Original Article

Evaluation the Levels of Pentosidine and Vitamin D3 in Diabetic Nephropathy

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Abstract

Background: Diabetic nephropathy (DN) is one of the most dangerous diabetic chronic microvascular complications and the major cause of end-stage renal disease. **Objectives:** The current study aimed not only to determine the levels of vitamin D3 and pentosidine as biomarkers in the serum of DN patients group and compared with diabetes mellitus individuals as controls, but also to investigate the variations in the levels of vitamin D3 and pentosidine by DN disease progression. **Materials and Methods:** This study comprised 120 type 1 diabetes mellitus patients who were separated into three groups based on estimated glomerular filtration rate (eGFR) and creatinine levels: early-stage DN patients (eGFR = 40–90), end-stage DN patients (eGFR < 40), and diabetic patients with normal renal function as a control group (eGFR > 90); 40 individuals for each. The concentration of pentosidine was measured using an enzyme-linked immunosorbent assay kit from the Al-Shkairate establishment for Medical Supply in Jordan. The vitamin D3 concentration was determined by a competitive chemiluminescence immunoassay using the biomedical engineering kit for the Maglumi 800 from Shenzhen New Industries. The fasting blood glucose (FBG), urea, and creatinine levels were assessed using an enzymatic method. **Results:** The level of pentosidine was found to be significantly higher in DN patients (early and end stages) than in the control (diabetic patients with normal renal function) group. Also the results revealed a negative correlation between pentosidine and eGFR in DN patients for both early- and end-stages. Vitamin D3 was declined in DN patients group. **Conclusion:** It is it possible to conclude that pentosidine is correlated with the progression of the disease and it might be used as one of the most reliable biomarkers for predicting renal function.

Keywords: Advanced glycosylation end product (AGEs), diabetic complications, diabetic nephropathy (DN), pentosidine, vitamin D

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases and is the leading cause of end-stage renal disease (ESRD) in patients , it is a metabolic disorder with inappropriate hyperglycemia either due to an absolute or relative deficiency of insulin secretion from beta cells of Langerhans of endocrine part of pancreas or reduction in the biologic effectiveness of insulin or both or the outer receptors are malfunctioning. It is one of the most common non-communicable diseases worldwide and is a growing concern.[1] Measuring C-peptide as in initial stages of diabetes gives information that may help distinguish type I from type II diabetes.[2] Type 1 diabetes is the most common type of diabetes in young people and adolescents, accounting for 5%–10% of all diabetes occurrences. It is uncommon throughout the first 9 months of life and is most common around the age of twelve. Diabetes type 1 is an autoimmune disorder wherein cytotoxic CD8-T lymphocytes target and destroy pancreatic B cell islands.[3] Diabetes, particularly when uncontrolled for an extended length of time, was already associated to the progression of both macrovascular and microvascular problems.[4] Diabetic retinopathy, diabetic nephropathy (DN), and diabetic peripheral neuropathy (DPN) are the three main types of microvascular problems. Diabetes is also associated with an increase in large vessel atherosclerotic disease, including cardiac, peripheral

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vascular disease, and cerebral diseases. Controlling dyslipidemia and hypertension is a critical step in lowering the risk of complications.[5]

Diabetes complications are caused by a number of the pathophysiological changes that occur inside the vascular wall. Among these pathways, the buildup of advanced glycation end-products (AGEs),[6] due to nonenzymatic glycation is thought to play a crucial role in increasing inflammation and endothelial dysfunction.[7]

DPN is a well-known consequence of diabetes. DPN is the most prevalent cause of neuropathy in the world, affecting up to 50% of patients along the clinical course. Patients with DPN are 10–20 times more likely than those without DPN to require limb amputation.[2]

DN is a primary cause of end-stage renal disease and is among the most recognized diabetic chronic microvascular consequences. The primary risk factors for the occurrence of DN are chronic hyperglycemia and elevated blood pressure.[8] Patients with cardiovascular disease and neurologic illnesses are at a significant risk of developing end-stage renal disease (ESRD) and dying. The traditional presentation of DN is defined by hyperfiltration and albuminuria in the early stages, followed by a gradual loss in renal function.

Advance glycation end-products are heterogeneous molecules composed of free amino groups from proteins, nucleic acids, or lipids coupled with reducing sugars. AGEs, a class of chemicals formed by a nonenzymatic interaction between reducing sugars and amine residues. Pentosidine is one of the most commonly chemically classified AGEs.[9] Pentosidine is an AGE that identifies oxidative damage produced by hyperglycemia exposure in tissues. The aggregation in AGEs and inflammatory also contribute to the development of diabetic nephropathies.[10]

Vitamin D is a fat-soluble vitamin and its role in the prevention of DN has received particular emphasis.[8] Both animal studies and clinical trials have shown a negative correlation of low vitamin D levels and DN risk, and vitamin D or its active derivatives have been shown to reduce endothelial cell damage, decrease proteinuria, decrease renal fibrosis, and therefore slow DN progression.[11]

The purpose of this study is to assess the levels of pentosidine and vitamin D in DN patients at both the early- and end-phases, and to compare them to non-nephropathy diabetic patients as a control group. In addition, the current study aims to assess the specificity and sensitivity of pentosidine like a biomarker for DN patients.

