Proteinuria as a Potential Biomarker for the Efficacy of Intravitreal Bevacizumab Injection in Patients with Diabetic Macular Edema

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Purpose: Anti-vascular endothelial growth factor (anti-VEGF) agents are good therapeutic options for diabetic macular edema (DME), especially in patients with DME that is refractory or shows delayed response to anti-VEGF treatment. We investigated the change of proteinuria following intravitreal bevacizumab injection (IVB) as a potential biomarker for treatment-responsiveness in patients with DME.

Methods: This pilot study was performed as secondary analysis of patients with diabetic retinopathy (DR) from prospectively enrolled patients scheduled for IVB from May 2018 to December 2018. Fifty-three patients with DR (30 with DME and 23 without DME) were initially included, and 46 eyes were finally included for analysis after propensity score matching. Urine tests were performed within 1 month before and 7 ± 1 days after IVB. The concentrations of urine protein and albumin were quantitatively measured, and the urinary albumin-to-creatinine ratio (UACR) was calculated from data before and after IVB.

Results: There were no significant differences in mean concentrations of urine albumin and protein between patients with and without DME. More patients in the DME group showed abnormal level of albuminuria both before and after IVB, but these differences were not statistically significant between the groups. A correlation analysis revealed no significant association between change in UACR and that of central retinal thickness.

Conclusion: Change in proteinuria was not a proper biomarker of the response to IVB in patients with DME.

Keywords: Albuminuria; Bevacizumab; Diabetic macular edema; Proteinuria
Introduction

Diabetic nephropathy (DN) and diabetic retinopathy (DR) are microvascular complications of diabetes that share a common pathogenesis. Previous studies have suggested a close association between DN and DR. They also share common pathogenic processes, such as endothelial dysfunction, thickening of the basement membrane, and chronic low-grade inflammation, which result in retinal and renal vascular damage [1,2]. Albuminuria and proteinuria are independent risk factors for cardiovascular morbidity or all-cause mortality regardless of diabetes [3,4], while they also appear to be associated with progression of DR [5-7]. A significant association has been reported between DN and DR [8,9], while DN did not markedly affect development of diabetic macular edema (DME) [10].

DME is one of the main causes of chronic visual impairment in patients with DR. The incidence of DME is associated with multiple systemic conditions, including poor glycemic control, elevated blood pressure, and dyslipidemia [11]. Vascular hyperpermeability from blood-retinal barrier (BRB) breakdown is a well-known pathogenesis of DME, followed by increased levels of vascular endothelial growth factor (VEGF) and ischemia [12]. Intravitreal injection of anti-VEGF agents has been widely used to treat DME, showing improvement of visual acuity in practice [13]. Intravitreal injection usually delivers a small amount of anti-VEGF agent into the vitreous body; this small amount can suppress plasma-free VEGF level and may have systemic effects in addition to local ocular effects [14].

A recent study identified a significant association between DN and DME [15], and there are also reports that impaired renal function is not associated with DME [16,17]. Urinary albumin-to-creatinine ratio (UACR) calculated from a random urine sample has been introduced as a marker with a good correlation to absolute urinary protein or albumin excretion collected over 24 hours [18,19]. The UACR can be measured in a random urine sample, which is easy to obtain in outpatient clinics, and is currently recommended for initial evaluation of proteinuria [20].

While anti-VEGF agents are good therapeutic options for DME, there are some patients with DME that is refractory or shows delayed response to anti-VEGF treatment [21,22]. However, there is currently no definite systemic biomarker that can be obtained non-invasively to identify these non-responders. The presence of DR in diabetic patients suggests that microvascular complications have manifested clinically. We hypothesized that change of proteinuria following intravitreal bevacizumab injection (IVB) might be different in patients with DME versus those without DME, as DME is the hallmark of BRB breakdown [23]. If there is any association between proteinuria and DME, then the potential of proteinuria as a biomarker for response of DME to IVB needs to be evaluated. Accordingly, we investigated the effect of IVB on proteinuria using UACR in patients with DME, along with the association of central retinal thickness change and UACR.

