Endocan and albuminuria in type 2 diabetes mellitus

Mehmet Ali Cikrikcioglu\textsuperscript{a}, Zeynep Erturk\textsuperscript{a}, Elif Kilic\textsuperscript{b}, Kenan Celik\textsuperscript{a}, Iskender Ekinci\textsuperscript{a}, Ayse Irem Yasin Cetin\textsuperscript{a}, Tuba Ozkan\textsuperscript{a}, Guven Cetin\textsuperscript{c}, Shute Ailia Daea\textsuperscript{a}, Rumeyza Kazancioglu\textsuperscript{d} and Reha Erkoc\textsuperscript{d}

\textsuperscript{a}Department of Internal Medicine, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey; \textsuperscript{b}Department of Biochemistry, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey; \textsuperscript{c}Department of Internal Medicine, Division of Hematology, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey; \textsuperscript{d}Department of Internal Medicine, Division of Nephrology, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey

\textbf{ABSTRACT}

\textbf{Background:} Endocan is a newly identified proteoglycan released from endothelium, stimulating angiogenesis and when increased, indicates endothelial activation (inflammation). Our aim was to examine the association between serum endocan levels and urine albumin-creatinine ratio (UACR).

\textbf{Method:} One hundred and thirty-seven patients with type 2 diabetes mellitus and normal serum creatinine who had no co-morbidities other than hypertension, diabetic nephropathy, retinopathy, or neuropathy were divided into normoalbuminuria (G1), microalbuminuria (G2), and macroalbuminuria (G3) groups and compared cross-sectionally regarding serum endocan levels.

\textbf{Result:} There were 55, 47, and 35 patients in G1, G2, and G3, respectively. The groups were comparable in terms of gender, age, duration of diabetes, diabetic neuropathy/retinopathy, fasting glucose, HbA1c, serum creatinine level, and eGFR. Patients in G3 had significantly higher blood pressure but lower serum albumin and endocan levels. UACR showed a negative bivariate correlation with serum endocan levels ($r = -0.282$, $p = 0.001$). There was bivariate positive correlation between endocan and systolic blood pressure ($r = 0.185$, $p = 0.030$). In linear regression analysis, UACR was negatively correlated with endocan while positively correlated with systolic blood pressure, duration of diabetes, and platelet distribution width.

\textbf{Conclusion:} Patients with macroalbuminuria had lower endocan levels, and increasing UACR was associated with decreasing serum endocan levels. Despite the occurrence of angiogenesis and glomerular hypertrophy in the early phase of diabetic nephropathy, ensuing significant renal injury over time may reduce the expression of endocan. Serum endocan levels may represent a novel marker for nephropathy progression.

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\textbf{Introduction}

Recent studies have shown that vascular endothelium plays a fundamental role in processes such as inflammation, coagulation, angiogenesis, and tumor invasion, through the release of a variety of mediators and through receptor/ligand interactions.\textsuperscript{1} One such molecule released by the endothelial cells is endocan (endothelial cell specific molecule-1), the increased tissue expression or serum levels of which may be an indicator of endothelial activation (inflammation) and neovascularization (tumor progression).\textsuperscript{2} Patients with diabetes mellitus (DM), chronic kidney disease (CKD), acute coronary syndrome, and hypertension (HT) have been shown to have elevated serum endocan levels.\textsuperscript{3–6} In a small study, patients with type 2 diabetes were found to have higher initial serum endocan compared to controls, and regulation of diabetes with diet, exercise, and medical treatment resulted in lowered serum endocan levels and albuminuria, with a positive correlation between the degree of decrease in albuminuria and endocan.\textsuperscript{6} In another study involving patients with CKD of varying etiology and severity (stages 1–5), plasma endocan levels were positively correlated with proteinuria and negatively correlated with estimated glomerular filtration rate (eGFR).\textsuperscript{5} There are only one published report on the association between endocan and albuminuria in DM.\textsuperscript{6} In the present study, association between serum endocan levels and albuminuria was investigated in patients with type 2 DM and normal serum creatinine levels who had no co-morbidities other than HT, diabetic nephropathy, retinopathy, or neuropathy. Our aim was to establish the role of...
endocan as a marker of diabetic nephropathy similar to the case with urine albumin–creatinine ratio (UACR).

Methods
Study design
This was a prospective cross-sectional observational study.

