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RESEARCH ARTICLE

Assessment of Endocan with Progression and Severity of Diabetic Nephropathy in Babylon City

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Abstract

Background: Diabetic nephropathy (DN) is one of risk complication in type2 diabetes mellitus (T2DM) that required for new biomarker for early detection and to decrease the progression in deterioration in kidney function. Excessive angiogenesis is a main factor in the development of diabetic nephropathy. Aim: Our aim was to examine the association between serum endocan levels and urine protein creatinine ratio (PCR) as biomarker for assessment DN This study focused on evalution of endocan as biomarker for establishing diagnosis of DN and evaluate its effect on progression and developing DN Methods: In this study we evaluated endocan and levels in DN and diabetes patients without nephropathy and compared them with healthy subjects. Poor glycemic control by assess HbA1c and duration of DM are proofed to be a risk factor for DN. Endocan estimation by ELISA technique. Blood urea and creatinine and protein in urine were estimated by spectrophotometric method. Results: Endocan levels in DN was 327.9±106.9 and in diabetic patient without nephropathy and healthy control were (206.3±91.5, 122.7±45.7), respectively which is statistically significant at p-value<0.05. Endocan is significantly correlated with protein to creatinine ratio (PCR) and estimated glomerulofilteration rate (eGFR) at p-value<0.05. Conclusion: Endocan could be used as reliable marker for assessment diabetic nephropathy

Keywords: *Diabetic nephropathy, Endocan, Type2 diabetes mellitus.*

Introduction

Diabetic nephropathy (DN) is the significant complication of diabetes, which is at the present time the major cause of chronic renal failure [1]. DM and its complications have become a public health problem, which is nowadays the main cause of chronic renal failure [2]. DN, the common reason of end stage renal disease, is characterised by glomerulosclerosis, the accumulation of extra cellular matrix in glomerular mesangium, and kidney interstitial tissue eventually leads to renal failure [3].

Three major histologic alterations happen in the glomeruli of persons with diabetic nephropathy, firstly; mesangial expansion is directly influenced by hyperglycemia, may be via increased matrix production or glycation of matrix protein [4]. Secondly; increase thickness of the glomerular basement membrane (GBM) takes place.

Thirdly; glomerular sclerosis is caused by intraglomerular hypertension, which is caused by dilatation of the afferent renal artery or from ischemic injury caused by hyaline narrowing of the vessels providing the glomeruli by blood, these variant histologic patterns appear to get similar prognostic significance [5]. These changes damage the kidney's glomeruli, which leads to the characteristic feature of albumin in urine (called albuminuria) [6].

DN usually causes no symptoms, and people who have this situation often produce normal amounts of urine. Symptoms of renal failure can take 5-10 years to appear after the beginning of kidney damage. These symptoms include itchy skin, headaches, severe tiredness, a general sensation of illness, nausea, vomiting, lack of appetite, and leg swelling [7]. Not everyone with diabetes will develop DN.

In people with T1DM, DN is more probable to progress 5 to 10 years or maybe more after the onset of diabetes. People with T2DM may discover that they previously have a small amount of protein in the urine at the time which diabetes is diagnosed because they may have had diabetes for several years [8].

Diagnosis is based on the measurement of abnormal levels of urinary albumin in a diabetic, coupled with exclusion of other causes of albuminuria [9]. Microalbuminuria is defined as levels of albumin ranging from 30 to 300 mg in a 24-h urine collection. Overt albuminuria, macroalbuminuria, or proteinuria is defined as a urinary albumin excretion of ≥300 mg/24 h. Urinary albuminuria comprises 20-70% or urinary total protein excretion [10].

Ethical Issues

The starting this work was need for following approval.

- Agreement of logical board in College of Medicine (University of Babylon/ Iraq).
- The study design and methods of this work were described to all subjects.

Material and Method

Study Design

This patient and control samples were collected from diabetes center in Merjan Medical City. The practical work was done in laboratory of biochemistry department in college of medicine, Babylon University. This case-control study includes (90) persons divided into three groups the first group includes (30) patients previously diagnosed withT2D with Nephropathy (DN.), the second group includes (30) patients diagnosed with T2D without nephropathy (DM.), the third group (30) persons apparently healthy control. Diagnoses were done by specialist consultation in Marjan medical hospital complete history was taken from all patients that include: smoking, age, residence, family history such as medical and drug therapy. Control group healthy persons, free of disease. Exclusion criteria: Type 1 diabetic patients and patients with UTI pregnant female were not excluded from this study.