Materials and Methods

This study was performed as secondary analysis of patients with DR from prospectively enrolled patients scheduled for IVB (Avastin®, Hoffmann-La Roche Ltd., Basel, Switzerland) due to various retinal diseases from 1 May 2018 to 31 December 2018. This study was approved by the Institutional Review Board of Ajou University Hospital, Suwon, Korea (IRB No. AJIRB-BMR-OBS-18-035) and complied with the Declaration of Helsinki. Written informed consent was obtained from all patients. Exclusion criteria were as follows: 1) age < 20 years; 2) history of vitrectomy; 3) prior intravitreal anti-VEGF injection within six months of the time of inclusion; 4) preexisting kidney disease or current dialysis, 5) withdrawn consent; and 6) loss to follow-up.

Medical history, clinical demographics, and information about current medications were obtained from the patients at the time of inclusion. Systolic and diastolic blood pressure values were also measured at each visit. Hemoglobin A1c (HbA1c) data from the past three months were collected, and grade of DR was assessed using fundus photographs and fluorescein angiography findings. IVB was administered as 1.25 mg/0.05 mL by one of three participating retinal specialists (Y.R.C., Y.H.K., or K.L.). DME was identified on optical coherence tomography (OCT). A Heidelberg Spectralis OCT device (Heidelberg Engineering, Heidelberg, Germany) was used for all measurements. Central retinal thickness (CRT) was measured as the distance from the hyperreflective line of the internal limiting membrane to the hyperreflective line of the retinal pigment epithelium/Bruch’s membrane complex [24] and was obtained both before and after IVB. The
retinal response to IVB in patients with DME was examined with OCT after five or six weeks of IVB. Among those with DME, an IVB responder was defined as one showing CRT reduction > 25% from baseline; others were classified as non-responders [25].

Random urine samples were collected before and after 7 ± 1 days of IVB, based on the report that bevacizumab injections show prominent suppression of plasma-free VEGF concentration at 1 week after injection [26]. Urine protein, albumin, and creatinine concentrations were measured, and UACR was calculated from these measurements using the following formula: UACR (mg/g) = urine albumin (mg/dL)/creatinine (g/dL).

UACR was categorized into three groups as follows: A1 (< 30 mg/g), A2 (≥ 30 and ≤ 300 mg/g), and A3 (> 300 mg/g), according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 clinical practice guidelines [20]. As patients with a higher value at baseline tended to have smaller changes on subsequent measurements, and vice versa (i.e., regression to the mean), we used the categorical analyses from the study by Jun et al. [27].

