

## ORIGINAL ARTICLE

# Evaluation of Endocan as a Novel Marker for Early Detection of Nephropathy in Patients with Type 2 Diabetes Mellitus

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## ABSTRACT

### Key words:

Type 2 diabetes mellitus, diabetic nephropathy, albuminuria, endocan

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**Background:** Diabetic nephropathy (DN) is a major complication of type 2 Diabetes Mellitus (T2DM). Screening for DN must be initiated once diagnosis of DM is confirmed. Persistent albuminuria is a hallmark of DN, while the risk for developing DN starts when urine albumin excretion (UAE) values are still within the normoalbuminuric range. **Objective:** The aim of this study was to assess serum endocan as a novel predictive marker for early detection of DN in T2DM and to investigate whether its level was parallel to the severity of DN as assessed by the degree of albuminuria. **Methodology:** The study included 60 patients with T2DM, were divided into three groups of normo, micro and macro albuminuria. They were compared to 20 controls. Endocan level in serum was measured by ELISA. **Results:** Serum endocan was increased in all patients compared to controls, and in macro and microalbuminuria compared to normoalbuminuria groups. Serum endocan was positively correlated with duration of DM, fasting blood glucose (FBG), hemoglobin (Hb) A1C, creatinine and urine albumin creatinine ratio (UACR), and negatively correlated with estimated glomerular filtration rate (eGFR). The receiver operating characteristic (ROC) analysis revealed that serum endocan has the highest diagnostic performance in discriminating between normoalbuminuria group and control group. **Conclusion:** Serum endocan was elevated early in patients with normoalbuminuria and its increase was parallel to the increase in UACR. Therefore, serum endocan may be used as a predictive marker for early detection of DN in T2DM and in monitoring of disease progression.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder characterized by hyperglycemia and the late development of micro and macrovascular complications. The chronic hyperglycemia of DM is characterized by long term damage and dysfunction of the kidneys, eyes, nerves, heart and blood vessels. Different mechanisms are involved in the development of diabetes. Type 1DM is caused by autoimmune destruction of  $\beta$ -cells of the pancreas leading to insulin deficiency, while type 2 is due to insulin resistance<sup>1</sup>.

Type 2 diabetes mellitus (T2DM) may remain several years undiagnosed because hyperglycemia occurs gradually and the classic symptoms of diabetes are not severing enough to be noticed. Nevertheless, even undiagnosed patients are at increased risk of developing complications<sup>2</sup>.

Diabetic nephropathy (DN) is a major microvascular diabetic complication and a leading cause to end stage renal disease (ESRD). The earliest clinical sign of DN is the occurrence of persistent microalbuminuria. The disease may progress from microalbuminuria to macroalbuminuria with urinary albumin excretion

(UAE) >300 mg/day. This is associated with progressive decline of glomerular filtration rate (GFR) and evolution of ESRD<sup>3</sup>.

However, the tests for detection of micro and macroalbuminuria are not sensitive enough to detect early diabetic kidney disease and so; they are used with some limitations<sup>4</sup>.

Therefore, the development of more sensitive and specific biomarkers that can reflect the onset of DN at an early reversible stage would be of considerable diagnostic value to prevent the condition not to be worsening further and stop the devastating outcomes of renal loss in DM<sup>5</sup>.

Endocan is a soluble dermatan sulfate proteoglycan derived from the endothelium. It was initially named as endothelial cell specific molecule-1(ESM-1). It is physiologically expressed and secreted as a soluble form in endothelial cells of the lung and kidney. The expression of endocan is up regulated by vascular endothelial growth factor (VEGF), interleukin (IL)-1, tumor necrosis factor (TNF)- $\alpha$ , transforming growth factor (TGF)- $\beta$  1 and fibroblast growth factor (FGF)-2, but down regulated by interferon (IFN) - $\gamma$ <sup>6</sup>.