Study population
Successful patients with DM type 2 attending to the internal medicine outpatient clinic of Bezmialem Vakif University Medical Faculty between November 2015 and February 2016 were included in this study if they met the inclusion and exclusion criteria and provided written informed consent.

Inclusion criteria
The patient group was between 18 and 75 years of age, which were newly or previously diagnosed as DM type 2 according to Criteria of American Diabetes Association.7

Exclusion criteria
Patients with the following conditions were excluded from the study: pregnancy, lactation, having serum creatinine >106.08 μmol/L, alcohol addiction, drug addiction, pituitary dysfunction, thyroid dysfunction, primary parathyroid dysfunction, adrenal dysfunction, use of oral contraceptives, use of postmenopausal hormone replacement therapy, being bed-ridden, history of major surgery, chronic or acute infections, cerebrovascular accident, ischemic heart disease, peripheral arterial disease, venous thrombosis, chronic venous insufficiency, heart failure, chronic obstructive pulmonary disease, asthma, obstructive sleep apnea syndrome, dementia syndromes, Parkinson’s disease, multiple sclerosis, inflammatory bowel disease, collagen tissue disorders, sarcoidosis, chronic hepatitis, portal HT, hemolytic anemia, hematological malignancy, solid tumors, use of corticosteroids, or immunosuppressive drugs.

Blood sampling
Venous blood sampling was carried out in the early morning after overnight fasting. CBC, routine biochemistry, 25-hydroxy vitamin D3, PTH, ferritin, and thyroid hormone measurements as well as hormone analyses were performed on the same day of sample collection. Sera were stored at −80°C for endocan assay, which was carried out in the whole group of samples at a later date.

Blood analysis
Complete blood count (CBC) analysis was performed using a Sysmex XT 1800i device (ROCHE-2011, Kobe, Japan). Biochemical analyses were carried out with a COBAS 8000 device (ROCHE-2007, Tokyo, Japan) and COBAS-C system kits. 25-hydroxy vitamin D3 levels were determined using commercial kits (ROCHE-2014, Mannheim, Germany) with a COBAS E 601 hormone analyzer (ROCHE, 2010, Tokyo, Japan). Thyroid hormone levels were determined using Advia Centaur (Advia-2013, Tarrytown, NY) kits, while parathyroid was assayed using intact parathormone kits (2014-Bayswater, Victoria, Australia). Ferritin assay was carried out using ferritin kits (2014-Bayswater, Victoria, Australia) with an Advia Centaur (2006, Dublin, Ireland) device.

Urinalysis estimated glomerular filtration rate
The first voided urine in the morning was used for routine urinalysis as well as for urine UACR and urine protein–creatinine ratio (UPCR) estimations. UACR and UPCR were measured only once and concurrently with venous blood sampling for serum endocan assay in our study. Urine albumin, urine protein, and urine creatinine were measured turbidimetrically using an ABBOTT architect 16200 (Princeton, NJ) device. In the absence of active urinary sediments, A UACR < 30 mg/g, 30 mg/g ≤ A UACR < 300, and A UACR ≥ 300 was considered as normoalbuminuria, microalbuminuria and macroalbuminuria, respectively. Estimated glomerular filtration rate was calculated using CKD-EPI formula.8

ELISA assay
Following overnight fasting, venous blood samples were collected into gel tubes. A 20-min time interval was allowed for coagulation, which was followed by centrifugation at 1500×g for 15 min. Sera stored at −80°C and on the day of the assay, concentrations of endocan in serum were measured with enzyme linked immunosorbent assay (ELISA) kit, according to protocols provided by manufacturer (Human Endocan Elisa Kit; lot no: 201506; Sunred Biological Technology, Shanghai, China). Multiskan FC® Microplate Photometer (ThermoScientific, Waltham, MA) was used for reading at 450 nm. The results were expressed in ng/L. Intra-assay coefficient of variation (CV) is smaller than 5.1%, inter-assay CV is smaller than 6.1%, sensitivity is
31.2 pg/mL, detection range is 31.2–2000 pg/mL for Endocan ELISA kit.