Estimation of Endocan

Assay of endocan was based on sandwich ELISA technique the kits were provide from Elabscience company (China), the protocol were done depending on protocol provided by manufacture.

Determination of Creatinine and Protein in Urine

Blood urea and creatinine and protein in urine were estimated by spectrophotometric method and estimation of protein to creatinine ratio, eGFR and assessment of glycemic control by estimation of HbA1c.

Statistical Analysis

The statistical analysis was done by using "SPSS version 22, (Med Calc Software bvba, Ostend, Belgium; 2014), GraphPad Prism version 8.0.0 for Windows, Graph Pad Software, San Diego, California USA, Data were expressed as Mean ± SD. Student′s t-test applied to evaluate the difference between case and control group. Statistical significant depend on value of P-value at less than 0.05.

Results and Discussion

The description analysis which contain full information and data obtained from this work were representing in table.1 which included full information about three groups participating in the study patients with DM without nephropathy and DN and healthy control groups.

Table 1: Baseline characteristic of diabetic and control group

Parameters	DN	T2DM	Control	P value
Age (years) Mean± SD	49.7±9.9	44.2±10.4	46.2±10.9	**0.123**
BMI (kg/m²) Mean± SD	28.6±5.2	27.8±4.9	27.8±4.7	**0.756**
HbA_{1c} (%) Mean± SD	10.0±1.6	7.7±2.1	5.7±0.5	**<0.001***
Blood Urea (mg/dl)	91.3±87.9	62.5±27.5	45.9±29.5	**0.003***
Creatinine (mg/dl)	1.5±0.2	0.7±0.2	0.6±0.2	**0.033***
Protien in urine (mg/dl)	180.2±17.5	83.9±14.2	22.5±5.5	**0.033***

eGFR **ml/min/1.73m²**	86.0±54.4	115.2±47.4	134.3±36.5	**0.004***
PCR	108.9±1	197.7±2	141.9±3	**0.000***

The results of this study were showing in table 1 were significant difference in estimated blood urea and creatinine between patients with DN and DM without nephropathy and control group beside poor glycemic control and positive correlation of DN with increase duration of DM. The developing of diabetic mellitus positively correlated with duration of DM in our study proved that the longer duration of DM has positive effect on developing the complication of DM as DN and this difference were statistically significant as shown in table .1and from analysis of this result there are great proof that chronic hyperglycemia and longer duration of DM are considered risk factor for developing diabetic complication .The estimated of HbA1c in three groups which participating in this study is shown in Figure 1.

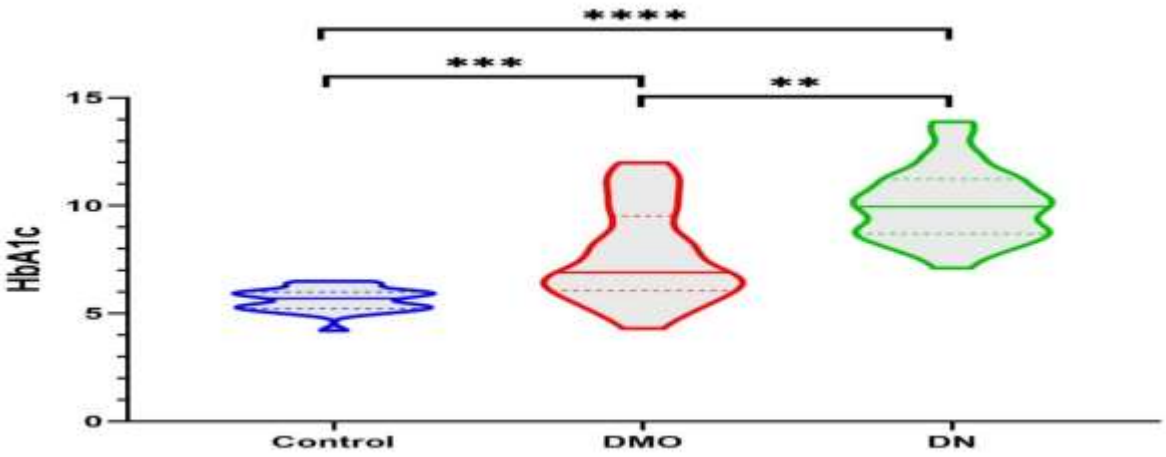

Figure -1: HbA1c distribution in diabetic patients and control

Duration of DM considered as risk factor association with increase susceptibility and progression of complication in DM, the effect of duration and developing of DN were representing in Figure 2.