Endocan can bind to a wide range of bioactive molecules included in cellular signaling and adhesion and thus can regulate proliferation, differentiation, migration and adhesion of different cells in health and disease. An increase in tissue expression or serum level of endocan indicates endothelial activation and neovascularization which are prominent pathophysiological changes associated with inflammation and tumor progression<sup>7</sup>.

Moreover, serum endocan was significantly associated with markers of inflammation [high-sensitivity C-reactive protein (hs-CRP)] and vascular defects (flow-mediated vasodilation and carotid intima-media thickness)<sup>8</sup>.

An inflammatory mechanism mediated by macrophages and angiogenesis may play important roles in the development of DN. Inflammatory cytokines and growth factors, mainly VEGF, TGF- $\beta$ , IL-1, IL-6, IL-18 and TNF- $\alpha$ , are also involved in the pathogenesis of DN<sup>9</sup>.

Studies reported that serum level of endocan was increased in DN owing to increase its production due to kidney inflammation or decrease its clearance due to deteriorating GFR<sup>10</sup>.

The aim of the study was to evaluate serum endocan level as a novel predictive marker for early detection of DN in patients with T2DM and to investigate whether its level was parallel to the severity of DN as assessed by the degree of albuminuria.

## METHODOLOGY

### Subjects:

The present study was conducted on 80 subjects, aged from (35 - 65) years, of both sexes (29 males and 51 females). They were divided as follows:

**1- Patients group:** included 60 patients diagnosed as T2DM according to the criteria of American Diabetes Association (ADA). They were selected from the Inpatients of Internal Medicine and Endocrinology Departments, Al Zahraa University Hospital in the period from October/2017 to September/2018. The patients were divided into three groups according to the type of albuminuria:

- a- Normoalbuminuria group: 20 patients with urine albumin creatinine ratio (UACR) < 30 mg/g creatinine.
- b- Microalbuminuria group: 20 patients with UACR 30-300 mg/g creatinine.
- c- Macroalbuminuria group: 20 patients with UACR >300 mg/g creatinine.

**2- Control group:** 20 sex and age matched apparently healthy individuals (8 males and 12 females). Their age ranged from (35 - 60) years.

Written informed consent had been obtained from all subjects and the study was approved by Research Ethics

Committee of Faculty of Medicine for Girls, Al-Azhar University.

### Exclusion criteria:

- Corticosteroid treatment and nephrotoxic drugs.
- Liver diseases and kidney diseases other than DN.
- Acute or chronic infections and inflammatory diseases.
- Hemolytic anemia or hematological malignancy.
- Solid tumors.

### Laboratory Methods:

**All subjects were submitted to the following examinations:**

Complete medical history and full clinical examination.

Radiological investigation: renal ultrasonography (US).

### Laboratory investigations:

- Complete blood count (CBC).
  - Erythrocyte sedimentation rate (ESR).
  - C-reactive protein (CRP).
  - Fasting blood glucose (FBG).
  - Hemoglobin (Hb) A1C.
  - Liver function tests: serum alanine transferase (ALT), aspartate transferase (AST) and serum albumin.
  - Kidney function tests: blood urea, serum creatinine and uric acid.
  - Estimated glomerular filtration rate (eGFR): was estimated by the Cockcroft and Gault equation and the result was obtained in ml/min/1.73 m<sup>2</sup>.
- $$\text{Cockcroft - Gault GFR} = \frac{72 \times \text{serum creatinine (mg/dl)}}{140 - \text{age (years)} \times \text{weight (kg)}} \times (0.85) \text{ if female}^{11}$$
- Urine albumin creatinine ratio: urine albumin and creatinine were measured and the UACR was calculated by dividing the urine albumin by urine creatinine and multiplying the result by 1000. The ratio was expressed in mg /g creatinine.

### Measurement of serum level of endocan:

#### Blood samples collection and preparation:

About 2 ml of venous blood were collected into plain test tube and left to clot for 30 minutes followed by centrifugation for 15 minutes at 1600 rpm. The serum was separated and stored at -20 till the time of analysis of endocan in a single assay to avoid repeated freeze - thaw cycles.

