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Original Research Article

The Function of Urinary Podocalyxin in the Early Detection of Diabetic Neuropathy

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Abstract:

Background: A series of metabolic disorders collectively known as diabetes mellitus (DM) are characterized by persistently elevated blood sugar levels. One of the most common side effects of diabetes and the main factor contributing to end-stage renal disease is diabetic nephropathy. Diabetes is a complicated chronic illness that calls for ongoing medical supervision along with multifactorial risk reduction techniques to achieve optimal glycemic control. Self-management education and care are essential for DM patients in order to avoid both short-term and long-term problems. One of the most common side effects of diabetes and the main factor contributing to end-stage renal disease is diabetic nephropathy. Clinically, proteinuria and increasing renal insufficiency are seen. Since podocyte injury has been linked to DN early on, estimating the amount of podocalyxin in urine can be used to determine podocyte injury.

Aim: The objective of this study was to quantify the amounts of urine podocalyxin and assess the biomarker's sensitivity and specificity for DN early detection.

Material and Method: The Department of Biochemistry carried out a cross-sectional investigation. This study comprised 75 participants with T2DM in total. The two categories that comprised the study population were patients with T2DM who were diagnosed with DN (n = 25) and patients with T2DM who were not diagnosed with DN (n = 50).

Results: According to our findings, there were significant differences between the subgroups in terms of all clinical and laboratory data that were looked at, as well as age, body mass index (BMI), glycated hemoglobin (HbA1c), blood glucose, UM/CR, total proteins, albumins, blood urea, serum creatinine, and eGFR. With the exception of albumin and GFR, which showed noticeably lower results, cases in the current study demonstrated significantly greater results for all examined parameters.

Conclusion: According to our findings, podocalyxin is a reliable and sensitive marker of diabetic nephropathy that can be used to both forecast the prognosis of the condition and detect it early. The study's findings, which include a large percentage of T2DM normoalbuminuric people with raised u-PDX levels and a higher diagnostic accuracy of u-PDX than microalbumin in DN patients, suggest that u-PDX may be useful in the early diagnosis of DN.

Keywords: Diabetic Nephropathy, Microalbumin, Podocalyxin, End-Stage Renal Disease and Glomerular Filtration Barrier (GFB).

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Introduction

The most prevalent and dangerous consequence in individuals with type 2 diabetes mellitus (T2DM) is diabetic nephropathy (DN). It is significant to remember that about 40% of T2DM patients get DN.

Proteinuria, hypertension, and a gradual decline in kidney function are the hallmarks of diabetic kidney disease (DN). Since DN is a major contributor to end-stage renal disease (ESRD), it has raised concerns about public health since more patients need renal replacement therapy, such as dialysis or transplantation. [1,2] Progress toward end-stage renal disease (ESRD) can be slowed down by early DN identification and treatments.

Since about 3% of individuals with newly diagnosed T2DM already have overt nephropathy, screening for DN should begin as soon as possible after the diagnosis. [3] The primary causes of death are non-communicable diseases, which mostly include diabetes mellitus, hypertension,

malignancies, and diseases of the heart and brain. Non-communicable illnesses account for 63% of deaths worldwide and 80 per cent of deaths in China annually. [4] China's population is aging at a rate that is rapidly increasing in the twenty-first century.

Approximately 20% of the elderly population has diabetes, and over 45% of the elderly population has prediabetes. Diabetes-related kidney disease is referred to as DKD. In China, between 20 and 40 percent of diabetic individuals have DKD. Additionally, DKD is now the primary cause of end-stage renal disease. [5,6]

Human podocytes, also known as glomerular visceral epithelial cells, are highly specialized, terminally differentiated cells that line the glomerular basement membrane (GBM) and help to determine the glomerular filter's final dimensions and charge barrier, which effectively stops protein leakage into the urine. [7]

Also, it has been demonstrated to be functionally and structurally injured in the natural history of diabetic nephropathy. [8] According to electron microscopy, podocyte injuries are characterized by distinct morphologic alterations, such as vacuolization, loss of slit diaphragms, effacement of the foot process, and the most serious lesion of podocytes, detachment from GBM into urine space. [9] Podocalyxin (PCX) is a transmembrane protein that is located in the apical wall of glomerular podocytes. [10] The function of PCX is to maintain the shape and slit diaphragm of the podocyte. [11] Hara et al. reported that is PCX shed from injured podocytes into urine, as small vesicles that appear on the tip of microvilli in podocytes. [12] One of the main contributing factors to the pathogenic pathways of DN is podocyte damage.