**Patient assessments**

From each patient, past medical history and family history were obtained. Duration of DM, smoking status, and menopausal status were recorded. A systemic physical examination was performed. Current medical treatments and comorbid conditions were determined. All patients had eye examination last year. All patients also had neurological examination for detection of polyneuropathy within last year. Blood pressure measurements were performed on the right arm in the early morning hours before blood sampling with the use a mechanic sphygmomanometer after 15 min of rest. Body mass index (BMI) was calculated using the following formula: $\text{BMI} = \frac{\text{body weight (in kg)}}{\text{height}^2 \text{ (in m)}}$.

**Statistical analyses**

Nominal variables were expressed as ratio, numeric variables were given as mean and standard deviation. Participants of the study were divided into three following groups: normoalbuminuria (group 1 = G1), microalbuminuria (group 2 = G2), and macroalbuminuria (group 3 = G3) groups. Nominal variables were compared with chi-square test among the groups. One sample Kolmogorov–Smirnov test was performed to determine if the continuous variables were normally distributed (ND). Whether a continuous variable was ND was indicated on the tables. Normally distributed independent continuous variables were compared with one-way ANOVA test, whereas non-normally distributed (NND) independent continuous variables were compared with Kruskal–Wallis test among the groups. Post hoc pairwise comparison was performed using LSD method after one-way ANOVA test but, post hoc pairwise comparison after Kruskal–Wallis test was carried out with Dunn’s test. Bivariate correlations were sought. Linear regression analysis (LRA) was carried out to show that endocan was still associated with UACR after controlling factors affecting or likely to affect UACR. A two-tailed $p$ value of <.05 was considered to be statistically significant.

**Ethics**

The study protocol was approved by Bezmialem Vakif University Medical Faculty Ethics Committee, and all participants recruited to the study provided informed consent. The study procedures were performed in accordance with 2009 Helsinki Declaration.

**Results**

The study population consisted of 137 patients; 55 patients with normoalbuminuria (G1), 47 patients with microalbuminuria (G2), and 35 patients with macroalbuminuria (G3). There were 42 male and 95 female participants. The mean age was 56.4 ± 8.4 years (range 36–75 years) and the mean BMI was 32.22 ± 6.42 kg/m² (BMI range: 16.7–62.1). The three groups were comparable in terms of age and gender, whereas BMI was significantly lower in G1 (Tables 1 and 2).

Systolic and diastolic blood pressure was significantly higher in G3 (Tables 1 and 2).

The average serum creatinine level and eGFR values in the study were 58.71 ± 11.44 μmol/L (range: 44.20–106.08) and 100.50 ± 20.85 mL/min/1.73 m² (range: 52–144), respectively. Three groups were similar in terms of serum creatinine level and eGFR (Table 1). The mean UACR and UPCR were 332.49 ± 673.44 mg/g (range 1.8–3448 mg/g) and 0.575 ± 0.897 mg/g (range 7–4470 mg/g), respectively. Three groups were significantly different with regard to UACR and UPCR (Table 1). The average fasting blood glucose was 9.43 ± 3.41 mmol/L (range: 4.50–21.59 mmol/L) and average HbA1c was 7.45 ± 1.48% (range: 5.6–14.20%). Again, no significant differences in fasting glucose and HbA1c were found between the groups (Table 3).

Frequency of acarbose and insulin use as well as the total duration insulin use was significantly higher in G3 than in G1 and G2. Dihydropyridine calcium antagonists were significantly less commonly used in G2 patients than in G3. Other than these, the groups were comparable in terms of the regular use of medications (Table 2).

Absolute blood neutrophil count, mean platelet volume (MPV), and triglyceride were significantly higher in G3 (Table 3). Serum albumin was significantly lower in G3 (Table 3).

Significantly lower serum endocan level was found in G3 than G1, endocan level was insignificantly lower in G2 than G1, and in G3 than G2 (Table 1). There was a negative bivariate correlation between UACR and serum endocan levels ($r = –.282$, $p = .001$) and between UPCR and serum endocan levels ($r = –.252$, $p = .006$). A bivariate positive correlation between endocan and systolic blood pressure was found ($r = .185$, $p = .030$).

