geometric means of UACR before and after IVB injection were higher in patients with diabetes than in control subjects, whereas paired t-tests revealed no difference between pre- and post-IVB UACR values within patients with diabetes (Table 3).

Table 3. Change of UACR before and after IVB

UACR	All Patients			Diabetic Patients		
	Control	DM	*P* Value	NPDR	PDR	*P* Value
Geometric mean (95% CI)						
Pre-IVB	9.0 (6.3–12.6)	94.4 (57.7–154.5)	<0.001	64.6 (31.6–132.2)	136.1 (68.1–272.3)	0.130
Post-IVB	8.9 (6.7–11.7)	101.4 (62.3–165.1)	<0.001	79.0 (39.8–156.7)	128.9 (62.4–266.5)	0.091
P value*	0.931	0.441		0.180	0.624	
Abnormal albuminuria†						
Abnormal range at pre-IVB	4 (11%)	36 (68%)	<0.001	16 (62%)	20 (74%)	0.328
Abnormal range at post-IVB	4 (11%)	38 (72%)	<0.001	19 (73%)	19 (70%)	0.698

Data are expressed as number (percentage) unless otherwise stated.
*P value by Wilcoxon signed rank test comparing pre-IVB and post-IVB data.
†Abnormal range of UACR ($\geq$ 30 mg/g).

Table 4. Categorical Distribution of UACR before and after IVB

Category of UACR	All Patients			Diabetic Patients		
	Control	DM	*P* Value	NPDR	PDR	*P* Value
Pre-IVB			<0.001*			0.120
A1 (< 30 mg/g, normal)	33 (89%)	17 (32%)		10 (38%)	7 (26%)	
A2 (≥ 30 and ≤ 300 mg/g)	4 (11%)	20 (38%)		11 (29%)	9 (33%)	
A3 (> 300 mg/g)	0	16 (30%)		5 (19%)	11 (41%)	
Post-IVB			<0.001*			0.712
A1 (< 30 mg/g, normal)	33 (89%)	16 (30%)		7 (27%)	9 (33%)	
A2 (≥ 30 and ≤ 300 mg/g)	4 (11%)	23 (43%)		14 (54%)	9 (33%)	
A3 (> 300 mg/g)	0	14 (26%)		5 (19%)	9 (33%)	
Categorical change†			0.782			0.021*
Improved change	0	5 (9%)		1 (4%)	4 (15%)	
No change of category	37 (100%)	44 (83%)		21 (81%)	23 (85%)	
Aggravated change	0	4 (8%)		4 (15%)	0	

Data are expressed as number (percentage).

*P value < 0.05 by χ^2 test.

†Improved change included change from A3 to A1 or A2 and A2 to A1, whereas aggravated changes included change from A1 to A2 or A3 and A2 to A3.

Compared with control subjects, more patients with diabetes presented abnormal levels of albuminuria both before and after IVB injection, whereas there was no difference in the number of patients presenting abnormal albuminuria before and after IVB injection (Table 3). Similarly, there was no difference in the number of patients with NPDR and PDR presenting abnormal albuminuria.

The changes in UACR according to categorical distribution are shown in Table 4. Compared with control subjects, more patients with diabetes showed abnormal levels of albuminuria (i.e., A2 and A3 categories) both before and after IVB injection, whereas there was no difference between patients with NPDR and PDR. Overall, about 80% of patients showed improved albuminuria, or at least no harmful effect in terms of albuminuria was observed among any of the patients with diabetes (Table 4). However, 15% of patients with NPDR showed aggravation of UACR ($P = 0.021$, Table 4).

The categorical distribution according to regression-to-the-mean did not differ between controls and patients with diabetes (Table 5). However, this categorical distribution was statistically significant

Table 5. Categorical Change of UACR before and after IVB (by Regression to the Mean)

Categorical Change of UACR		All Patients			Diabetic Patients		
		Control	DM	*P* Value	NPDR	PDR	*P* Value
A1	Residual decrease	19 (51%)	13 (25%)	0.187	7 (27%)	6 (22%)	0.452
	Regression to the mean	14 (38%)	4 (8%)		3 (12%)	1 (4%)	
A2	Residual decrease	3 (8%)	7 (13%)	0.237	5 (19%)	2 (7%)	0.085
	Regression to the mean	0	8 (15%)		2 (8%)	6 (22%)	
	Residual increase	1 (3%)	5 (9%)		4 (15%)	1 (4%)	
A3	Regression to the mean	0	3 (6%)	N/A	1 (4%)	2 (7%)	0.931
	Residual increase	0	13 (25%)		4 (15%)	9 (33%)	
P value		0.006*	<0.001*		0.026*	<0.001*	

Data are expressed as number (percentage). Categorical change of UACR by baseline categories is as follows: 1) for A1, residual decrease implied minor change or decreased of UACR ≥ 30%, and regression-to-the-mean implies increase of UACR ≥ 30%; 2) for A2, residual decrease implied decrease of UACR ≥ 30%, regression-to-the-mean implied minor change, and residual increase implied increase of UACR ≥ 30%; and 3) for A3, regression-to-the-mean implied decrease of UACR ≥ 30%, and residual increase implied increase of UACR ≥ 30% or minor change.

*P value < 0.05 by χ^2 test.

within each group, indicating that patients with deteriorated baseline UACR were associated with more residual increase after IVB injection ($P < 0.05$ in all groups).