The serum level of endocan was measured by enzyme linked immunosorbent assay (ELISA) using Human Endothelial Cell –specific Molecule 1 ELISA kit (Bioassay Technology Laboratory, Shanghai, China), and according to the manufacturer's instructions.

### Statistical Analysis:

The data were coded and entered to the Statistical Package for Social Science (SPSS) version 23. The quantitative data were expressed as mean, standard deviations (SD) and ranges when their distributions were found parametric, and as median with inter-quartile range (IQR) when their distributions were found non parametric. Also qualitative variables were presented as numbers and percentages.

The comparison between the qualitative data in two groups was done by Chi-square test and Fisher exact test instead of Chi-square test when the expected count in any cell was found less than 5.

The comparison between the quantitative data and parametric distribution in two independent groups was done by using Independent t-test while the comparison between the quantitative data and non-parametric distribution in two independent groups was done by using Mann-Whitney test.

The comparison between quantitative data and parametric distribution in more than two independent groups was done by using One Way ANOVA followed by post hoc analysis using least significant difference (LSD) test, while the comparison between quantitative data and non-parametric distribution in more than two independent groups was done by using Kruskal-Wallis test followed by post hoc analysis using Mann-Whitney test.

Spearman correlation coefficients assessed the correlation between two quantitative parameters in the

same group. Multivariate linear regression analysis detected the factors most affecting serum endocan level.

Receiver operating characteristic (ROC) curve assessed the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC).

The confidence interval (CI) was set to 95% and the margin of error accepted was set to 5%.

P-value > 0.05: Non significant (NS)

P-value < 0.05: Significant (S)

P-value < 0.01: Highly significant (HS)

## RESULTS

The study included sixty patients with T2DM who were classified into three groups of normo (UACR < 30 mg/g creatinine), micro (UACR = 30-300 mg/g creatinine) and macroalbuminuria (UACR > 300 mg/g creatinine).

Twenty apparently healthy controls were involved in the study. The demographic and clinical data of all participants were illustrated in table 1.

**Table 1: Comparison between studied groups regarding demographic data**

Demographic data		Control	Normo albuminuria	Micro albuminuria	Macro albuminuria	One way ANOVA	*p-value
		n = 20	n = 20	n = 20	n = 20	Test value	
Age (years)	Mean ± SD	54.24 ± 8.93	52.80 ± 8.65	55.95 ± 10.67	55.75 ± 8.47	0.873•	0.459
	Range	45 – 65	37 – 65	35 – 65	36 – 65		
Sex	Female	12 (60.0%)	14 (70.0%)	13 (65.0%)	12 (60.0%)	0.595"	0.898
	Male	8 (40.0%)	6 (30.0%)	7 (35.0%)	8 (40.0%)		
Duration of DM (years)	Mean ± SD	-	7.45 ± 3.38	13.25 ± 5.12	18.35 ± 4.36	31.534•	<0.001
	Range	-	1 – 10	6 – 21	15 – 29		
<b>Post hoc analysis: by LSD test</b>							
		Normo Vs Micro albuminuria		Normo Vs Macro albuminuria		Micro Vs Macro albuminuria	
Duration of DM (years)		<0.001		<0.001		<0.001	

\*P-value >0.05: Non significant; P-value <0.05: Significant; P-value < 0.01: Highly significant.

"Chi-square test; • One Way ANOVA test

The ANOVA test among patients and control groups (Table 1) showed a non-significant difference in age (P=0.459) and sex (P=0.898), and a significant difference in duration of DM between the patients groups (P<0.001).

Comparison between patients groups regarding the biochemical data (Table 2) showed a significant increase in FBS, Creatinine and UACR in macro and microalbuminuria groups as compared to normoalbuminuria group, and in macroalbuminuria group as compared to microalbuminuria group. The

eGFR was decreased significantly in macro and microalbuminuria groups as compared to normoalbuminuria group (P<0.001), and in macroalbuminuria group as compared to microalbuminuria group (P<0.001). While the level of HbA1C showed a significant increase in macro and microalbuminuria groups as compared to normoalbuminuria group (P<0.001), and a non-significant difference between macro and microalbuminuria groups (P=0.598).