Patients with or without retinopathy and patients with or without neuropathy were comparable in terms of serum endocan levels.
Table 1. Between-group comparisons of demographic data, blood pressures, albuminuria, proteinuria, eGFR, and endocan levels.

| Variable | Group 1 mean ± SD | Group 2 mean ± SD | Group 3 mean ± SD | Normal value | \(p\) values | Type of distribution |
|----------|-------------------|-------------------|-------------------|--------------|--------------|---------------------|
| Age      | 56.78 ± 7.48      | 54.47 ± 8.60      | 58.28 ± 9.21      | .110         | ND           | ND                  |
| BMI (kg/m^2) | 29.96 ± 6.12      | 33.28 ± 6.18      | 33.14 ± 7.90      | 17–25        | .010         | ND                  |
| Post hoc \(p\) values for BMI: G1–G2: \(p < .005\), G1–G3: \(p < .035\), G2–G3: \(p < .626\) | | | | | | |
| UACR (mg/g) | 9.77 ± 6.58       | 85.30 ± 66.31     | 1171.54 ± 911.25  | < .30        | < .001       | NND                 |
| Post hoc adjusted \(p\) values for UACR: G1–G2: \(p < .001\), G1–G3: \(p < .001\), G2–G3: \(p < .001\) | | | | | | |
| UPCR (mg/g) | 98.34 ± 42.89     | 211.72 ± 104.19   | 1615.20 ± 1124.30 | < .200       | < .001       | NND                 |
| Post hoc adjusted \(p\) values for UPCR: G1–G2: \(p < .001\), G1–G3: \(p < .001\), G2–G3: \(p < .001\) | | | | | | |
| eGFR (mL/min/1.73 m^2) | 99.85 ± 16.82     | 103.72 ± 24.03    | 97.20 ± 21.95     | > .90        | .361         | ND                  |
| Urea (mmol/L) | 11.47 ± 2.86      | 11.18 ± 3.57      | 11.79 ± 3.86      | 2.9–8.2      | .720         | ND                  |
| Creatinine (umol/L) | 66.3 ± 11.49      | 68.07 ± 15.91     | 69.84 ± 12.38     | 53–106       | .500         | ND                  |
| Endocan (ng/L) | 495.45 ± 344.82   | 450.73 ± 311.16   | 379.96 ± 189.95   | Unknown      | .029         | NND                 |
| Post hoc adjusted \(p\) values for endocan: G1–G2: \(p < .159\), G1–G3: \(p < .029\), G2–G3: \(p < .001\) | | | | | | |
| Duration of diabetes (years) | 10.15 ± 6.56      | 9.85 ± 5.91       | 13.91 ± 9.45      | .072         | ND           | ND                  |
| Duration of insulin use (years) | 2.13 ± 4.74      | 2.89 ± 3.78       | 4.37 ± 5.25       | .018         | ND           | ND                  |
| Post hoc adjusted \(p\) values for duration of insulin use: G1–G2: \(p < .019\), G1–G3: \(p < .096\) | | | | | | |
| SBP (mmHg) | 133.27 ± 24.04    | 132.12 ± 18.28    | 145.71 ± 23.42    | < .135       | .015         | ND                  |
| Post hoc adjusted \(p\) values for systolic blood pressure: G1–G2: \(p < .043\) | | | | | | |
| DBP (mmHg) | 75.54 ± 8.95      | 74.78 ± 9.60      | 79.71 ± 7.06      | < .85        | .037         | ND                  |
| Post hoc adjusted \(p\) values for diastolic blood pressure: G1–G2: \(p < .110\), G1–G3: \(p < .044\) | | | | | | |

ND: normal distribution; NND: non-normal distribution; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Demographic data, regular use of medications, and diabetic complications.

