Evaluation of serum endocan as a marker of diabetic nephropathy

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Introduction
Diabetic nephropathy (DN) is a significant medical problem because of its increasing incidence, morbidity and mortality. DN is a microvascular complication of diabetes mellitus (DM) that has been observed in 30%–40% of type 1 DM and 10%–20% of type 2 DM patients. Recent studies focus on novel diagnosis and treatment strategies for DN to decrease its mortality and morbidity. New biomarkers such as endocan are considered to be associated with endothelial dysfunction, angiogenesis and inflammation and may be reliable markers for early detection and progression of DN.

Aim
This study aimed to establish the role of endocan as a marker of DN similar to the case with the urine albumin–creatinine ratio.

Patients and methods
This study has been carried out on 60 diabetic patients selected from the inpatient department and outpatient clinics of the Department of Internal Medicine in Dikirnis General Hospital and 30 healthy controls who fulfilled the inclusion and exclusion criteria. The selected participants were divided into three groups: group 1 included 30 healthy controls, group 2 included 30 diabetic patients with normoalbuminuria and group 3 included 30 diabetic patients with microalbuminuria or macroalbuminuria.

Results
In this study, there was no correlation between endocan and serum creatinine levels as well as estimated glomerular filtration rate in diabetic patients with proteinuria. Patients with microalbuminuria in this study had insignificantly lower endocan levels (111.9±85.7) than patients with normoalbuminuria (130.7±76.3).

Conclusion
Here, in this study, serum endocan did not have considerable specificity or sensitivity in early detection or progression of DN.

Keywords:
diabetic nephropathy, serum endocan, urine albumin–creatinine ratio

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Authors' contributions: Amr Elprawy: (1st author): data collection from the case group and the control group, and contributed to writing. Mohamad s.Abd Alhamid Aladlany contributed to writing contributed to the clinical part of the research, statistical analysis and data reviewing and interpretation. He is the main supervisor of the work. Mohamed Ali Atwa: responsible for the laboratory part of the research, contributed to writing and data interpretation. Rania Bahriz: (Corresponding author): gave the idea of the work, contributed to writing (main role), contributed to the clinical part, and follow-up of patients and responsible for statistical analysis, and data reviewing and interpretation.

Background

Diabetes mellitus (DM) may cause severe microvascular and macrovascular complications that impair the quality of life of diabetic patients [1]. The vascular complications of diabetes are classified as either microvascular (retinopathy, nephropathy and neuropathy) or macrovascular, which include coronary artery, peripheral and cerebral vascular disease [2].

Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease, which may require hemodialysis or even kidney transplantation. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt DN. This progression occurs in patients with type 1 and type 2 diabetes [3].

Among the risk factors for the progression of kidney disease and cardiovascular disease is albumin excretion in urine, which indicates kidney damage. The role of urinary albumin measurements has focused attention on the clinical need for accurate and clearly reported results. Historically, albuminuria has been defined in terms of urinary excretion of albumin per unit time, typically 24 h. The difficulty of collecting 24-h urine samples has led to surrogate measurements of the albumin excretion rate. A commonly used surrogate is the ratio of urinary concentrations of the albumin and creatinine ratio (UACR) [4].

Various methods are used to measure and define proteinuria and albuminuria [5]. The 'The National Kidney Foundation–Kidney Disease Outcomes Quality Initiative guideline for evaluation, classification, and stratification of chronic kidney disease (CKD)' recommends UACR in spot samples of urine as a better measure rather than urine protein or albumin. The rationale for this recommendation is that UACR is a more sensitive and specific measure of kidney damage [6].

Developing new biomarkers for early and appropriate detection of adverse events such as renal failure, cardiovascular events and all-cause mortality in CKD patients remains a huge challenge [7].

Diabetic kidney disease is defined by characteristic structural and functional changes. The predominant structural changes include mesangial expansion, glomerular basement membrane thickening, podocyte injury and, ultimately, glomerular sclerosis [8]. Hyperglycemia, increased advanced glycation end products, increased pro-inflammatory response, upregulation of the polyol pathway, an altered blood flow and oxidative/nitrative stress are considered to cause endothelial dysfunction and impaired angiogenesis in DN aetiology [9].

It was shown in previous studies that vascular endothelium plays a fundamental role in processes such as inflammation, coagulation, angiogenesis and tumour invasion, through the release of a variety of mediators and through receptor/ligand interactions [7]. One such molecule released by the endothelial cells is endocan. It is a novel protein encoded by endothelial cell-specific molecule-1 gene, leads to endothelial dysfuction and neovascularization [10]. In normal physiological conditions, it promotes vasodilation, protects the endothelium from migration and proliferation of inflammatory cells, and plays a central role in the regulation of inflammation-induced endothelial dysfunction [10,11].

Patients with DM, CKD, acute coronary syndrome and hypertension (HTN) have been shown to have elevated serum endocan levels [12]. In this study, the association between endocan levels and DN (variant grades) will be investigated in diabetic patients.

Aim

The aim of this study is to establish the role of serum endocan as a marker of DN similar to the case with UACR.

Procedure

Study setting

This study was carried out on 60 diabetic patients selected from the inpatient department and outpatient diabetes clinics of the Internal Medicine Department in Mansoura Specialized Medicine Hospital, Mansoura University, Egypt, and 30 healthy controls. The selected participants were divided into normoalbuminuric patients, low-grade albuminuria (microalbuminuric) patients, and high-grade albuminuria (macroalbuminuric) patients versus healthy controls.

Study period

This study was carried out from March 2019 to January 2020.

Study design

This is a case–control study and was carried out on 60 diabetic patients and 30 healthy controls.

The selected participants were divided into three groups:

(1) Group 1 included 30 healthy controls.
(2) Group 2 included 30 diabetic patients with normoalbuminuria.
(3) Group 3 included 30 diabetic patients with microalbuminuria or macroalbuminuria; microalbuminuria was defined as UACR more than 2.5 mg/mmol in men and more than 3.5 mg/mmol in women, and macroalbuminuria was defined as UACR more than 25 mg/mmol [13].

The inclusion criteria included any type 2 diabetic patient between 18 and 75 years of age, who had been newly or previously diagnosed with DM, whereas the exclusion criteria included end-stage chronic illnesses (cardiac, hepatic, pulmonary), any

malignancy, cerebrovascular accident, any degree of cognitive impairment and psychological diseases, patients with ketoacidosis, vomiting, dehydration, convulsions and pregnant and lactating women. An informed consent was obtained from all participants before participation in the study.