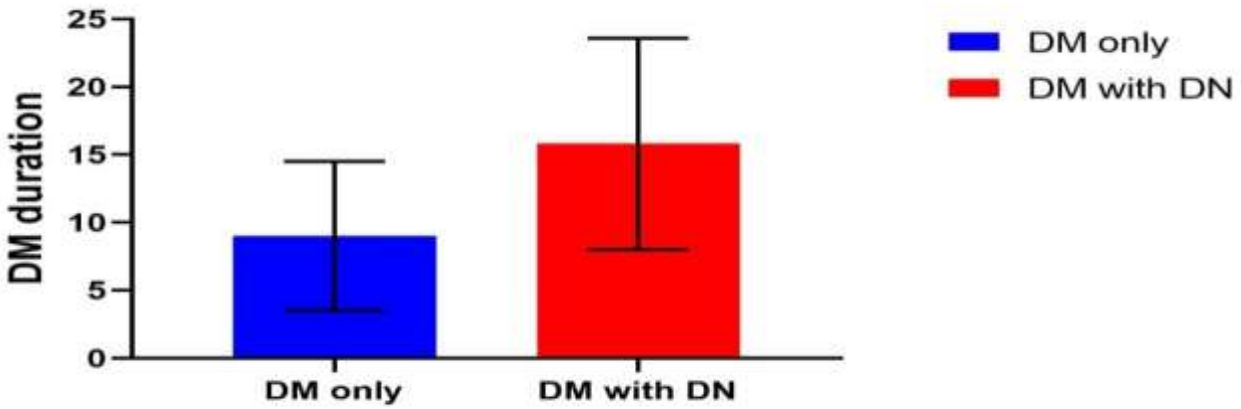

Figure -2: The relationship between DN and duration of DM

There are other Studies which confirmed the participating of chronic hyperglycemia and poor glycemic control as risk factor for developing diabetic complication [11, 16]. The result of present study revealed that deterioration in eGFR in patient with DM give indication for developing complication of DM as in DN. The decline in eGFR between patient with DN and DM without DN are representing in Figure 3:

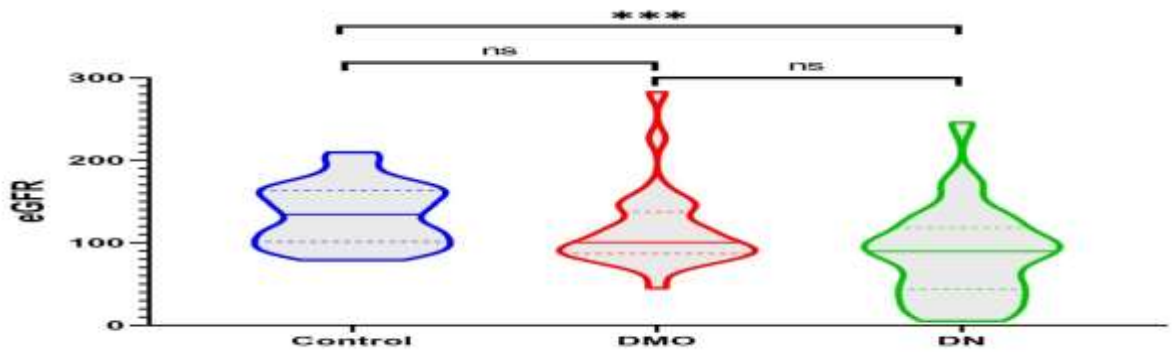

Figure- 3: Violin plot of eGFR (individual p-value calculated using Dunn's multiple comparisons test)

Most studies revealed that decline in eGFR among diabetic patient considered as hallmark for deter ion in kidney function [17, 18].In this study estimation of protein in urine revealed significant difference in DN with compares DM patients without DN as representing in Figure 4:

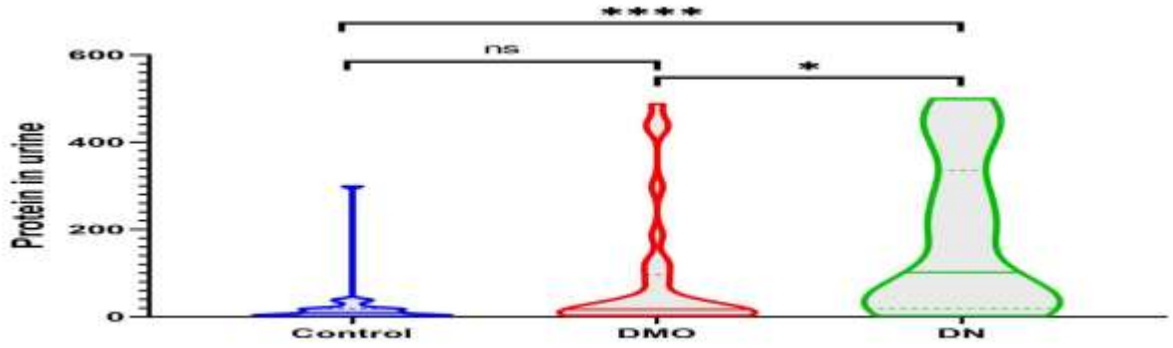

Figure -4: Violin plot of protein in urine (individual p-value calculated using Dunn's multiple comparisons test)

The assessment of endocan in DN and DM without nephropathy and control group were representing in Table 2.

Table 2: Assessment of ednocan in studied groups

Variables	Control	DM only	DM with DN	p-value
Number	30	30	30	-
Endocan (pg/ml)				
Mean ± SD	122.7±45.7	206.3±91.5	327.9±106.9	<0.001
Median (IQR)	111.0 (84.75-157.0)	177.5 (135.8-248.8)	323.5 (254.3-381.0)	

IQR: interquartile range (25%-75% percentile), GFR: glomerular filtration rate, DM: diabetic mellitus, DN: diabetic nephropathy, SD: standard deviation
p-value calculated using Kruskal – Wallis test

The difference in the level of endocan among group participating in the study were representing in Figure 5.

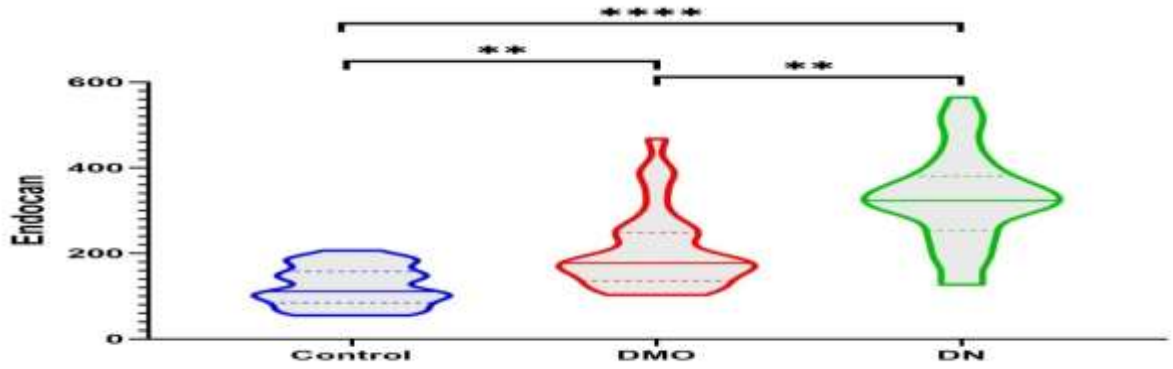

Figure 5: Violin plot of endocan (individual p-value calculated using Dunn's multiple comparisons test

The markers which were studied in this work and could be used to predict DN were revealed in Table 3:

Table 3: validity analysis of various markers as predictor of DN from control

Marker	Cut-off	SN	SP	AC	PPV	NPV
Endocan	>207	86.7	100	93.1	100	87.5
HbA1c	>6.5	83.3	100	91.4	84.8	100
Protein in urine	>40	73.3	96.4	84.5	95.7	77.1
Protein creatinine ratio	>239	76.7	96.4	86.2	95.8	79.4
eGFR	≤90	56.7	89.3	73.0	85.0	65.8
SN: sensitivity, SP: specificity, AC: accuracy, PPV: positive predictive value, NPV: negative predictive value						