Table 1. Baseline characteristics of included patients with diabetes

| Variable                      | DME (n = 30) | No DME (n = 23) | p-value |
|-------------------------------|--------------|-----------------|---------|
| Age (years)                   | 59.1 ± 10.7  | 53.4 ± 9.9      | 0.050†  |
| Male (sex)                    | 20 (67)      | 9 (39)          | 0.046†  |
| HbA1c (%)                     | 8.0 ± 1.8    | 9.1 ± 2.2       | 0.055‡  |
| Medications                   |              |                 |         |
| ACEI/ARB                      | 11 (37)      | 10 (43)         | 0.716‡  |
| β-Blocker                     | 5 (17)       | 2 (9)           | 0.353†  |
| CCB                           | 11 (37)      | 8 (35)          | 0.770‡  |
| Diuretics                     | 4 (13)       | 4 (17)          | 0.740‡  |
| Statins                       | 13 (43)      | 13 (57)         | 0.414†  |
| Antidiabetic medications      |              |                 |         |
| Metformin                     | 18 (60)      | 15 (65)         | 0.870†  |
| Sulfonylurea                  | 10 (33)      | 12 (52)         | 0.202†  |
| SGLT-2 inhibitor              | 2 (7)        | 1 (4)           | 0.683†  |
| DPP-4 inhibitor               | 15 (50)      | 14 (61)         | 0.529†  |
| Thiazolidinedione             | 2 (7)        | 4 (17)          | 0.246†  |
| Insulin                       | 10 (33)      | 12 (52)         | 0.202†  |
| Systolic BP (mmHg)            | 136.3 ± 21.6 | 134.1 ± 20.5    | 0.781†  |
| Diastolic BP (mmHg)           | 75.6 ± 11.9  | 79.6 ± 13.3     | 0.285†  |
| p-value                       |              |                 |         |
| Geometric                     |              |                 |         |
| Pre-IVB lab                   | 109.1 (56.3-211.3) | 78.2 (35.5-172.3) | 0.473* |
| Post-IVB lab                  | 119.4 (65.4-217.7) | 81.9 (34.9-192.4) | 0.430* |
| p-value‡                      | 0.441        | 0.693           |         |
| Abnormal albuminuria‡          |              |                 |         |
| Pre-IVB                       | 21 (70)      | 15 (65)         | 0.712‡  |
| Post-IVB                      | 22 (73)      | 15 (65)         | 0.524‡  |

Values are presented as mean ± standard deviation unless otherwise indicated.

DME = diabetic macular edema; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; β-blocker = β-adrenergic receptor-blocker; CCB = calcium channel blocker; SGLT-2 = sodium glucose cotransporter-2; DPP-4 = dipeptidyl peptidase-4; BP = blood pressure; NPDR/PDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

Table 2. Urine analysis of proteinuria before and after IVB

| Variable (mg/dL) | DME (n = 30) | No DME (n = 23) | p-value* |
|------------------|--------------|-----------------|---------|
| Pre-IVB lab      |              |                 |         |
| Microalbumin     | 25.1 ± 51.1  | 20.9 ± 31.1     | 0.597   |
| Protein          | 48.9 ± 87.4  | 35.3 ± 37.4     | 0.971   |
| Creatinine       | 95.1 ± 75.1  | 81.9 ± 56.5     | 0.615   |
| Post-IVB lab     |              |                 |         |
| Microalbumin     | 22.1 ± 38.8  | 21.1 ± 33.8     | 0.399   |
| Protein          | 35.6 ± 47.9  | 30.4 ± 34.0     | 0.712   |
| Creatinine       | 80.9 ± 53.2  | 70.5 ± 49.7     | 0.419   |

Values are presented as mean ± standard deviation unless otherwise indicated.

IVB = intravitreal bevacizumab injection; DME = diabetic macular edema.

Table 3. Change in UACR both before and after IVB

| Variable                | DME (n = 30) | No DME (n = 23) | p-value† |
|-------------------------|--------------|-----------------|---------|
| Geometric               |              |                 |         |
| Pre-IVB                 | 0.441        | 0.693           |         |
| Abnormal albuminuria‡   |              |                 |         |
| Pre-IVB                 | 21 (70)      | 15 (65)         | 0.712‡  |
| Post-IVB                | 22 (73)      | 15 (65)         | 0.524‡  |

Values are presented as mean (95% confidence interval) or number (%).

UACR = urinary albumin-to-creatinine ratio; DME = diabetic macular edema; IVB = intravitreal bevacizumab injection.

†Mann-Whitney U test; †Wilcoxon signed rank test comparing pre-IVB and post-IVB data; ‡abnormal range of UACR (≥ 30 mg/g); §chi-square test.
All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS, IBM Corp., Armonk, NY, USA).