All patients were subjected to the following:

(1) Clinical assessment:
History taking: age, sex, duration of diabetes, smoking, presence of HTN, medication history (use of insulin, oral antidiabetic, antihypertensive) and presence or absence of specific diabetic complications.
Clinical examination: general condition assessment, cardiac, respiratory, abdominal and renal examination, including weight, height and signs of diabetic complications.
Diabetic complications were assessed using history and clinical judgment. Also, the presence or absence of neuropathy was assessed using the Neuropathy Symptom Score and the Neuropathy Disability Score [14].
(2) Laboratory assessment:
(a) This included complete blood count, glycated hemoglobin (HBA1c), fasting and 2-h postprandial plasma glucose (2hPP), liver function tests (aspartate aminotransferase, alanine aminotransferase, albumin), C-reactive protein (CRP), renal function tests (serum creatinine, serum urea), UACR and estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration equation. Serum endocan was determined using ELISA kits and the serum specimens were allowed to clot for 10–20 min at room temperature and centrifuged at 2000–3000 RPM for 20 min.

Statistical analysis

Data were analyzed using the statistical package for the social science software computer program version 22 (SPSS).

Results

Analysis of the demographic data showed that in the group of diabetic patients without proteinuria, there were 15 male patients (50%) and 15 female patients (50%), with a mean age of 51.1±8.1 years. Also, in the group of diabetic patients with proteinuria, there were 18 male patients (60%) and 12 female patients (40%), with a mean age of 52.63±10.2 years. However, the mean body mass index (BMI) was 33.6±5.9 and 33.2 ±6.6 kg/m^2, respectively. Among participants of the control group, there were 11 male patients (36.7%) and 19 female patients (63.3%), with a mean age of 50.8 ±10.2 years. In our study, only 3% of all diabetic patients were smokers and around 2% of the participants in the control group were smokers. According to the duration of DM, the group without proteinuria had diabetes for a period of 5.8 ±4.8 years, while the group with proteinuria had diabetes for a period of 7.7±7.6, showing an insignificant difference between the 2 groups. HTN was present in 5 (16.7), 11 (36.7) and 12 (40) patients, respectively, showing an insignificant difference between groups (χ^2=4.6, P=0.1) (Table 1).

Diabetes with proteinuria included both micro- and macroalbuminuria. Only 3 randomly chosen patients had macroalbuminuria in whom UACR was equal to or exceeding 2.5 mg/dl and the rest had microalbuminuria (Table 2).

Table 1 Comparison of demographic and clinical data among the studied groups

		Diabetic group		
	Control group n=30	Without proteinuria n=30	With proteinuria n=30	Significance
Age in years mean±SD	50.8±10.2	51.1±8.1	52.63±10.2	F=0.3, P=0.7
Sex No (%)				
Female	19 (63.3)	15 (50)	12 (40)	χ^2=3.3, P=0.2
Male	11 (36.7)	15 (50)	18 (60)	
BMI	30.9±5.5	33.6±5.9	33.2±6.6	F=1.8, P=0.2
Smoking history No (%)				
Smoker	2 (6.7)	–	3 (10)	P=0.4
Nonsmoker	28 (93.3)	30 (100)	27 (90)	
Medical history				
DM duration	–	5.8±4.8	7.7±7.6	Z=1.1, P=0.3
Hypertension	5 (16.7)	11 (36.7)	12 (40)	χ^2=4.6, P=0.1

BMI, body mass index; DM, diabetes mellitus. Data presented as No (%), except for age, which is presented as Mean±SD. Data in bold are statistically significant at $P\leq0.05$. χ^2, Mann–Whitney, and Monte Carlo tests were used.

In the diabetic group with proteinuria, diabetic neuropathy was more frequent (70%) compared with the diabetic group without proteinuria (40%), and this difference was statistically significant at *P*=0.02. Other diabetic complications were not frequently reported (Fig. 1).

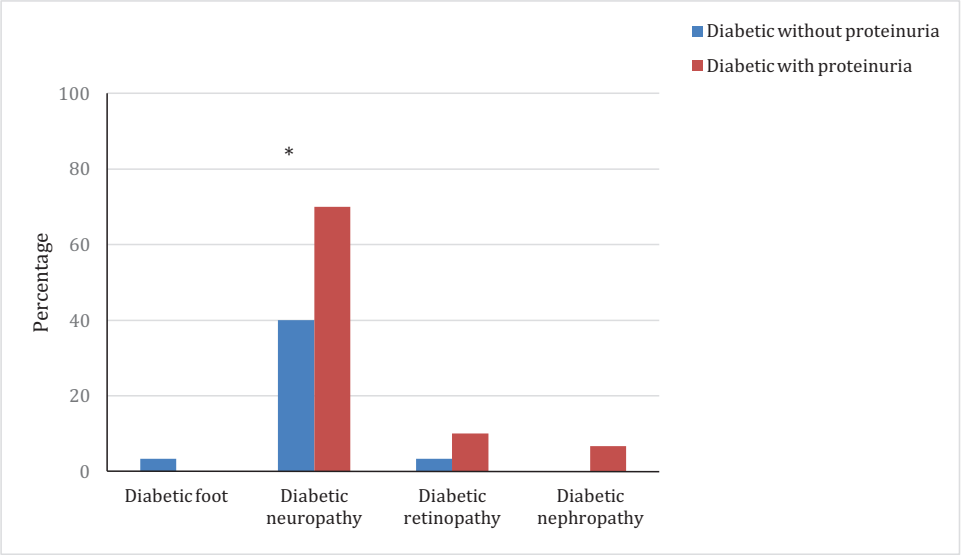
In the diabetic group without proteinuria, angiotensin-converting-enzyme inhibitor/angiotensin II receptor blockers were used more frequently, whereas the diabetic group with proteinuria showed more usage of diuretics. They were used mainly to control HTN; this may affect UACR, making it more controlled in those without proteinuria than those with proteinuria. Again, oral hypoglycemics were used more frequently in diabetics without proteinuria, while those with proteinuria were more dependent on insulin. In our study population, diabetics without proteinuria were users of insulin with relatively equal proportions and doses to those with proteinuria as follows: 30% with a mean dose of 50 (30–60) units/day, and 46.7% with a mean dose of 45 (6–100) units/day, respectively (Table 3).

Table 2 Micro- and macroalbuminuria in diabetic patients with proteinuria

Diabetics with microalbuminuria	Diabetics with macroalbuminuria
87 (96.7%)	3 (3.3%)

In terms of the laboratory results in the studied group, fasting blood sugar (FBG) levels were higher in the diabetic group with proteinuria (mean=191.8, SD=35.1) compared with the control group and the diabetic group without proteinuria (mean=93.6, SD=21.1 and mean=144.7, SD=52.9, respectively)

Figure 1



Diabetic complications among patients with and without proteinuria.