| Variable                     | G1 (n = 55) | G2 (n = 47) | G3 (n = 35) | \(p\) values |
|------------------------------|-------------|-------------|-------------|--------------|
| Male/female, no/no           | 37/18       | 33/14       | 25/10       | .905         |
| Smoker, no                   | 11/8        | 10/7        | 10/8        | .487         |
| Ex-smoker, no                | 2/7         | 6/7         | 4/7         | .676         |
| Menopause, no                | 33/24       | 24/20       | 20/12       | .212         |
| Pretibial edema, no          | 8/10        | 9/10        | 9/10        | .648         |
| Hypertensive patients, no    | 39/37       | 39/37       | 39/37       | .253         |
| Retinopathy, no              | 9/10        | 10/15       | 9/15        | .071         |
| Neuropathy, no               | 20/21       | 20/20       | 20/19       | .150         |
| ACE inhibitor or angiotensin receptor blockers, no | 29/21       | 21/21       | 21/21       | .769         |
| Thiazide, no                 | 15/10       | 8/10        | 8/10        | .764         |
| Beta-blocker, no             | 5/9         | 4/9         | 4/9         | .306         |
| Alpha-blocker, no            | 0/1         | 0/1         | 0/1         | .381         |
| Dihydropyridine CCB, no      | 7/4         | 10/4        | 10/4        | .035         |
| Oral anti diabetic, no       | 53/45       | 34/25       | 34/25       | .217         |
| Insulin, no                  | 15/20       | 20/20       | 20/20       | .017         |
| Fenoibrate                   | 4/5         | 2/5         | 2/5         | .387         |
| Statin                       | 6/4         | 1/4         | 1/4         | .694         |
| Acetyl salicylic acid        | 29/26       | 25/26       | 25/26       | .187         |
| Gabapentin or pregabalgin    | 12/10       | 12/12       | 12/12       | .209         |
| Alpha lipoic acid            | 11/12       | 12/10       | 10/12       | .625         |

No bivariate correlation was found between serum endocan level and duration of diabetes, BMI, diastolic blood pressure, serum creatinine, eGFR, erythrocyte sedimentation rate (ESR), sensitive C-reactive protein (sCRP), HbA1c, fasting glucose, HDL-cholesterol, LDL-cholesterol, ferritin, transferrin saturation rate (TSR), leukocyte count, absolute blood neutrophil count, MPV, platelet count, platelet distribution width (PDW), red cell distribution width (RDW), and 25 OH vitamin D3.

In LRA, UACR was a dependent variable and 30 independent variables were used. These independent variables were as follows: age, gender, BMI, duration of DM type 2, retinopathy, neuropathy, systolic blood pressure, diastolic blood pressure, use of diihydropyridine calcium channel blocker, use of ACE inhibitor or angiotensin receptor blocker, insulin use, duration of insulin use, statin use, fenofibrate use, smoking, being an ex-smoker, 25 OH vitamin D3, HbA1c, fasting glucose, triglycerides, HDL-cholesterol, LDL-cholesterol, eGFR, sodium, absolute neutrophil count, sCRP, MPV, PDW, RDW, and endocan. There were four independent variables in total with a significant association with UACR, according to LRA.

Duration of DM type 2, systolic blood pressure, and PDW correlated positively with UACR, while endocan was negatively correlated with UACR (Table 4).

Discussion

Surprisingly, patients with macroalbuminuria had significantly lower serum endocan levels than normoalbuminuric subjects in our study. Furthermore, when UACR increased, we observed that serum endocan levels decreased. In the only previous study examining endocan in type 2 diabetics, endocan showed a parallel increase with increased UACR and a parallel decrease with lowering UACR. In another study with no diabetic or non-diabetic distinction in CKD patients, again plasma endocan showed a positive correlation with proteinuria. Also, despite the reported strong negative correlation between endocan and GFR in CKD, there was no such correlation between endocan and serum creatinine level as well as eGFR.

In early phases of diabetic nephropathy, hyperglycemia leads to abnormal angiogenesis and glomerular hypertrophy either directly or through stimulation of vascular endothelial growth factor (VEGF-A). VEGF-A is known to be a potent endothelial cell mitogen.
Furthermore, it is both an angiogenic factor and enhancer of vascular permeability. On the other hand, in diabetic patients renal injury may ensue as a result of polyol pathway activation, renin–angiotensin system activation, reactive oxygen species (ROS) production and advanced glycation end-products (AGE). With increasing renal injury, VEGF expression is reduced and further angiogenesis cannot be achieved.

In diabetic nephropathy, VEGF-A was shown to correlate negatively with the renal injury. In severely injured glomeruli as confirmed by the loss of endothelial cells and reduction in podocyte markers (WT1, nephrin, and podocin mRNA), a 2.5-fold decrease in VEGF-A expression has been detected. When one considers the fact that podocyte and tubular cells represent the primary source of VEGF-A, the reduced VEGF-A expression in diabetic nephropathy may explain the impaired angiogenic response.