Podocyte enlargement, foot process effacement, separation from the glomerular basal membrane, epithelial-mesenchymal trans-differentiation (EMT), and apoptosis are common symptoms of podocytopathy. [13,14] Proteinuria results from the early stages of diabetic kidney disease (DN), which are marked by a progressive decrease in podocyte numbers, the loss of their foot processes, podocyte shedding through urine, and damage to the filtration slit-diaphragm. [15,16] As a result, podocytes and the particular proteins they produce in urine may be thought of as possible indicators for the early diagnosis of DN. The majority of studies examining novel markers for the early diagnosis of DN are concentrated on podocyte-specific proteins, including mindin, nephrin, synaptopodin, podocin, and podocalyxin. [17,18] Podocytes are visceral epithelial cells that contribute to the formation of the glomerular filtration barrier (GFB).[19] As a sialo glycoprotein and a significant part of the podocyte glycocalyx, PDX creates the electrostatic barrier that separates plasma proteins from GBM. [20,21] Urine contains PDX due to podocyte damage and shedding, which makes it a potentially helpful marker for DN early detection. [22]

Material and Methods

The study was a cross-sectional study has been conducted in the Department of Biochemistry. A total of 75 patients with T2DM were included in this study. The study population consisted of two subgroups, the first subgroup of patients with T2DM who were diagnosed with DN (n = 25), and the second subgroup of patients with T2DM who were not diagnosed with DN (n = 50). As a control group was used a total of 25 healthy subjects. Inclusion criteria for patients with T2DM and DN were clinically manifested DN, represent by certain levels of kidney function – an abnormality in serum creatinine levels and glomerular filtration rate (GFR), presence of macroalbuminuria or microalbuminuria and elevated arterial blood pressure. All Participants received clear instructions from the investigators about the study and signed the written form of informed consent.

Inclusion Criteria

- Age: 20 -70 years.
- Sex: males and females. Known clinically and biochemically diabetic patients with diabetic nephropathy.

Exclusion Criteria

- Patients with severe organ dysfunction such as heart, liver, kidney, and others. The criteria for evaluating organ dysfunction were put as follows: cardiac function grade III or higher NYHA, chronic kidney disease stage 3 or higher (according to the criteria by Kidney Diseases Improving Global Outcomes, KDIGO), alanine aminotransferase ALT above the upper limit of normal 2.5 times;
- Comorbidities such as hypertension
- Type 1 diabetes, special types of diabetes, and gestational diabetes;
- Acute complications such as ketoacidosis, hypertonic coma, infection, and other states of stress.

All patients with T2DM (n=75) were divided into three subgroups according to urinary microalbumin to creatinine ratio (UM/CR):

- patients with normoalbuminuria – UM/CR <30 mg/g (n = 50),
- patients with microalbuminuria – UM/CR 30–300 mg/g (n = 20)
- patients with macroalbuminuria – UM/CR >300 mg/g (n = 5)

The classification was performed according to guidelines of KDIGO – kidney disease: Improving Global Outcomes guidelines from 2012. [23] The subgroup of patients with T2DM without diagnosed DN consisted of 23 patients with microalbuminuria and 26 with normoalbuminuria, while a subgroup of patients with T2DM with diagnosed DN consisted of 5 patients with macroalbuminuria and 21 with microalbuminuria.

Urine Sample Collection:

Urine samples (10 ml) were collected in plastic tubes, without additives. First in fresh urine samples was performed chemical analysis by using urinary strips, then microalbumin levels were by turbidometric method, and creatinine levels by Jaffe reaction were measured on the biochemical analyzer Chem Well (2910 Awareness Technology, Inc.). The rest of the urine samples were clarified by centrifugation at 3,000 rpm, 10 min, and frozen in Eppendorf tubes at –80°C until estimation.

Blood Sample Collection:

Blood samples were drawn from an antecubital vein in the morning after overnight fasting. Samples were centrifuged at 3,000 rpm, for 10 minutes, and collected in Eppendorf tubes. The UM/CR was defined as the urinary albumin value divided by the urinary creatinine concentration (mg/g). Glomerular filtration rate (eGFR) was estimated using the Cocroft and Gault formula. [24]

The blood samples were collected for the estimation of urea, creatinine, glucose, total protein, and albumin. Blood parameters were measured photometrically on the biochemical analyzer Chem Well.

Medical history and information on sex, age, height, weight, duration of the disease, blood pressure, and glycemic control were obtained by completing patients' questionnaires.

Statistical Analysis

The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using the SPSS program (version 18 for windows, SPSS Inc. Chicago, IL, USA).