**Table 2: Comparison between the biochemical data in patients groups**

Parameters		Normo	Micro	Macro	One way ANOVA Test value	*p-value
		albuminuria (n = 20)	albuminuria (n = 20)	albuminuria (n = 20)		
FBG (mg/dl)	Mean ±SD	144.55 ± 21.51	179.20 ± 51.46	223.50 ± 64.11	13.012	<0.001
	Range	125 – 215	130 – 278	145 – 350		
HbA1C (%)	Mean ±SD	7.92 ± 0.95	9.56 ± 1.08	9.38 ± 1.07	15.170	<0.001
	Range	6.8 – 9.5	7.57 – 10.89	8.1 – 11.3		
Creatinine (mg/dl)	Mean ±SD	0.97 ± 0.16	1.50 ± 0.29	2.46 ± 0.27	183.392	<0.001
	Range	0.7 – 1.2	0.8 – 1.9	2.1 – 2.8		
eGFR (ml/min/1.73m <sup>2</sup> )	Mean ±SD	91.90 ± 6.37	63.20 ± 7.83	43.40 ± 6.81	240.754	<0.001
	Range	85 – 105	54 – 80	36 – 58		
UACR (mg /g creatinine)	Mean ±SD	21.08 ± 4.22	161.02 ± 71.63	932.65 ± 365.18	104.396	<0.001
	Range	11.4 – 29.1	87.5 – 292.3	523 – 1727.2		

**Post hoc analysis: by LSD test**

Parameters	Normo Vs Micro albuminuria	Normo Vs Macro albuminuria	Micro Vs Macro albuminuria
FBG (mg/dl)	0.029	<0.001	0.006
HbA1C (%)	<0.001	<0.001	0.598
Creatinine (mg/dl)	<0.001	<0.001	<0.001
eGFR (ml/min/1.73m <sup>2</sup> )	<0.001	<0.001	<0.001
UACR (mg /g creatinine)	0.044	<0.001	<0.001

\*P-value >0.05: Non significant; P-value <0.05: Significant; P-value < 0.01: Highly significant.

As regards serum endocan, its level was increased significantly in normo, micro and macroalbuminuria groups as compared to control group (P<0.001), in macro and microalbuminuria groups as compared to

normoalbuminuria group (P<0.001), and in macroalbuminuria group as compared to microalbuminuria group (P<0.001) (Table 3).

**Table 3: Comparison between all studied groups regarding serum endocan**

Endocan(ng/L)	Control	Normo	Micro	Macro	Kruskal-Wallis test Test value	*p-value
	(n = 20)	albuminuria (n = 20)	albuminuria (n = 20)	albuminuria (n = 20)		
Median(IQR)	71.6(58.2 - 84.9)	169.4 (103.7 – 188.5)	217.0 (207.1- 253.2)	462.3(300.9-865.4)	70.142	<0.001
Range	36.1 – 115.2	50.2 – 213.9	139.8 – 359.5	210.8 – 3001.7		

**Post hoc analysis: by LSD test**

	Normo albuminuria Vs Control	Micro albuminuria Vs Control	Macro albuminuria Vs Control	Normo Vs Micro albuminuria	Normo Vs Macro albuminuria	Micro Vs Macro albuminuria
Endocan (ng/L)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

\*P-value >0.05: Non significant; P-value <0.05: Significant; P-value < 0.01: Highly significant.

Correlations between endocan and the studied parameters revealed a significant positive correlations between its level with duration of DM, FBG, HbA1C, creatinine and UACR (P<0.001), and a significant

negative correlation with eGFR (P<0.001). While neither age (P=0.249) nor sex (P=0.325) were correlated with serum endocan (Table 4-5).