Discussion

DR is one of the leading causes of severe vision loss and blindness, and it shares common pathogenesis with DN.[24,25] There have been studies on correlations between DR and DN. Albuminuria and gross proteinuria are known to be independent risk factors for cardiovascular morbidity or all-cause death, regardless of diabetes, and for the development of PDR or DN in patients with diabetes.[26,27] Furthermore, DR itself is a risk factor not only for DN but also for chronic kidney disease.[28,29] DR and albuminuria also reflect systemic endothelial dysfunction.[30] These conditions share common pathogenic processes such as endothelial dysfunction, thickening of basement membrane, and chronic low-grade inflammation, resulting in retinal and renal vascular damages.[28,31]

Among the VEGF family, VEGF-A is a key regulator of angiogenesis and vascular hyperpermeability, which play a main role in the pathogenesis of DR. VEGF is synthesized and released mainly by retinal Müller cells in response to hypoxia, and the vitreous levels of VEGF were markedly increased in patients with PDR and other ischemic retinal diseases.[32–34] Anti-VEGF agents have been shown to be effective in the treatment of these retinal diseases via intravitreal injections. Bevacizumab and aflibercept were reported to suppress the concentration of plasma-free VEGF, which was lowest at 1 week after injection.[6] There has always been a concern about the possible systemic effects of intravitreal anti-VEGF injections on systemic VEGF levels, especially in the kidney, owing to the high expression of VEGF and its receptors. VEGF is expressed mainly by podocytes in the kidney, and it regulates the functions of glomerular capillary cells and tubular endothelial cells through bidirectional communication.[35] VEGF plays an important role in the homeostasis of renal physiology, and there are many similarities in the aspects of developmental and structural features between retinal pigment epithelia and podocytes of the kidney.[36]

In an experimental study, upregulation of VEGF and its receptors contributed to early diabetic renal dysfunction, and anti-VEGF treatment reduced albuminuria and hyperfiltration in rats with diabetes, whereas no such effect was observed in control rats.[37] Our study showed that there were more patients with abnormal albuminuria among patients with diabetes than in those without diabetes at baseline, whereas no significant change was noted in any of the groups after 1 week of IVB injection. All patients without diabetes and 92% of those with diabetes showed no aggravation of albuminuria, which suggests the safety of IVB. However, a tendency of worsening albuminuria, that is, residual increase in UACR, was noted with higher baseline UACR, irrespective of whether the patient had diabetes. This suggests that patients with preexisting renal dysfunction have a relatively higher risk of worsening albuminuria. Although IVB injection is generally safe in absolute change of albuminuria, caution is required in patients with impaired kidney function. A recent case series reported that patients with diabetes showed aggravated proteinuria after multiple IVB injections.[14] Comparing the results of that study with those of the current study, aggravation of proteinuria was commonly observed in patients with abnormal proteinuria and UACR at baseline. The blood pressure of the patients was unaffected by IVB injection in the study by Hanna et al.[14] and in the previous study conducted by our group.[38]

Glomerular endothelial damage under hyperglycemia leads to podocyte damage, and subsequent podocyte loss further exacerbates endothelial injury, forming a vicious cycle of diabetic renal dysfunction.[36,39] Early DN is characterized by glomerular hypertrophy and hyperfiltration maintained by increased levels of VEGF and VEGF receptors, followed by thickening of glomerular basement membrane and mesangial expansion.[37] Anti-VEGF therapy might be protective at this early stage, that is, in the A1 group in this study, because 25% of patients with diabetes showed residual decrease of UACR. Meanwhile, more advanced DN is typically associated with increased albuminuria that progresses to glomerular sclerosis, with decreased level of VEGF owing to podocyte loss.[37] When vascular rarefication and renal fibrosis result in advanced DN, VEGF levels and receptor activation are diminished in the glomeruli.[40] In this advanced stage, that is, in the A3 group of this study, anti-VEGF therapy may have suppressed the already-diminished VEGF levels so that 25% of patients with diabetes showed residual increase of UACR.

Although this study had a prospective design, the small number of included patients is the major limitation of this study. Although we fulfilled the required sample size for patients with DM and control groups, the number was small for the analysis between NPDR and PDR groups. Second, UACR was calculated by urine testing performed once in clinical practice, and urine samples of pre-IVB and post-IVB might

not have been collected at similar hours of the day. This might lead to less-accurate results than those obtained through analysis of urine samples collected for 24 hours, which help prevent diurnal variation. We attempted to perform urine tests of each patient by making visits at similar times, if possible, to reduce this bias. Third, we were unable to determine specific levels of severity of DN owing to lack of data regarding estimated glomerular filtration rate. This should be evaluated in further studies with serum creatinine levels. Fourth, the possibility of any undiagnosed systemic diseases might exist in the control group, especially in those with retinal vein occlusions. However, the presence of long-lasting undiagnosed diabetes or hypertension affecting renal function in the control group is highly unlikely, as the National Health Insurance Service of Korea which covers 98% of the whole population undergoes a national health screening examination every 2 years in all insured individuals aged ≥ 40 years.[41] Last, bevacizumab appeared to accumulate in the system with repeated dosing, while only a single intravitreal injection of bevacizumab was investigated in this study. A further prospective study on the effect of proteinuria when repeated injections of bevacizumab are administered in patients with diabetes is required.

In conclusion, close monitoring of renal function after IVB might be needed according to the severity of DN. Although IVB injection is not harmful in the majority of patients with diabetes, patients with poor pre-existing renal dysfunction still have a relatively higher risk of worsening albuminuria.

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