Endocan, according to analysis of the above data in table 4 can be considered a good marker to predict diabetic nephropathy and has correlation with eGFR and PCR, as shown in Figure 6 and 7:

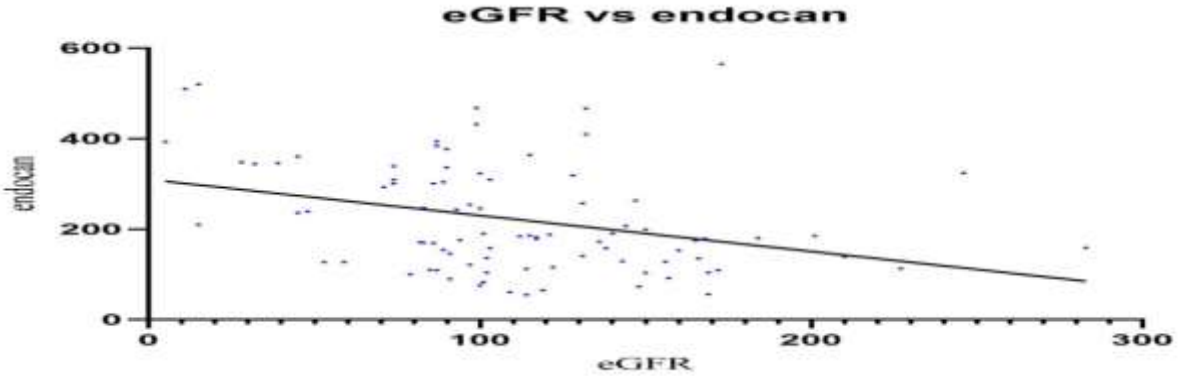

Figure-6: Negative correlation between eGFR with endocan

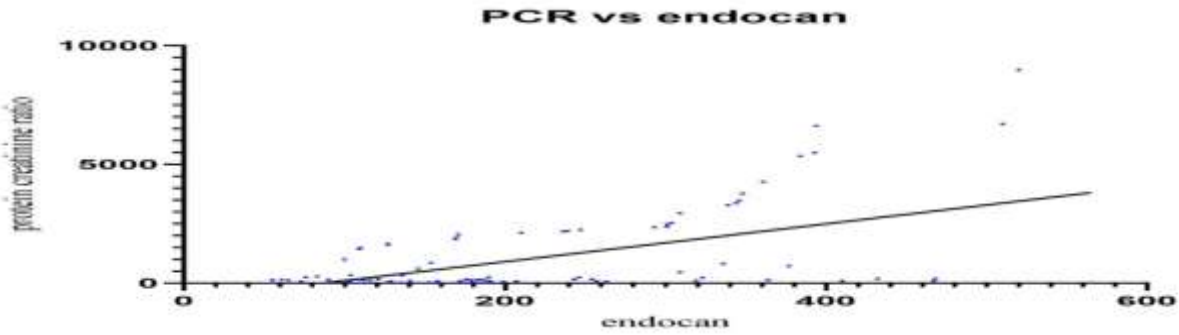

Figure-7: Positive correlation between PCR with endocan

Proteinuria is considered as hallmark for detection and evalution damage to kidney and considered valid estimation for assessment kidney damage in disease as recognized as an independent risk factor for cardiovascular and renal disease and as a predictor of end organ damage used in the early detection of several specific conditions, e.g., preeclampsia, diabetic nephropathy, and nephrotoxicity attributable to drugs. Studies indicate that protein in urine are hallmark for DN in which in patients with type 2 diabetes with heavy proteinuria revealed the rapid decline of renal function [19, 20].

Endocan is a newly identified proteoglycan released from endothelium, stimulating angiogenesis and when increased, indicates endothelial activation (inflammation).These findings implied that endocan was involved in important pathophysiologic processes in the kidney. Normally, the negatively charged basement membrane in a healthy glomerulus can prevent endocan from passing through the glomerular filtration barrier because of the presence of dermatan sulfate, an important component of endocan, which is also highly negatively charged [21]. The assessment of DN through application of endocan to predict the changes in glomerulo filtration in kidney due to type 2DM to evaluate the degree of severity and progression of DN among patient with type 2 DM in this study were in agreement with other studies [22, 25].

Acknowledgement

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