One-way analysis of variance (ANOVA) was used to compare the results of all examined groups followed by an LSD test to compare statistical differences between groups. P value ≤ 0.05 was considered statistically significant.

Result:

Our results showed that there was a significant difference among subgroups regarding all examined clinical and laboratory data, age, duration of disease, body mass index (BMI), blood glucose, glycated hemoglobin (HbA1c), UM/CR, total proteins, albumins, blood urea, serum creatinine, and eGFR.

Table 1: Comparison of clinical and laboratory data among subgroups of patients with T2DM divided according to UM/CR and healthy subjects

	Macro albuminuria	Microalbuminuria	Normo-albuminuria	Healthy subjects
Age (years)	52.1 ± 9.3	54.1 ± 5.2	55.3 ± 5.7	45.6 ± 8.2
Duration of disease (years)	12 ± 5.7	6.3 ± 5.3	3.7 ± 3.1	/
BMI (kg/m^2)	26.4 ± 4.3	26.7 ± 2.3	26.6 ± 3.0	22.5 ± 2.7
Glucose (mmol/L)	7.7 ± 2.7	6.1 ± 1.5	5.7± 1.2	3.1 ± 1.1
UM/CR (mg/g)	328.6 ± 150.5	80.4 ± 53.7	10.1 ± 5.6	13.1 ± 13.2
HbA1c (%)	6.8 ± 0.7	6.2 ± 1.3	5.5 ± 1.1	3.5 ± 0.3
Total proteins (g/L)	68.1 ± 6.1	62.1 ± 10.4	60.2 ±09.3	70.5 ± 3.5
Albumin (g/L)	37 ± 3.1	35.2 ± 6.1	34.3 ± 5.3	44.2 ± 1.8
Urea (mmol/L)	6.7 ± 1.1	7.2 ± 4.2	5.6 ± 1.5	3.2 ± 1.1
Creatinine (µmol/L)	104.3 ± 5.3	78.4 ± 13.1	72.6 ± 11	73.1 ± 12.3
eGFR (ml min^{-1} 1.73 m^{-2})	46.1 ± 10.4	62.1 ± 17.5	66.4 ± 10.4	88.2 ± 3.8
u-PDX (ng/ml)	66.4 ± 15.3	55.5 ± 24.1	48.1 ± 52.3	25.1 ± 10.7

With the exception of albumin and GFR, which showed noticeably lower results, cases in the current study demonstrated significantly greater results for all examined parameters.

When comparing cases with respect to the degree of albuminuria, it was found that those with macroalbuminuria had significantly greater levels of every measure, with the exception of serum albumin and GFR, which had significantly lower levels.

Additionally, all values were significantly greater in instances with microalbuminuria, with the exception of serum albumin and GFR, which were significantly lower.

Table 2: Comparison of clinical and laboratory data among patients with T2DM divided according to diagnosed DN and healthy subjects

	T2DM patients with diagnosed DN	T2DM patients without diagnosed DN	Healthy subjects
Age (years)	54.6 ± 8.1	56.8 ± 5.6	45.7 ± 8.2
Duration of disease (years)	9.5 ± 5.8	3.7 ± 2.3	-
BMI (kg/m^2)	25.7 ± 3.1	26.5 ± 3.1	23.5 ± 2.6
Glucose (mmol/L)	7.2 ± 2.5	5.7 ± 1.2	3.1 ± 1.1
UM/CR (mg/g)	203.3 ± 204.1	15.7 ± 16.8	13.1 ± 14.2
HbA1c (%)	6.5 ± 1.4	5.7 ± 1.1	3.6 ± 0.3
Total proteins (g/L)	66.4 ± 8.8	58.2 ± 9.6	70.5 ± 3.6
Albumin (g/L)	37.2 ± 8.2	34.5 ± 5.2	44.3 ± 1.6
Urea (mmol/L)	6.2 ± 4.2	5.6 ± 1.1	3.2 ± 1.1
Creatinine (µmol/L)	91.1 ± 16.1	71.8 ± 10.1	72.1 ± 12.4
eGFR (ml min^{-1} 1.73 m^{-2})	58.1 ± 19.6	65.1 ± 10.3	89.2 ± 4.7
u-PDX (ng/ml)	68.5 ± 72.1	43.2 ± 20.1	25.1 ± 10.8

Table 2 displays the comparison of clinical and laboratory data between patients classified as healthy subjects and those with DN diagnoses. The study found statistical significance in the comparison of u-PDX levels between subgroups of patients based on the diagnosis of diffuse gingivitis and healthy volunteers. When comparing the level of u-PDX to healthy persons, all patient categories split based on UM/CR had considerably higher levels.