Table 3 Medication history in studied groups

	Control group *n*=30	Diabetic without proteinuria *n*=30	Diabetic with proteinuria *n*=30	*P* value
Insulin (%)	–	9 (30)	14 (46.7)	χ^2=1.8. *P*=0.2
Insulin dose (median (min–max))	–	50 (30–60)	45 (6–100)	Z=1.2, *P*=0.2**
Oral hypoglycemic No (%)	–	23 (76.7)	15 (50)	χ^2=4.6. **P=0.03**
Combination insulin/oral hypoglycemic No (%)	–	2 (6.7)	1 (3.3)	*P*=0.6*
ACEIs/ARBs No (%)	4 (13.3)	10 (33.3)	3 (10)	**P=0.04**
Diuretics No (%)	1 (3.3)	4 (13.3)	10 (33.3)	**P=0.006**
Aspirin/NSAIDs No (%)	4 (13.3)	2 (6.7)	3 (10)	*P*=0.9*

ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blockers. Data presented as No (%), χ^2 test. *Fisher's exact test. **Mann–Whitney Z test. The bold values are means significant values.

at $P< 0.001$. Also, 2hPP and HBA1c levels were higher in the diabetic group with proteinuria (mean=289.4, SD=57.4 and mean=8.3, SD=1.3, respectively), compared with the control group and the diabetic group without proteinuria at $P< 0.001$. Among diabetic patients, diabetics without proteinuria were well controlled (30 patients), with FBG levels with a mean level of 144.7±52.9, whereas diabetics with proteinuria were uncontrolled, with FBG levels with a mean level of 191.8±35.1. All the participants in the control group were normal, with FBG levels ranging from 76 to 123, with a mean level of 93.6±21.1 ($P<0.001$). Albumin and serum creatinine levels were significantly different in the diabetic group with proteinuria (4.1±0.3 and 1.3±0.6, respectively) compared with other groups at $P=0.002$ and $P=0.001$, respectively. No statistically significant differences were found between the three groups in serum urea, triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL)/LDL, eGFR and serum endocan levels (Table 4).

In the diabetic group with proteinuria, UACR levels were significantly higher (mean=1.04, SD=1.1) compared with the other two groups at $P< 0.001$. Serum creatinine levels were significantly different in the diabetic group with proteinuria (1.3±0.6) compared with other groups at $P=0.001$. However, no statistically significant difference was observed between the groups in serum urea and eGFR (Table 5, Fig. 2).

As we looked into indicators of kidney function 'serum endocan, albumin, total bilirubin and serum creatinine and s. urea' in the diabetic group with no HTN, apart from albumin, no statistically significant differences were found in any of the other indicators. Albumin levels were higher in nonhypertensive diabetic patients without proteinuria (mean=4.4, SD=0.3) compared with nonhypertensive diabetic patients with proteinuria at $P=0.004$. Looking into differences in serum albumin, total bilirubin, urea, creatinine and endocan within each group according to HTN status, there was no statistically significant difference, except for

Table 4 Comparison of laboratory results in the studied groups

	Group 1 *n*=30	Group 2 *n*=30	Group 3 *n*=30	Significance test
Hemoglobin	10.9±1.4	10.9±1.1	11.4±1.6	F=0.9, *P* =0.4
TLC	7.3±2.7	7.1±1.8	7.7±1.9	F=0.6, *P* =0.6
Platelets	208.7±64.1	230.3±55.0	236.7±67.5	F=1.7, *P*=0.2
FBG	93.6±21.1*	144.7±52.9*	191.8±35.1*	F=48.6, *P*< **0.001**
2hPP	129.3±21.4*	198.2±68.1*	289.4±57.4*	F=69.2, *P*< **0.001**
A1C	5.3±0.6*	6.7±1.3*	8.3±1.3*	F=53.1, *P*< **0.001**
AST	35±10.9	35.4±6.2	35.9±9.6	F=0.07, *P*=0.9
ALT	30.3±6.5	29.9±5.6	30.0±6.9	F=0.02, *P*=0.9
Albumin	4.3±0.3	4.4±0.3*	4.1±0.3*	F=6.9, ***P*=0.002**
Total bilirubin	0.86±0.11	0.93±0.11	0.92±0.15	F=2.6, *P*=0.08
Serum creatinine	0.9±0.2*	0.9±0.26∞	1.3±0.6*∞	F=8.3, ***P*=0.001**
Serum urea	33.7±6.9*	36.4±9.9	44.8±17.1*	F=2.3, *P*=0.002
TGs	149.3±17.9	145.2±25.9	158.6±43.3	F=1.5, *P*=0.2
Cholesterol	197.1±41.4	194.7±24.8	195.1±28.9	F=0.05, *P*=0.9
LDL	122.9±43.3	120.4±24.9	119.5±27.9	F=0.09, *P*=0.9
HDL	45.8±8.0	54.2±5.2	43.9±7.5	F=0.6, *P*=0.6
HDL/LDL	0.4±0.2	0.4±0.2	0.4±0.3	F=0.1, *P*=0.9
eGFR	78.8±16.9	78.2±18.4	67.9±27.5	F=2.4, *P* =0.09
UACR	0.16±0.08*	0.16±0.04∞	1.04±1.1*∞	F=18.8, *P*< **0.001**
Serum endocan	100.1±23.8	130.7±76.3	111.9±85.7	F=1.6, *P*=0.2

2hPP, 2-h postprandial blood sugar; A1C, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; F, Fisher's exact test; FBG, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TLC, total leukocyte count; UACR, urine albumin-to-creatinine ratio. Data in bold are statistically significant at $P\leq0.05$. *and ∞ indicate that the difference between the groups is statistically significant.

Table 5 Comparison of kidney function indicators between groups

	Group 1 *n*=30	Group 2 *n*=30	Group 3 *n*=30	Significance test
eGFR	78.8±16.9	78.2±18.4	67.9±27.5	F=2.4, *P*=0.09
UACR	0.16±0.08*	0.16±0.04∞	1.04±1.1*∞	F=18.8, *P*< **0.001**
Serum endocan	100.1±23.8	130.7±76.3	111.9±85.7	F=1.6, *P*=0.2

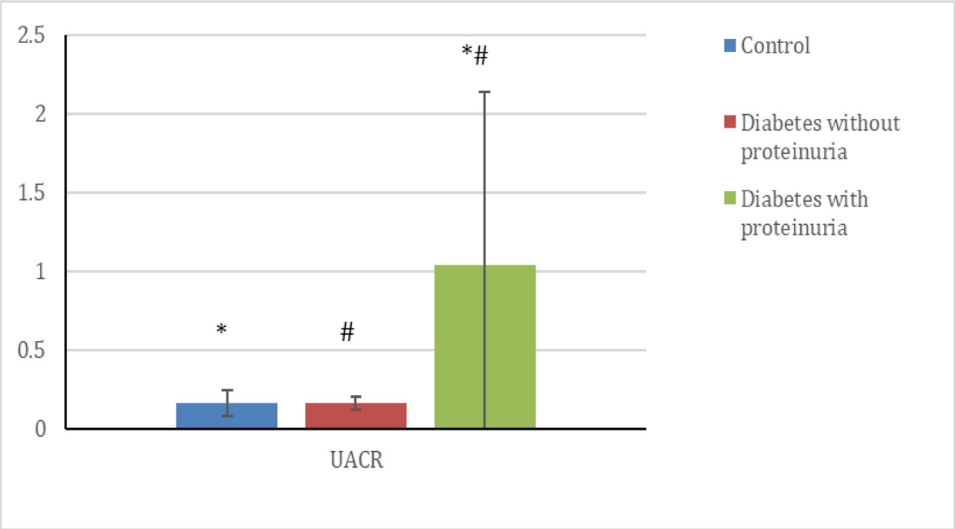
eGFR, estimated glomerular filtration rate; F, Fisher's exact test; UACR, urine albumin-to-creatinine ratio. Data in bold are statistically significant at $P\leq0.05$. *and ∞ indicate that the difference between the groups is statistically significant.

the total bilirubin level, which was significantly higher in nonhypertensive diabetics without proteinuria (Figs 3 and 4).