**Table 4: Correlations between serum endocan and the studied parameters in all patients**

	Endocan (ng/L)	
	r	*p-value
Age (years)	0.151	0.249
Duration of DM (years)	0.666	<0.001
FBG (mg/dl)	0.610	<0.001
HbA1C (%)	0.465	<0.001
Creatinine (mg/dl)	0.886	<0.001
eGFR (ml/min/1.73m <sup>2</sup> )	-0.897	<0.001
UACR (mg /g creatinine)	0.905	<0.001

\*P-value >0.05: Non significant; P-value <0.05: Significant; P-value < 0.01: Highly significant.

**Table 5: Relation of serum endocan with sex of all patients.**

Sex	Endocan(ng/L)		Test value	*P-value
	Median (IQR)	Range		
Female	213.5 (183.6 – 310.8)	45.6 – 1305	0.984	0.325
Male	221.1 (195.6 – 507.4)	60.3 – 3001.7		

\*P-value >0.05: Non significant; P-value <0.05: Significant; P-value < 0.01: Highly significant.

Based on multivariate linear regression analysis, UACR was the most factor affecting serum endocan level (P<0.001), followed by serum creatinine level (P=0.002) (Table 6).

**Table 6: Multivariate linear regression analysis for parameters most affecting the serum endocan level.**

	Unstandardized Coefficients		Standardized Coefficients	t	Significance
	B	Standard error	Beta		
(Constant)	321.572	542.949		0.592	0.556
Duration of DM (years)	6.995	7.142	0.082	0.979	0.332
FBG (mg/dl)	-1.151	0.758	-0.127	-1.519	0.135
HbA1C (%)	25.136	34.036	0.060	0.738	0.463
Creatinine (mg/dl)	-465.034	143.445	-0.588	-3.242	0.002
eGFR (ml/min/1.73m <sup>2</sup> )	1.840	3.745	0.074	0.491	0.625
UACR (mg /g creatinine)	1.611	0.146	1.390	11.000	<0.001

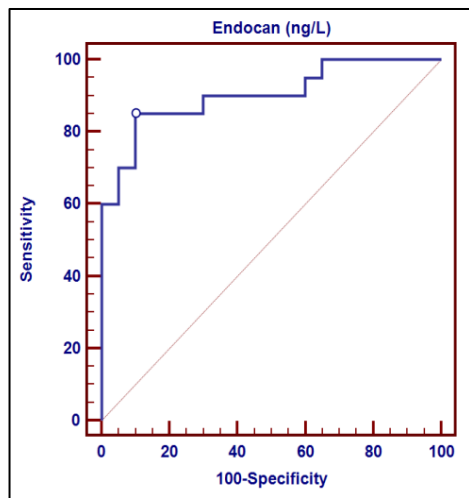
The diagnostic performance of endocan in discriminating between normoalbuminuria group and control group has the highest AUC value (0.940) with 95% sensitivity and specificity, followed by discrimination between micro and normoalbuminuria

groups with AUC value (0.903), 85% sensitivity and 90% specificity. The least AUC value (0.885) was for discrimination between macro and microalbuminuria groups with 80% sensitivity and 85% specificity (Table7).

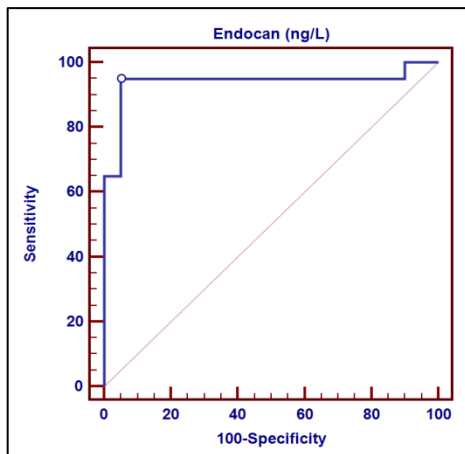
**Table 7: Diagnostic performance of endocan in discriminating between studied groups.**

Studied groups	AUC (95% CI)	Cut of Point	Sensitivity %	Specificity %	PPV %	NPV %	p-value
Control& normoalbuminuria	0.940	>93.9	95.00	95.00	95.0	95.0	<0.01
Normoalbuminuria & microalbuminuria	0.903	>195.6	85.00	90.00	89.5	85.7	<0.01
Microalbuminuria & macroalbuminuria	0.885	>290.6	80.00	85.00	84.2	81.0	<0.01