Discussion

A steady decline in GFR, high arterial blood pressure, and chronic albuminuria (>300 mg/d or >200 µg/min) verified at least twice, 3-6 months apart, characterize the clinical syndrome known as DN. [25] Microalbuminuria has long been regarded as the gold standard for DN early detection. But because of a few drawbacks, it cannot forecast DN with enough accuracy. Microalbuminuria is neither sensitive nor specific enough to identify DN in its early stages. Moreover, 30% of people with T2DM who have microalbuminuria may actually have normoalbuminuria; not all of them may develop ESRD.

According to recent research, microalbuminuria manifests itself when a considerable renal damage has already taken place. [26,27] For the purpose of early DN identification, it is therefore imperative to find more sensitive and specific indicators than microalbuminuria. Elevated levels of particular urine biomarkers can be utilized as early indicators of diabetic DN in T2DM patients, even prior to the onset of severe microalbuminuria. [28] Since DN is thought to be a podocytopathy, measuring particular podocyte proteins, including u-PDX, can be used to evaluate podocyte damage. All three of these components are injured when microalbuminuria is present in GFB; however, podocyte injury is indicated specifically by u-PDX, a particular podocyte marker that is independent of the other two components (glomerular basement membrane and endothelial cells). It seems plausible that podocyte cell destruction and the onset of PDX in urine occur prior to microalbuminuria in diabetic kidney disease. [29]

Over the past 15 years, the prevalence of diabetes has increased fivefold, making it a rapidly growing global health concern. Nephropathy, one of the primary consequences of diabetes, is also getting worse concurrently. Prolonged proteinuria, high arterial blood pressure, and a reduction in renal function are the hallmarks of diabetic nephropathy, a debilitating chronic condition. When the albumin excretion rate (AER) continuously surpasses 300 mg in a 24-hour urine collection, overt nephropathy is diagnosed. [30]

Damage to the glomerular filtration barrier is the cause of the decrease in renal function and glomerular filtration rate. This is accompanied morphologically by the thickening of the basement membrane and build-up of extracellular matrix within the glomeruli, as well as a decline in tubular reabsorption and a rise in circulating creatinine. In the end, diabetic nephropathy results in severe scarring and an end-stage kidney that is non-functional and necessitates dialysis and kidney transplantation treatments. The first identifiable sign of nephropathy, known as microalbuminuria, is defined as a persistent AER between 30 and 300 mg/24 hours. It occurs before overt nephropathy. It is also a highly reliable indicator of type 1 diabetes-related nephropathy down the road. [31]

Lioudaki, et al.2015 [32] conducted a study at the Diabetes Clinic of the University Hospital of Heraklion, Crete. They studied 80 patients with type 2 DM and stated that both serum creatinine and urea levels were significantly correlated with DM Shoji et al.2016 [33] evaluated whether urinary podocalyxin levels were associated with the urinary albumin-to-creatinine ratio (ACR). They found that these levels were significantly associated with ACR.Urinary podocalyxin levels were considerably

higher in patients with microalbuminuria than in those with normoalbuminuria when the participants were split based on their levels of normo albuminuria, microalbuminuria, or macro albuminuria. Patients with macroalbuminuria had greater urinary podocalyxin levels than those with normoalbuminuria, but not higher levels than those with microalbuminuria.

We may have two limits to our research. The study's cross-sectional design and its small sample size are the first two factors. Furthermore, this work doesn't address whether u-PDX is a component of a causative process or if it will be able to predict DN in the future. We require a prospective calculation of u-PDX in patients with T2DM and normoalbuminuria in order to respond to these queries.

More extensive, prospective research is advised to validate the clinical usefulness of u-PDX as a marker for the early identification of DN. If further large prospective studies research confirms that u-PDX is an earlier and more sensitive and specific marker of pre-clinical DN than microalbuminuria, it could be implemented in laboratory practice as a routine marker for early detection of DN.

Conclusion

According to our findings, podocalyxin is a reliable and sensitive marker of diabetic nephropathy that can be used to both forecast the prognosis of the condition and detect it early. The study's findings, which include a large percentage of T2DM normoalbuminuric people with raised u-PDX levels and a higher diagnostic accuracy of u-PDX than microalbumin in DN patients, suggest that u-PDX may be useful in the early diagnosis of DN. We can draw the conclusion that, when it comes to the early detection of DN, u-PDX may be a more helpful, sensitive, and specific marker than microalbuminuria.

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