### Table 3. Comparison of complete blood count and biochemistry data between three groups.

| Variable            | G1 mean ± SD | G2 mean ± SD | G3 mean ± SD | Normal value | p Values | Type of distribution |
|---------------------|--------------|--------------|--------------|--------------|----------|----------------------|
| Glucose, mmol/L     | 9.25 ± 3.50  | 9.77 ± 3.63  | 9.24 ± 3.01  | 3.9–6.1      | .700     | ND                   |
| HbA1c               | 0.07 ± 0.02  | 0.08 ± 0.02  | 0.07 ± 0.01  | 0.04–0.07    | .737     | ND                   |
| Total protein, g/L  | 73.20 ± 5.10 | 75.10 ± 3.90 | 73.60 ± 4.80 | 60–80        | .120     | ND                   |
| Albumin, g/L        | 43.20 ± 2.50 | 44 ± 2.10    | 42.20 ± 2.50 | 35–50        | .003     | NND                  |

Post hoc adjusted p values for albumin: G1–G2 p: .436, G1–G3 p: .082, G2–G3 p: .002

| AST, µkat/L         | 0.39 ± 0.27  | 0.31 ± 0.15  | 0.30 ± 0.12  | 0.17–0.51    | .059     | NND                  |
| ALT, µkat/L         | 0.52 ± 0.41  | 0.42 ± 0.30  | 0.38 ± 0.18  | 0.17–0.68    | .200     | NND                  |
| ALP, µkat/L         | 1.34 ± 0.39  | 85.49 ± 22.23| 1.43 ± 0.46  | 0.5–2.0      | .440     | ND                   |
| GGT, µkat/L         | 0.53 ± 0.59  | 0.55 ± 0.40  | 0.52 ± 26.78 | 0.03–0.51    | .379     | NND                  |
| Calcium, mmol/L     | 2.43 ± 0.07  | 2.45 ± 0.07  | 2.41 ± 0.07  | 2.05–2.55    | .050     | NND                  |
| Phosphorus, mmol/L  | 1.19 ± 0.17  | 1.33 ± 0.19  | 1.19 ± 0.15  | 0.74–1.52    | .061     | NND                  |
| Ferritin, pmol/L    | 132.60 ± 119.05 | 126.62 ± 172.28 | 120.08 ± 84.02 | 33.450 .900 ND |
| TSH, mIU/L          | 1.66 ± 0.99  | 1.72 ± 0.90  | 1.93 ± 0.89  | 0.4–4.2      | .400     | ND                   |
| Vitamin B12, pmol/L | 347.28 ± 252.48 | 291.17 ± 151.62 | 312.64 ± 179.76 | 118–701     | .617     | NND                  |
| Potassium, mmol/L   | 4.18 ± 0.62  | 17.11 ± 7.64 | 7.36 ± 0.45  | 3.2–8.3      | .390     | NND                  |
| Sodium, mmol/L      | 139.96 ± 2.30 | 139.14 ± 1.96 | 139.44 ± 2.47 | 136–150     | .205     | ND                   |
| HDL, mmol/L         | 1.34 ± 0.33  | 1.25 ± 0.25  | 1.24 ± 0.18  | 1.0–2.7      | .032     | NND                  |

### Table 4. UACR as the dependent variable in LRA.

| Independent variables | Unstandardized coefficients | Standardized coefficients | t | Sig. |
|-----------------------|-----------------------------|---------------------------|---|------|
| (Constant)            | −2054.150                   | 762.131                   | −2.695 | .008 |
| Duration of diabetes  | 20.683                      | 7.721                     | .219  | .679 |
| Systolic blood pressure| 6.793                       | 2.475                     | .227  | .745 |
| Endocan               | −392                        | 1.83                      | −1.16 | .237 |
| PDW (platelet distribution width) | 79.276                     | 39.100                    | .166  | .028 |

R² = .157, adjusted R² = .130; F: 5.898.
renal injury may be accounted for by the loss of the ability of these cells to secrete VEGF-A. In some other studies, a decreased VEGF-A expression in the sclerotic renal areas as well as in the nodular lesions of the diabetic nephropathy has been found. Decrease in VEGF-A expression may lead to further renal injury, resulting in a more marked loss of renal functions, producing a vicious cycle. In line with these observations, intraocular anti-VEGF treatment was reported to cause acute renal failure in diabetic patients.