In our study, there was no correlation between endocan and serum creatinine levels as well as eGFR in diabetic patients with proteinuria.

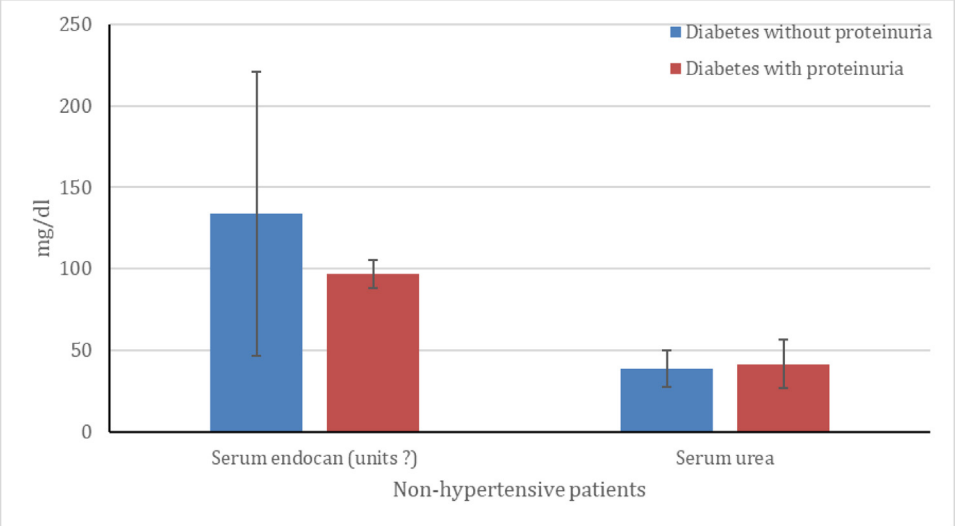
Although eGFR did not show a significant difference between groups, eGFR has the lowest average (67.9 ±27.5) in diabetic patients with proteinuria comparable with other groups and similarly, serum urea level has the highest average (44.8±17.1); it should be kept in mind that in the group of diabetic patients with proteinuria, the majority had microalbuminuria and only 3 patients had macroalbuminuria. S. endocan levels in diabetic patients without proteinuria correlated poorly with S. urea (r=0.4, P=0.02). In the group of diabetic patients with proteinuria, serum endocan was significantly moderately correlated with CRP (r=0.58, P=0.001). Among the patients diabetic without proteinuria, s. endocan levels were poorly inversely correlated with DM duration. Also, in the group of diabetic patients with proteinuria, a poor positive correlation with DM duration was observed. Among diabetic patients, no correlation was observed between serum endocan levels and HA1C (Figs 5–10).

Figure 2



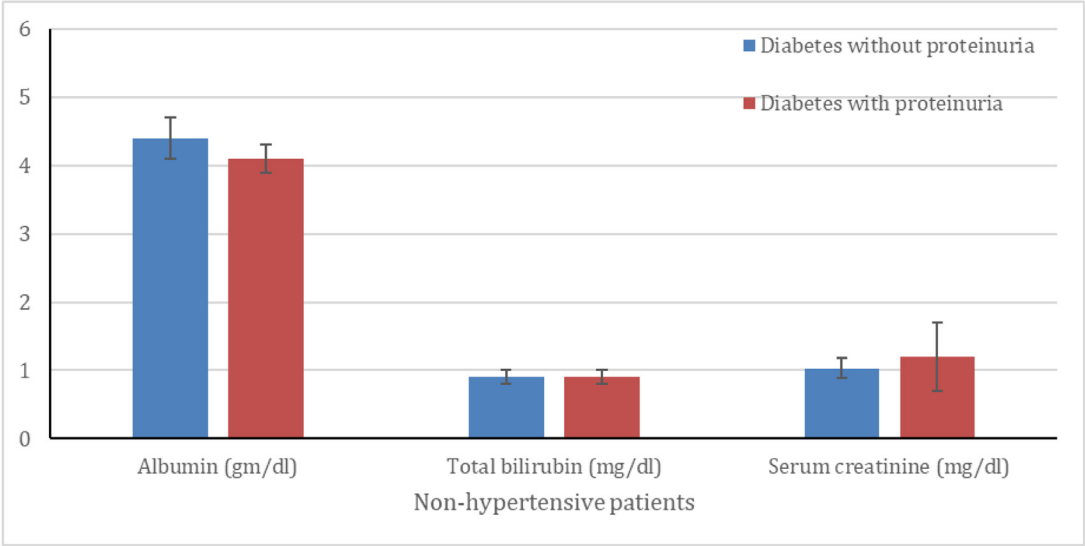
Urine albumin–creatinine ratio in all groups.

Figure 3



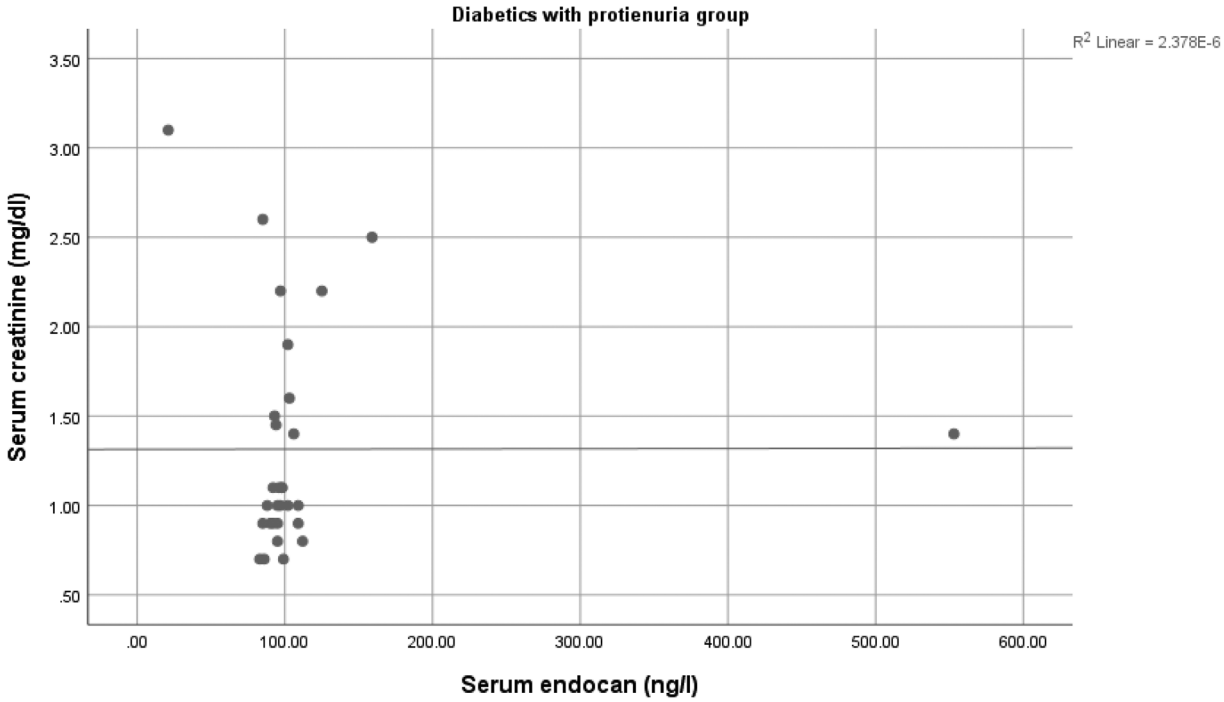
Endocan and urea levels in nonhypertensive patients.