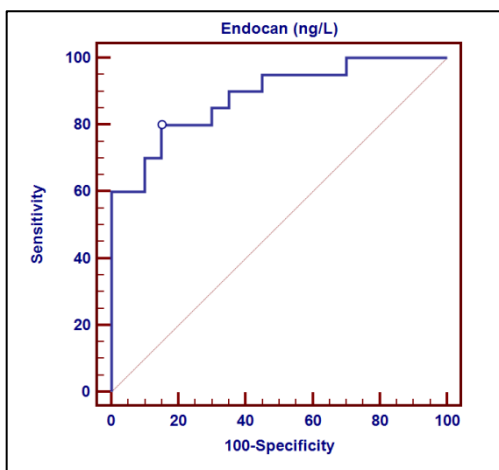




**Fig. 1:** Roc analysis of endocan in discriminating between control& normoalbuminuria.



**Fig. 2:** Roc analysis of endocan in discriminating between normoalbuminuria & microalbuminuria.



**Fig. 3:** Roc analysis of endocan in discriminating between microalbuminuria & macroalbuminuria.

## DISCUSSION

Nephropathy is one of the major microvascular complications of DM and leading cause of morbidity and mortality. No adequate therapy is currently available for patients with nephropathy. 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<sup>12</sup>.

Persistent microalbuminuria is the first clinical evidence of DN. However, increased UAE lacks the sufficient diagnostic sensitivity as the diabetes-related kidney damage occurs early when UAE value is still within the normoalbuminuric range before the detection of microalbuminuria which becomes evident only, when significant structural damage has occurred<sup>5</sup>.

Furthermore, UAE can be affected by plasma concentrations of several factors such as atrial natriuretic peptide, angiotensin II, aldosterone and arterial blood pressure. UAE also varies with physiological factors like exercise, posture and diuresis. Thus measurement of albumin in urine is not standardized and has significant imprecision<sup>13</sup>.

In spite of using decreased creatinine clearance and increased serum creatinine level as clinical markers of renal dysfunction in DM, but we cannot depend on them only to diagnose DN or to follow up the patients because serum creatinine levels rise very late after significant structural and functional abnormalities of the kidney have occurred<sup>13</sup>.

Therefore, we aimed to evaluate serum endocan as a novel predictive marker for early detection of DN in T2DM and to investigate whether its level was parallel to the severity of DN as assessed by the degree of albuminuria.

In our study, a significant difference in duration of diabetes between the three patients groups was observed, with the highest duration in macroalbuminuria group followed by micro then normoalbuminuria groups. That was agreed with Wu et al.<sup>14</sup> who stated that the duration of diabetes was the second most powerful risk factor for DN in patients with T2DM after hypertension.

In addition, all studied diabetic groups showed poor glycemic control as indicated by the significantly elevated HbA<sub>1c</sub>% particularly in patient group with macroalbuminuria. It is well known that poor glycemic control is a risk factor for most diabetic complications including DN<sup>15</sup>.

Serum creatinine and UACR were significantly increased, and eGFR was significantly decreased in macro and microalbuminuria groups as compared to normoalbuminuria group. These results indicated that early deterioration of kidney function in our diabetic patients was coincided with the development of

microalbuminuria and became exacerbated with progression to macroalbuminuria.

It is known that DM is among the systemic diseases that affect the kidney functions as manifested by decreased eGFR, decreased creatinine clearance leading to increased serum creatinine level, and decreased capability of the kidney to preserve albumin and hence, increased UAE<sup>16</sup>.

Our results were in accordance with the findings of Awnya et al.<sup>17</sup> who detected such impairment of kidney functions among diabetic patients with microalbuminuria. While Sheira et al.<sup>18</sup> and Sharaf et al.<sup>19</sup> found significant alteration of kidney functions in their studied diabetic patients with normoalbuminuria.