The lower endocan level in macroalbuminuric patients than in normoalbuminurics in our study can be probably explained on the basis of the mechanism explained above, since VEGF is the most important molecule that increases endocan expression. Endocan itself is a strong stimulator of angiogenesis. Diabetics were found to have higher endocan levels than non-diabetics, both before and after regulation of the diabetes. This may be due to the stimulation of endocan release by hyperglycemia via VEGF. In early phase diabetic nephropathy, endocan may be elevated, but once nephropathy progresses leading to severe renal injury, that is, during the proteinuria phase, serum endocan levels may be lowered due to the reduced VEGF release. In our study, despite similar fasting blood glucose and HbA1c between the groups, patients in group 3 had lower endocan, since this group was supposed to include the patients with most severe renal injury. Increasing endocan levels with increasing blood pressure has been demonstrated in another study, similar to the positive correlation between endocan and blood pressure in our study. Interestingly, although patients in group 3 had higher blood pressure, endocan levels were lower in group 3. In the only study examining relationship between endocan and albuminuria in diabetic patients, the participants were not subgrouped based on the severity of the renal damage; thus, since most patients were in the early phase of diabetic nephropathy, endocan was initially higher due to hyperglycemia before regulation of diabetes, with a subsequent decrease in serum endocan with correction of hyperglycemia. This observation may be explained on the basis of the increased VEGF production with hyperglycemia leading to increased endocan release, followed by a reduced VEGF and serum endocan after hyperglycemia was treated. Of course, during this phase albuminuria may also be reduced. Therefore, in contrast with our findings, a positive correlation between UACR and endocan could have been detected. Patients with microalbuminuria in our study had insignificantly lower endocan than patients with normoalbuminuria. Since renal injury occurs when early phase diabetic nephropathy proceeds to the microalbuminuric phase, endocan release may also have been lowered. The absence of a statistical significance between G1 and G2 may be due to the fact that some proportion of the patients in G2 (i.e., microalbuminuria group) could have microalbuminuria independent of the diabetic nephropathy. Serum endocan levels in G3 were also insignificantly lower than G2. This may be due to low number of patients in the macroalbuminuric group. Furthermore, the ratio of patients using dihydropyridine calcium antagonists in G2 was lower compared to that of G3 which may increase UACR in G2. Therefore, serum endocan levels in G2 may have been found lower, thus statistical significance between G2 and G3 according to serum endocan level might disappear. Also, since UACR was measured only once, some macroalbuminuric patients may have been classified as microalbuminuric and vice versa. Therefore, we may have failed to detect the statistical difference in all post hoc pairwise comparisons with respect to endocan between three groups.

The results of LRA, endocan was still associated with UACR after controlling factors affecting or potentially affecting UACR. Duration of DM, systolic blood pressure, and PDW were other factors which were associated with UACR. Association between these three factors and diabetic nephropathy is already known. Endocan was emerged as a new factor associated with diabetic nephropathy. As known, only an association between UACR or diabetic nephropathy and these four independent variables can be mentioned. These independent variables may worsen or lead to the development of diabetic nephropathy, or they may represent a subsequent result of diabetic nephropathy. One has to propose different mechanisms to explain the association between diabetic nephropathy and each of these variables, in accordance with statistical principles. Diabetic nephropathy is probably a multi-factorial pathology. Therefore, endocan may be considered to represent another distinct factor that leads to the vicious cycle of progressively worsened diabetic nephropathy.

The negative association of endocan with UACR may have certain practical implications. Although albuminuria is the cornerstone of the diagnosis of diabetic nephropathy, serum endocan levels may have a utility in the detection of diabetic nephropathy even before the development of micro- or macro-albuminuria. Occurrence of progressive lowering of serum endocan in a patient with diabetes may signify a worsening in diabetic nephropathy. Therefore, serum endocan level may be measured along with UACR in the follow up of diabetic patients, and when there is a progressive decline in serum endocan in the absence of other factors that may impact serum endocan, these patients may be included in a more intensive therapy program.
with early initiation of protective strategies against diabetic nephropathy.

In conclusion, macroalbuminuric patients had significantly lower endocan levels as compared to those with normoalbuminuria. Since endocan is an angiogenic molecule, it may be produced excessively in the early phase of diabetic nephropathy followed by a decline in its secretion in more advanced nephropathy. Therefore, endocan may have role as a marker in the monitoring of the progression of diabetic nephropathy.

Disclosure statement
All authors declare that they have no conflict of interest.

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