Figure 4



Albumin, bilirubin, and creatinine levels in nonhypertensive patients.

Figure 5



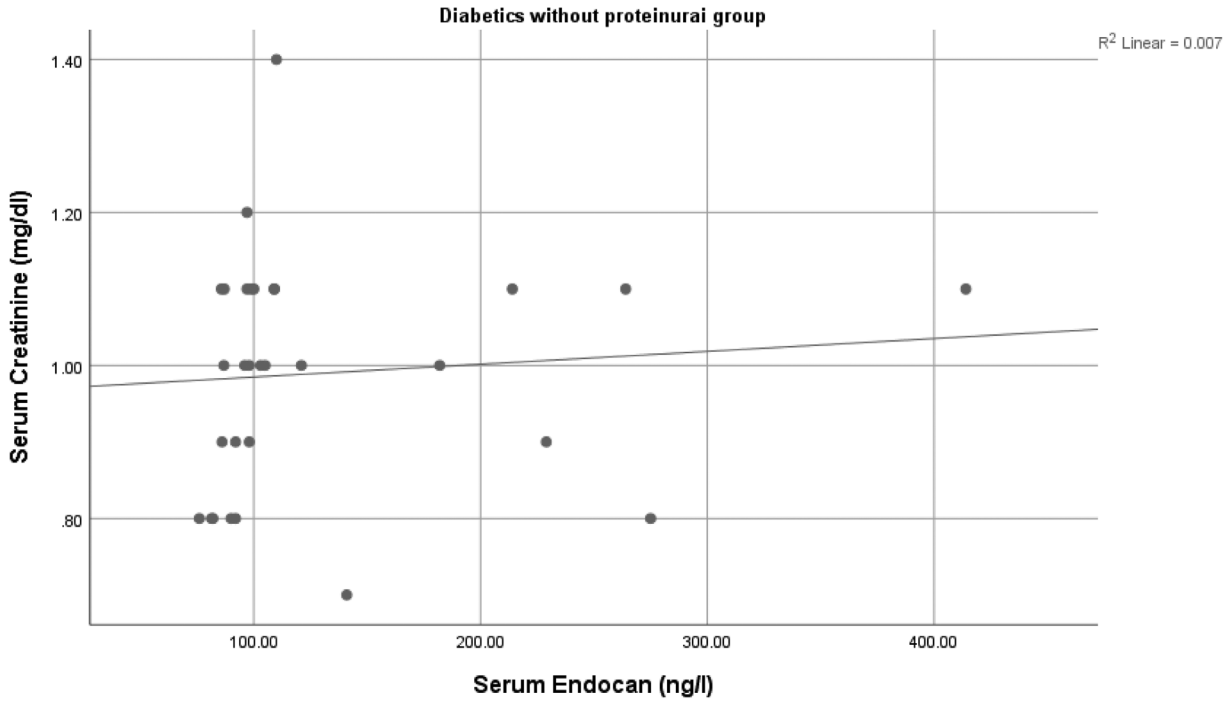
Correlation of endocan with creatinine in diabetics with proteinuria.

Patients with microalbuminuria in our study had insignificantly lower endocan levels (111.9±85.7) than patients with normoalbuminuria (130.7±76.3) (Table 6).

Our study found a nonsignificant correlation of S. endocan with age and BMI. Also, we found a nonsignificant poor negative correlation between endocan and cholesterol in the proteinuric group. Generally, there was a nonsignificant correlation between serum endocan levels and blood lipid profile (Table 7).

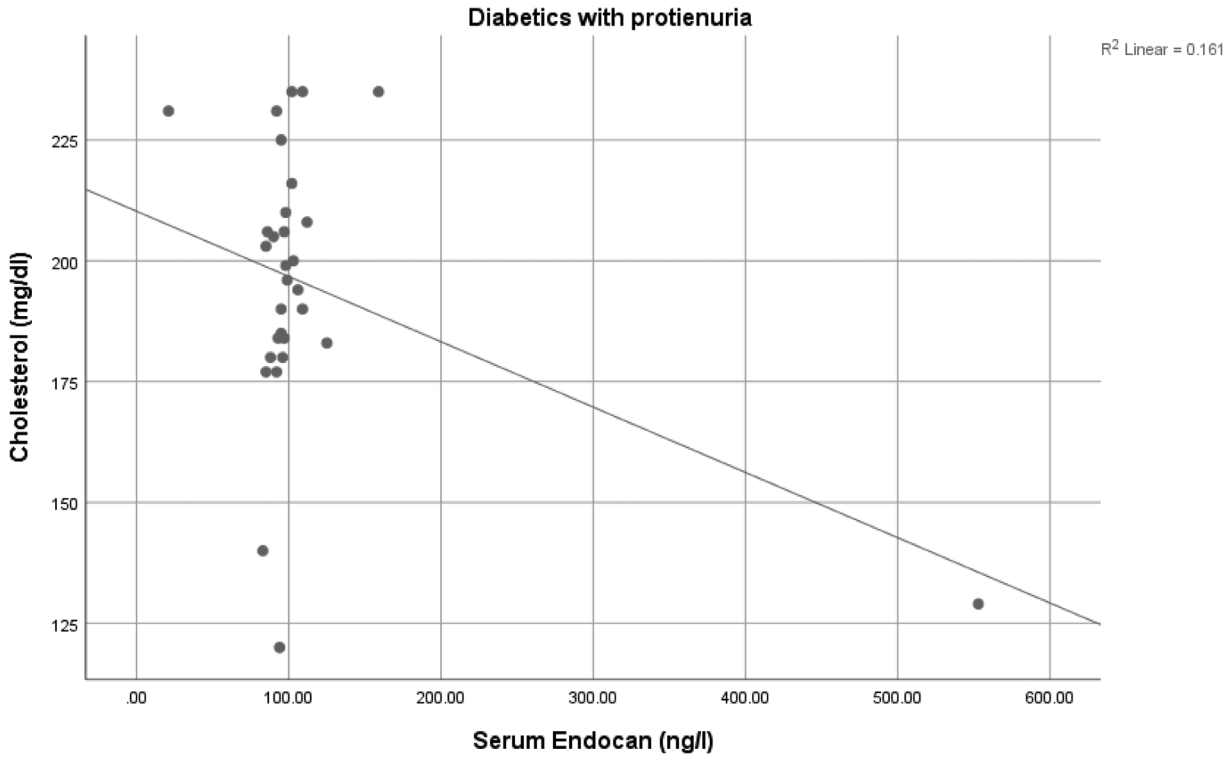
Our study population did not include many patients with active diabetic retinopathy or advanced peripheral diabetic neuropathy, which may explain the insignificant effect (t=1.1, *P*=0.3) of complications other than DN on endocan expression (Table 8).