Table 4. Categorical distribution of UACR both before and after IVB

| Variable (mg/dL) | DME (n = 30) | No DME (n = 23) | p-value* |
|-----------------|-------------|----------------|---------|
| Pre-IVB A1 (< 30 mg/g, normal) | 9 (30) | 8 (35) | 0.844 |
| A2 (≥ 30 and ≤ 300 mg/g) | 11 (37) | 9 (39) |
| A3 (> 300 mg/g) | 10 (33) | 6 (26) |
| Post-IVB A1 (< 30 mg/g, normal) | 8 (27) | 8 (35) | 0.796 |
| A2 (≥ 30 and ≤ 300 mg/g) | 14 (46) | 9 (39) |
| A3 (> 300 mg/g) | 8 (27) | 6 (26) |
| Categorical change† | 4 (13) | 1 (4) | 0.368 |
| Improved change | 23 (77) | 21 (82) |
| No change in category | 3 (10) | 1 (4) |

Values are presented as number (%). UACR = urinary albumin-to-creatinine ratio; IVB = intravitreal bevacizumab injection; DME = diabetic macular edema.

*Chi-square test; †improved changes were from A3 to A1 or A2 and from A2 to A1, while aggravated changes were from A1 to A2 or A3 and from A2 to A3.

Table 5. Categorical change in UACR both before and after IVB (by regression to the mean)

| Categorical change of UACR | DME (n = 30) | No DME (n = 23) | p-value* |
|---------------------------|-------------|----------------|---------|
| A1 Residual decrease | 6 (20) | 7 (30) | 0.576 |
| A1 Regression to the mean | 3 (10) | 1 (4) |
| A2 Residual decrease | 4 (13) | 3 (13) | 0.930 |
| A2 Regression to the mean | 4 (13) | 4 (17) |
| A2 Residual increase | 3 (10) | 2 (9) |
| A3 Regression to the mean | 3 (10) | 0 |
| A3 Residual increase | 7 (23) | 6 (26) | 0.250 |

Values are presented as number (%). Categorical change of UACR by baseline category is as follows: 1) for A1, residual decrease implied minor change or decrease of UACR ≥ 30%, and regression to the mean implies increase of UACR ≥ 30%; 2) for A2, residual decrease indicated decrease of UACR ≥ 30%, regression to the mean implied a minor change, and residual increase implied increase of UACR ≥ 30%; and 3) for A3, regression to the mean suggested decrease of UACR ≥ 30%, and residual increase implied increase of UACR ≥ 30% or minor change.

UACR = urinary albumin-to-creatinine ratio; IVB = intravitreal bevacizumab injection; DME = diabetic macular edema.

†Fisher’s exact test; ‡chi-square test.

Table 6. Change following IVB according to OCT-based type of DME

| Variable | Diffuse type (n = 13) | CME type (n = 8) | SRF type (n = 9) | p-value |
|----------|-----------------------|-----------------|-----------------|---------|
| CRT Pre-IVB | 388.3 ± 85.6 | 473.8 ± 290.1 | 461.8 ± 121.0 | 0.294 |
| Post-IVB | 352.9 ± 70.6 | 341.8 ± 68.7 | 285.6 ± 84.0 | 0.017 |
| p-value† | 0.017 | 0.093 | 0.017 |
| Geometric Pre-IVB UACR | 118.2 (34.8-401.5) | 117.4 (35.7-386.1) | 90.9 (22.0-375.6) | 0.093 |
| Post-IVB UACR | 144.6 (55.8-374.9) | 104.2 (26.1-415.6) | 102.0 (26.0-400.8) | 0.784 |
| p-value† | 0.279 | 0.484 | 0.374 |
| Categorical change of UACR‡ | | | | 0.685 |
| Improved change | 1 (8) | 1 (13) | 2 (22) |
| No change in category | 10 (77) | 7 (87) | 6 (67) |
| Aggravated change | 2 (15) | 0 (0) | 1 (11) |

Values are presented as mean ± standard deviation, mean (95% confidence interval) or number (%). CRT = central retinal thickness; UACR = urinary albumin-to-creatinine ratio.