The serum endocan level in our study was significantly increased in all patients in comparison to control. The explanation was that in T2DM, the endothelium is stimulated by hyperglycemia -induced inflammation. Thus, hyperglycemia may cause increased expression of endocan in endothelial cells in response to proangiogenic factors such as VEGF and proinflammatory cytokines, such as TNF -  $\alpha$ , IL-1 $\beta$  and lipopolysaccharide<sup>6</sup>.

Our results were consistent with Arman et al.<sup>20</sup> who found that serum endocan increased in patients with T2DM with poor glycemic regulation in comparison to controls. The regulation of diabetes with diet, exercise and medical treatment resulted in lowered serum endocan level indicating improvement in endothelial function.

Our study showed that serum endocan level was increased significantly in normoalbuminuria group in comparison to control that might be due to hyperglycemia which increases the expression of VEGF which is the most important factor that increases endocan expression. The high expression of VEGF induces DN by increasing the vascular permeability, migration and proliferation of endothelial cells, reducing trans-endothelial electrical resistance, and activation of matrix-degrading protease<sup>21</sup>.

We suggested that elevated serum endocan level early in normoalbuminuria group with normal renal function might help in early detection of DN in patients with T2DM even before the development of micro or macroalbuminuria.

Serum endocan level was also found increased with increased UAE in DN patients being higher in macroalbuminuria than microalbuminuria and in microalbuminuria than normoalbuminuria groups. This could be explained by that elevated levels of uremic toxins in chronic kidney disease (CKD) causes endothelial injury by increasing production of the inflammatory cytokines, such as TNF-  $\alpha$  and IL-1 $\beta$ . Thus serum endocan level increased in response to endothelial injury via inflammation<sup>22</sup>.

In line with our finding, Kanesaki et al.<sup>23</sup> found that the level of serum endocan was increased parallel with

increase UACR in patients with DN. While in contrary with our findings, Cikrikcioglu et al.<sup>6</sup> found that patients with macroalbuminuria had significantly lower serum endocan levels than normoalbuminuria patients. Furthermore, when UACR increased, a decrease in serum endocan level was observed. They explained their results by the stimulation of endocan release in early phase of DN by hyperglycemia via VEGF, but as nephropathy progresses and causes severe renal injury, a decrease in serum endocan levels might occur due to reduced VEGF release. The reduced VEGF expression in renal injury may be caused by loss of the ability of these cells to secrete VEGF<sup>24</sup>.

Our study demonstrated that serum endocan level was positively correlated with serum creatinine and UACR, and negatively correlated with eGFR. Multivariate linear regression analysis showed that UACR was the most factor independently associated with change in serum endocan level.

In alignment with our results, Arman et al.<sup>20</sup>, and Yilmaz et al.<sup>25</sup> found a positive correlation between serum endocan and UACR and a negative correlation with eGFR. They stated that as renal function declined, serum endocan level increased which may be due to increased production or decreased clearance. They suggested that serum endocan might be used as a marker of negative prognosis in patients with DN, as the rate of increase of endocan was directly proportional to the degree of kidney damage demonstrated by progression of albuminuria.

We further investigated the diagnostic performance of endocan in discrimination between the studied groups through ROC analysis. Endocan showed a significant discriminative power to distinguish between normoalbuminuria and control with the highest AUC (0.940) and cutoff value >93.9 ng/L, followed by normoalbuminuria & microalbuminuria with AUC (0.903) and cutoff value >195.6 ng/L, and finally between microalbuminuria & macroalbuminuria with AUC (0.885) and cutoff value >290.6 ng/L.

**In conclusion**, the level of serum endocan was significantly elevated early in diabetic patients with normoalbuminuria and its increase was parallel to the increase in UACR. Therefore, serum endocan may be used as a marker in early detection of DN in patients with T2DM and in monitoring of the progression of the disease. Further studies are recommended to assess the effect of long-term treatment on serum endocan level to determine its exact role as a prognostic marker in patients with DN.

#### Conflicts of interest:

- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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