Figure 6



Correlation of endocan with creatinine in diabetics without proteinuria.

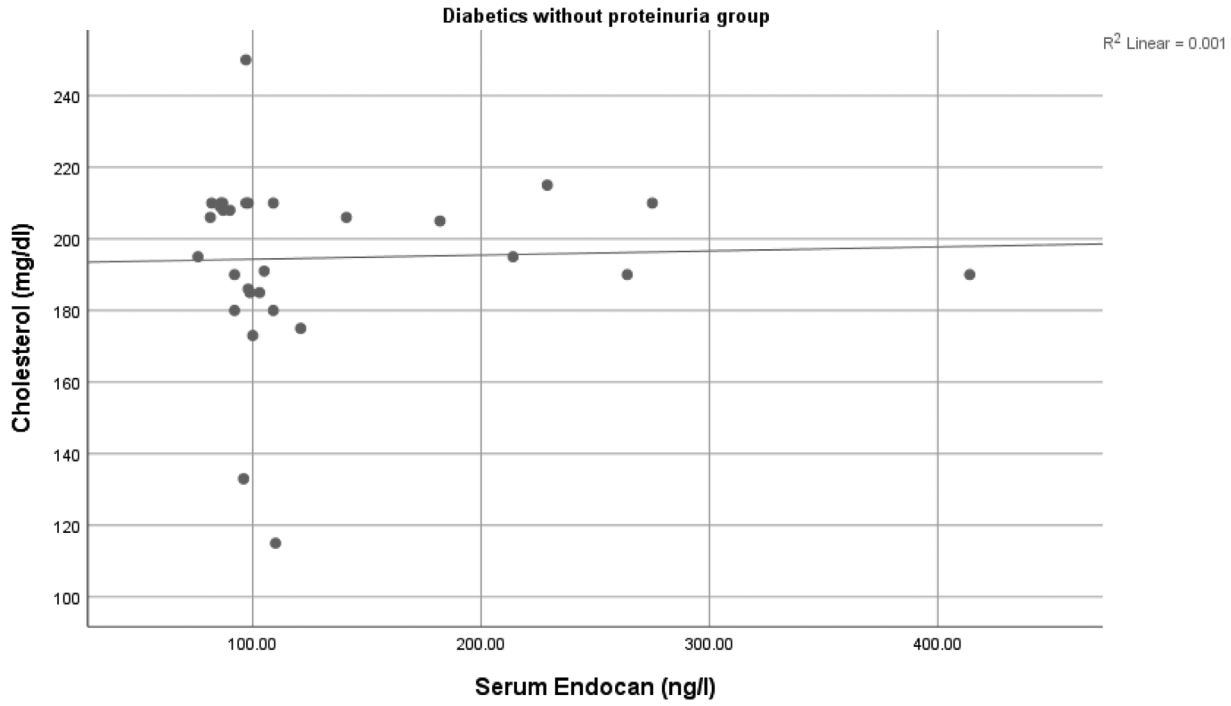
Figure 7



Correlation of endocan with cholesterol in diabetics with proteinuria.

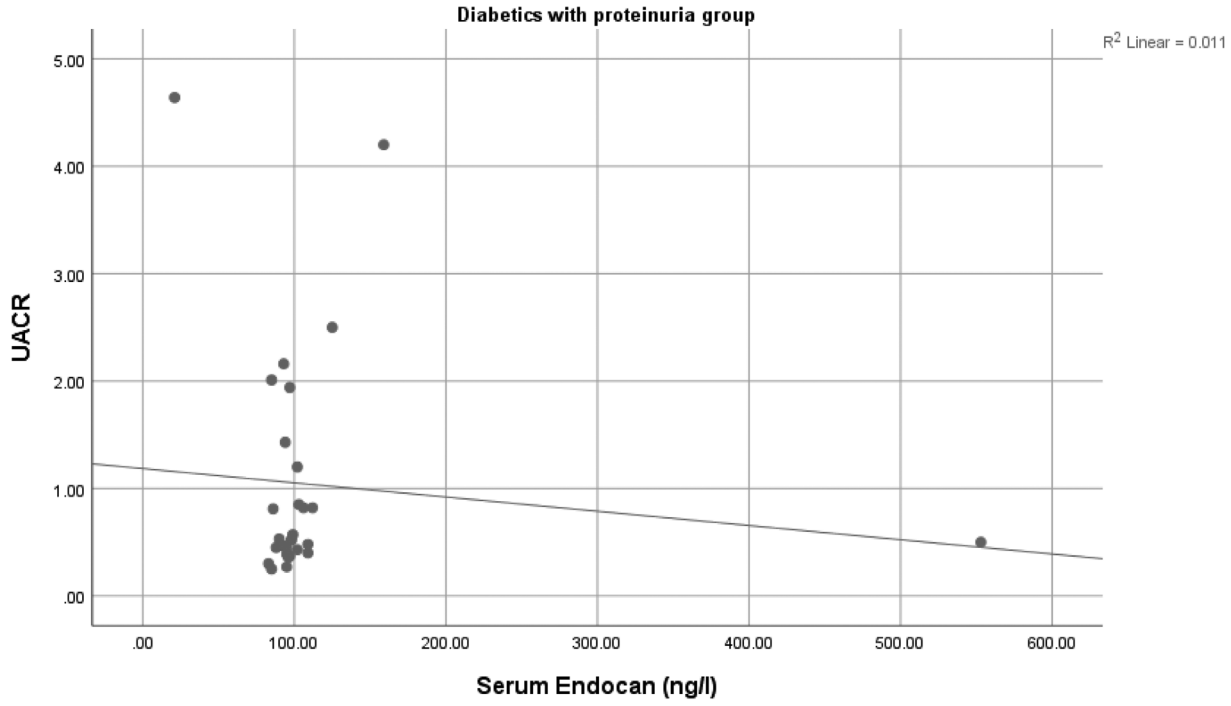
Our results showed an insignificant correlation between endocan levels and HTN (t=0.7, *P*=0.5), but slightly higher levels of endocan were found in hypertensive patients (130.3±103.2) compared with nonhypertensive patients (115.7±64.6) (Table 9).