*Kruskal-Wallis test; †Wilcoxon signed rank test comparing pre-IVB and post-IVB data; ‡improved changes were from A3 to A1 or A2 and from A2 to A1, while aggravated changes were from A1 to A2 or A3 and from A2 to A3; §chi-square test.
variables were compared using the chi-square test or Fisher’s exact test, and continuous variables were assessed using the Mann-Whitney U test or Kruskal-Wallis test. The Wilcoxon signed rank test was used to compare pre-IVB and post-IVB values. As UACR showed an extremely skewed distribution, log10-transformed values were calculated and used for statistical analysis. For log10 transformed data expression, the values were back-transformed to geometric mean and presented as geometric mean with 95% confidence interval. Pearson’s correlation analysis was used to analyze the association of DME and proteinuria. A p-value less than 0.05 was considered statistically significant.

**Results**

Fifty-three patients with DR (30 with DME and 23 without DME) were included in the analysis. The baseline characteristics of the included patients with or without DME are summarized in Table 1. The mean age of these participants was 59.8 ± 14.3 years, and there were more male patients in the DME group. The HbA1c level was higher, and more DME patients showed advanced DR (i.e., proliferative DR [PDR]) compared to the DME group, although this difference was not statistically significant. There were no significant differences among the other variables between groups.

In terms of proteinuria, there were no differences in mean concentrations of urine albumin and urine protein between groups with and without DME and before and after IVB (Table 2). More patients in the DME group showed abnormal level of albuminuria both before and after IVB, although the differences between groups were not statistically significant. The geometric mean of UACR both before and after IVB showed no differences between the groups, and the paired t-test revealed no difference between pre- and post-IVB UACR values within groups (Table 3). Among patients with DME, pre-IVB UACR was higher in IVB non-responders than IVB responders, but this difference was not statistically significant (128.9 mg/g vs. 68.3 mg/g, p = 0.104).

The changes in UACR according to categorical distribution are shown in Table 4. There was no difference in number of patients with newly developed albuminuria or in severity of albuminuria both before and after IVB. The categorical distribution from improvement to aggravation was not different between patients with or without DME, although the mean CRT in patients with DME was significantly reduced following IVB (446.7 ± 182.1 μm to 328.6 ± 79.0 μm, p < 0.001). The categorical distribution according to regression to the mean was not different between patients with and without DME (Table 5). However, this categorical distribution was statistically significant within either group, showing that patients with deteriorated baseline UACR were associated with greater residual increase following IVB (p < 0.05 in all groups).

Among the types of DME based on OCT-associated morphologic classification, DME eyes were classified as follows: diffuse macular edema (n = 13), cystoid macular edema (n = 8), and DME with subretinal fluid (n = 9). There was no difference in UACR or CRT pre- or post-IVB or in categorical distribution among these subtypes of DME (Table 6). In correlation analysis performed in eyes with DME, there was no significant association between change in UACR and that of central retinal thickness (r = -0.116, p = 0.589, Fig. 1).

**Discussion**

DME, which is a clinically significant complication of DR, can occur at any stage of DR [28]. In recent years, the mainstay of treatment for DME has shifted from laser photocoagulation to intravitreal anti-VEGF injections, as they are anatomically and functionally superior [28]. Among the
various types of anti-VEGF agents available for intravitreal injections, systemic exposure was higher with bevacizumab compared to ranibizumab or aflibercept [29]. Pharmacokinetic studies have confirmed systemic absorption of anti-VEGF agents after intravitreal injections, as well as an associated decrease in serum VEGF level [30]. Proteinuria is the second most common complication after hypertension following systemic administration of bevacizumab [31,32]; therefore, the systemic effects of IVB on proteinuria need to be evaluated.

DR and DN share a common pathogenesis, but there is discordance in the risk factors for progression [33]. Song et al. [33] reported that average glycemic level was associated with DR progression, whereas glycemic variability was associated with DN. In contrast, another study reported association of ratio of glycated albumin to HbA1c, an indicator reflecting fluctuations in plasma glucose, with DR but not with DN [34]. Although controversies remain, a relationship between DR and kidney function has been identified [35,36]. Comorbidity of DR and DN is common in diabetic patients; the two entities have a similar pathogenesis, including endothelial dysfunction, thickened basement membrane, and/or chronic inflammation [37].