Figure 8



Correlation of endocan with cholesterol in diabetics without proteinuria.

Figure 9



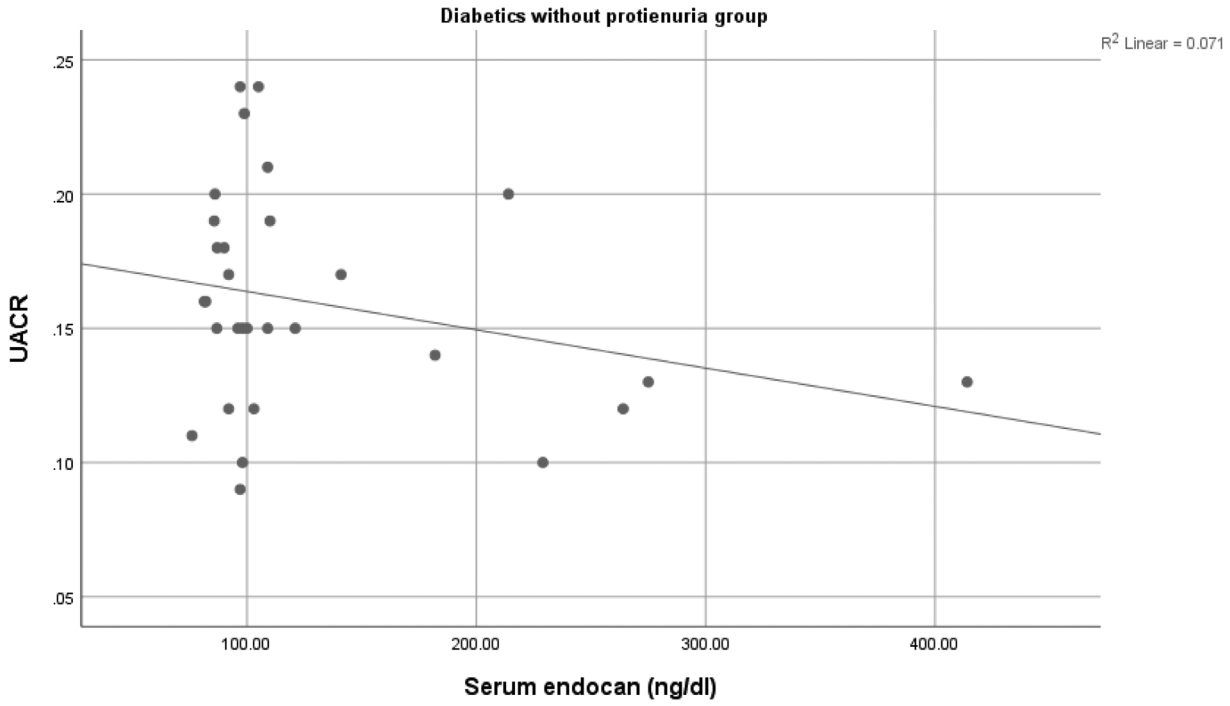
Correlation of endocan with urine albumin–creatinine ratio in diabetics with proteinuria.

This is the worst situation. When the AUC is ~0.5, the model has no discrimination capacity to distinguish between positive class and negative class. Thus, here, in our study, serum endocan did not have considerable specificity or sensitivity in the diagnosis of proteinuria as a marker of DN (Fig. 11).

Discussion

The main cause of CKD in the world currently is DM. The increasing incidence, morbidity, and mortality of DN make it a significant medical problem as one of the microvascular complications of DM that has been

Figure 10



Correlation of endocan with urine albumin–creatinine ratio in diabetics without proteinuria.

Table 6 Comparison of endocan levels between groups

	Control group	Diabetic without proteinuria	Diabetic with proteinuria	
Serum endocan	100.1±23.8	130.7±76.3	111.9±85.7	F=1.6, *P*=0.2

F, Fisher's exact test.

Table 7 Correlation between S. endocan with age and BMI

	Age	BMI	Insulin dose	HB	Albumin	Cholesterol
All diabetics (N=60)	*r*=–0.08, *P*=0.5	*r*=0.02, *P*=0.9	*r*=–0.05, *P*=0.8	*r*=0.04, *P*=0.7	*r*=0.04, *P*=0.7	*r*=–0.18, *P*=0.09
S. endocan						
Diabetics without proteinuria	*r*=–0.44, ***P*=0.02**	*r*=0.12, *P*=0.5	*r*=–0.33, *P*=0.4	*r*=–0.19, *P*=0.3	*r*=0.17, *P*=0.4	*r*=0.04, *P*=0.9
Diabetics with proteinuria	*r*=0.25, *P*=0.2	*r*=–0.09, *P*=0.6	*r*= 0.05, *P*=0.9	*r*=0. 18, *P*=0.4	*r*=–0.15, *P*=0.4	*r*=–0.4, ***P*=0.03**

BMI, body mass index; HB, hemoglobin. Data in bold are statistically significant at $P \leq 0.05$.

detected in 30%–40% of type 1 DM and 10%–20% of type 2 DM patients [15]. Therefore, early prediction of DN enables the timely administration of the most appropriate protective treatments and can significantly improve the prognosis of diabetic kidney disease [16].

The pathogenesis of DN is very complex. Elevated blood sugar levels and hemodynamic changes are the important contributing factors that act together. One of the principal pathogenic mechanisms of DN is increased angiogenesis. There is increasing evidence that angiogenic growth factors lead to DN. Normally, there is a strict balance between proangiogenic and antiangiogenic factors, but in some pathological conditions like DM, there is an imbalance between them in such a way that pro-angiogenic factors have dominance over downregulated antiangiogenic molecules. Therefore, this imbalance leads to increased proliferation and migration of endothelial cells and results in immature and leaky vessels [17].

Recent studies have focused on novel diagnosis and treatment strategies for DN to decrease its mortality and morbidity. Therefore, reliable biomarkers that aid in the detection and progression of DN are necessary along with molecular targets for personal treatment. Therefore, we aimed to establish the role of serum

Table 8 Endocan with diabetes mellitus complications

	All diabetics (*n*=60) Serum endocan (ng/l)	Significance test
Diabetic complications		
Yes (*n*=33)	110.5±79.9	t=1.1, *P*=0.3
No (*n*=27)	134.5±81.9	

t, Student's *t* test.

Table 9 Endocan with hypertension

	All diabetics (*n*=60) Serum endocan (ng/l)	Significance test
Hypertension		
Yes (*n*= 23)	130.3±103.2	t=0.7, *P*=0.5
No (*n*=37)	115.7±64.6	

t, Student's *t* test.

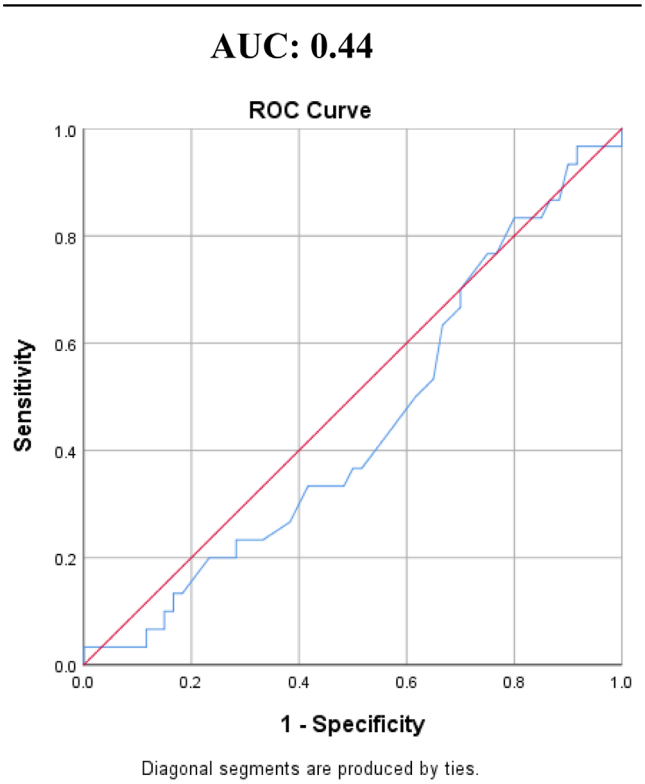
endocan as a marker of DN similar to the case with UACR.

Our study found a positive correlation of proteinuria grade with the duration of diabetes; this is similar to the results of Chowta *et al.*, who studied microalbuminuria in DM. A positive correlation was found between duration of DM and microalbuminuria, which is in line with many previous reports. Duration of diabetes has a significant contribution to the development of microalbuminuria due to prolonged exposure to advanced glycosylation end products induced by hyperglycemia [18,19].

Fasting blood glucose levels in this study were higher in the diabetic group with proteinuria (mean=191.8, SD=35.1) compared with the control group and the diabetic group without proteinuria (mean=93.6, SD=21.1 and mean=144.7, SD=52.9, respectively) at $P< 0.001$. Also, 2hPP and A1C levels were higher in the diabetic group with proteinuria (mean=289.4, SD=57.4 and mean=8.3, SD=1.3, respectively) compared with the control group and the diabetic group without proteinuria at $P< 0.001$. This finding is in accordance with Varghese *et al.* [20], who detected a correlation of FBG and HbA1c levels with the prevalence of microalbuminuria. However, the Afkhami-Ardekani *et al.* [21] study showed no statistically significant correlation between the prevalence of microalbuminuria and the FBG or HbA1c; this discrepancy might be attributable to differences in the study population and sample size between their and our studies.

In the current study, we found a nonsignificant poor negative correlation between endocan and total cholesterol in the proteinuric group. Generally, there is a nonsignificant correlation between serum endocan

Figure 11



A receiver operating characteristic curve, or ROC curve.

levels and blood lipid profile. This is in line with a study carried out by Kadayıfçı and Karada [22], in which there was no significant correlation between serum endocan levels and blood lipid measurement. In the future, more studies are needed to confirm the detected relationship and to determine its causal nature.

Among diabetic patients, no correlation was observed between endocan levels and HbA1c in our study. Similar to our results, in a study carried out by Lee *et al.* [7], the authors reported that HbA1c values did not have much effect on the outcome. In contrast, Klisic *et al.* [23] concluded that endocan is independently correlated with HbA1c in patients with type 2 diabetes and prediabetes and they attributed this to the fact that as the endocan concentration increased by one unit, the probability of a higher HbA1c concentration increased by more than three times.

There was no correlation between serum endocan and serum creatinine level as well as eGFR in our study in the diabetic group with proteinuria. Although eGFR did not show a significant difference between groups, eGFR has the lowest average (67.9±27.5) in diabetics with proteinuria comparable to other groups, and the same was found for serum urea level having the highest average (44.8±17.1), keeping in mind that the majority

of diabetics with proteinuria had microalbuminuria and only 3 patients had macroalbuminuria. In line with our results, a study carried out by Lee *et al.* [7] titled 'Endocan as a potential diagnostic or prognostic biomarker for chronic kidney disease' also found no correlation between serum endocan and serum creatinine level as well as eGFR. In contrast, Yilmaz *et al.* [24] and Arman *et al.* [12] found that endocan levels were inversely correlated with eGFR; they stated that as renal function declined, serum endocan levels increased, which may be due to the increased production or decreased clearance. The fact that its levels are increased in a variety of inflammatory diseases without renal involvement could reinforce increased secretion [7].

Focusing on the correlation of endocan with UACR in our study, the group of diabetic patients with proteinuria had both micro- and macroalbuminuria. Among the randomly chosen patients, only three patients had macroalbuminuria, in whom UACR was equal to or exceeded 25 mg/dl, and the rest had microalbuminuria. The absence of a high number of macroalbuminuric patients in our study population may be why serum endocan did not show a significant difference as was found in the study of Cikrikcioglu *et al.* [25], who studied endocan and albuminuria in 137 patients with Type 2 DM including 47 microalbuminuric and 35 macroalbuminuric patients. This may be due to the stimulation of endocan release by hyperglycemia through vascular endothelial growth factor (VEGF), which is one of the important molecules that increases endocan expression, and endocan itself is a potent stimulator of angiogenesis [1]. Thus, patients with diabetes were found to have higher serum endocan levels than nondiabetic patients, both before and after regulation of the diabetes [5]. In early-phase DN, endocan may be elevated, but once nephropathy progresses, leading to severe renal injury, that is, during the proteinuria phase, serum endocan levels may decrease due to the reduced VEGF release [26].

Among diabetic patients without proteinuria, serum endocan levels were poorly inversely correlated with the duration of DM. Also, among diabetic patients with proteinuria, a poor positive correlation with DM duration was observed. However, Cikrikcioglu *et al.* [25] showed no correlation between endocan levels and duration of diabetes. This could be explained by the fact that endocan was relatively high initially in the early stages of hyperglycemia at the time of DM diagnosis, but decreased with longer duration, which may be justified as endocan expression is controlled due to the effect of antidiabetic drugs. While once proteinuria occurred means renal injury progressed and positive correlation can be interpreted through insulin usage as endocan increase with insulin dose reword of what between brackets (using insulin in controlling blood sugar lead to increasing VEGF and thus higher endocan level) and or poor glycemic control of proteinuric patients.

In conclusion, serum endocan levels show fluctuations according to the glycemic state and various other situations (systemic disorders, medications, etc.). Initial hyperglycemia in newly diagnosed or poorly controlled diabetes is associated with high endocan expression, but the same patients may also show low endocan expression if treated promptly and according to their lifestyle, HTN and other comorbidities that affect endocan expression. Also, macroalbuminuric patients have a wide range of kidney insult according to KDIGO and so they may show a parallel increase in endocan expression with increased proteinuria, while when the kidney function deteriorates, endocan expression may become exhausted and decrease again.

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Conflicts of interest

There are no conflicts of interest.

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