In this study of diabetic patients, there was no significant effect of IVB on proteinuria regardless of presence of DME in patients with DR. This tendency was consistent after propensity score matching based on age, sex, glycemic control (i.e., HbA1c level), and DR severity (NPDR vs. PDR). This finding suggests a weak correlation between kidney function and DME, unlike DR. This tendency was also noted by previous studies. Impaired renal function assessed using glomerular filtration rate was associated with increased prevalence of DR, severe DR, and severe visual impairment, while no significance was noted with DME [16,17]. Similarly, secondary analysis of a randomized clinical trial comparing different anti-VEGF agents reported no treatment group differences in UACR as a reflection of kidney function in patients with DME [38].

Furthermore, the finding of no definite association between renal function and DME followed by IVB suggests that change in proteinuria may not be a proper biomarker for DME or its anatomic response to IVB. A recent prospective study assessing serologic inflammatory factors as biomarkers for DME by enzyme-linked immunosorbent assay (ELISA) kits reported that serum high-sensitivity C-reactive protein (hsCRP) and intercellular adhesion molecule 1 levels were associated with early response to IVB (mean number of IVB injections: 4.8 ± 0.6), while serum VEGF level was associated with 6-month CRT change [39]. The previously mentioned study suggested hsCRP and IACM1 as significant biomarkers for response to anti-VEGF treatment [39]. However, these measurements require blood sampling from patients as well as ELISA kits for evaluation, which may be invasive and is inconvenient for clinical practice. We tried to identify the clinical significance of UACR as a biomarker for DME due to its non-invasive and convenient method of collection from patients, but these results suggest that UACR was not a proper biomarker in DME. Although not statistically significant, pre-IVB UACR was higher in those with DME, and these patients did not respond as well to IVB. However, this result suggests the potential of UACR as a systemic biomarker in DME, which may need further investigation with a larger number of patients.

Investigations on renal function and retinal thickness have been mostly limited to patients with end-stage renal disease who are on dialysis. Studies of change in OCT findings related to hemodialysis have reported decreased retinal thickness with improved DME without treatment [40], while others found no effect of hemodialysis in diabetic patients with or without DME [41]. In this study, we excluded patients undergoing dialysis, as renal function cannot be correctly assessed in these patients; in addition, no correlation existed between renal function and central retinal thickness. Furthermore, IVB did not affect any change in albuminuria, from improvement to aggravation, regardless of presence of DME. However, a real tendency of worsening albuminuria (i.e., residual increase in UACR) was noted with higher baseline UACR with or without DME. This finding suggests a relatively higher risk of worsening albuminuria for those with preexisting renal dysfunction. Although generally safe in cases with absolute change in albuminuria, caution is needed in patients with impaired kidney function. Taken together, these findings suggest that IVB is safe in terms of proteinuria without affecting retinal thickness in diabetic patients, which is a possible complication following systemic release of bevacizumab.

The small number of included patients was the major limitation of this study despite its prospective design. This study investigated only the effect of a single IVB, while repeated treatments are often needed in clinical practice. Additional-
ly, UACR was calculated with a one-time random urine test performed in a clinical setting, so the urine samples for pre- and post-IVB might not have been collected during similar times of day. This method might therefore be less accurate than a 24-hour urine collection. To reduce this bias, we tried to perform urine tests in each patient at the same time of day by scheduling their clinic visits at similar times when possible.

In conclusion, proteinuria was not affected by IVB regardless of presence of DME in patients with DR. IVB can be safely used in patients with DR regardless of baseline renal function. However, UACR was not a proper biomarker for the effect of IVB in DME.

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Conflicts of Interest
All authors have no potential conflicts of interest to disclose.

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