Materials and Methods

Study design

The research was planned as a case–control study.

Fisher's exact test for sample size was used to determine sample size.

The patients were divided into three groups: 40 with diabetes who were receiving insulin treatment without complications, 40 with diabetes who were receiving insulin treatment along with a renal drug, and 40 with diabetes who were receiving insulin prescribed for dialysis prior to the first kidney wash. Subjects with T2DM, pregnancy, congestive heart failure, systemic lupus erythematous, and polycystic kidney disease were excluded from the study.

Population under investigation

The research featured 120 participants divided into three groups:

1) Group 1: 40 type 1 diabetes patients with early-stage nephropathy (20 male and 20 female) participants with age ranging from 30 to 60 years.
2) Group 2: 40 type 1 diabetes patients with end-stage nephropathy (20 male and 20 female) participants with age ranging from 30 to 60 years.
3) Group 3: 40 type 1 diabetes patients with normal renal function (20 male and 20 female) participants with ages ranging from 30 to 60 years.

Statistical analysis

To detect the effect of the various groups (end, early, and control) on research parameters, the Statistical Analysis System 2018 program was utilized. To make a meaningful comparison between means, the *t* test was performed. In this study, the correlation coefficient between variables was estimated, as well as the sensitivity and specificity of parameters in different groups (ROC analysis).

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 4 in 06/07/2022 to get this approval.

Determination of serum pentosidine (PTSD) level

Principle

Using ELIZA kit, serum pentosidine was determined.

Results

General characteristics of the study

Age and gender distribution in patients and controls

The present study included 120 people. They were separated into three groups: type 1 diabetes patients with early-stage

nephropathy (20 male and 20 female patients) with age ranging from 30 to 60 years (52.36±1.08). T1 DM patients with end-stage nephropathy (20 male and 20 female) with age (30–60) years range (59.40±0.94) and type 1 DM patients with normal renal functions (47.94±1.01) as shown in Figure 1.

Body mass index of participants

The current study's findings demonstrated a significantly higher *P*-value ≤ 0.001 for body mass index (BMI) among end-stage diabetic nephropathies and early-stage diabetic nephropathies patients when compared to non-nephropathy diabetic individuals as controls, as shown in Table 1.

The current investigation found a significant rise in blood glucose levels *P*-value ≤ 0.001 in the patient groups compared to control patients. Also, substantial increases *P*-value ≤ 0.001 in urea and creatinine levels were reported in the diabetic nephropathies groups for both end- and early-stages when compared to diabetic non-nephropathy individuals with normal renal function as control groups. When DN early- and end-stages patients are compared with diabetic non-nephropathy individuals control group, their eGFR values decline significantly [Figures 2 and 3].

Comparison of pentosidine value between different stages of diabetics nephropathies patients and diabetic with normal renal function control

Table 2 shows the mean ± SE for pentosidine values in different phases of DN patients and diabetics with normal renal function control.

Vitamin D3 among diabetes nephropathies patients group and control groups

According to the current study findings, DN early- and end-stages patients group had significantly lower blood concentrations of vitamin D3 than the non-nephropathy diabetic control group (*P*-value ≤ 0.001), as shown in Table 3.

Correlation between studied biochemical parameters

The result of the current study shows a statistically significant negative correlation between eGFR and pentosidine at two stages of patients group (early and end) ($P \leq 0.01$), $R = -0.44, -0.74$.

Discussion

DN is one major complication of uncontrolled diabetic mellitus.

As indicated in Table 1, the current study's findings revealed a significant difference (*P*-value < 0.01) in the ages of DN patients in both the early- and late-stages when compared to non-nephropathies persons in the control groups. The diabetics complication progresses when the disease duration increases. That supports the assertion made by Baek *et al.*[12] that renal function diminishes with disease duration in persons with type 1

Table 1: Mean of BMI among diabetes nephropathies patients group and control groups

Variable	Group	Mean ± SE	*P*-value
BMI (kg/m^2)	Early-stage	24.08±0.42	0.879
	Control	24.01±0.64	
	End-stage	21.75±0.24	0.0001**
	Control	24.01±0.64	
	Early--stage	24.08±0.42	0.0001**
	End-stage	21.75±0.24	

** $P \leq 0.01$

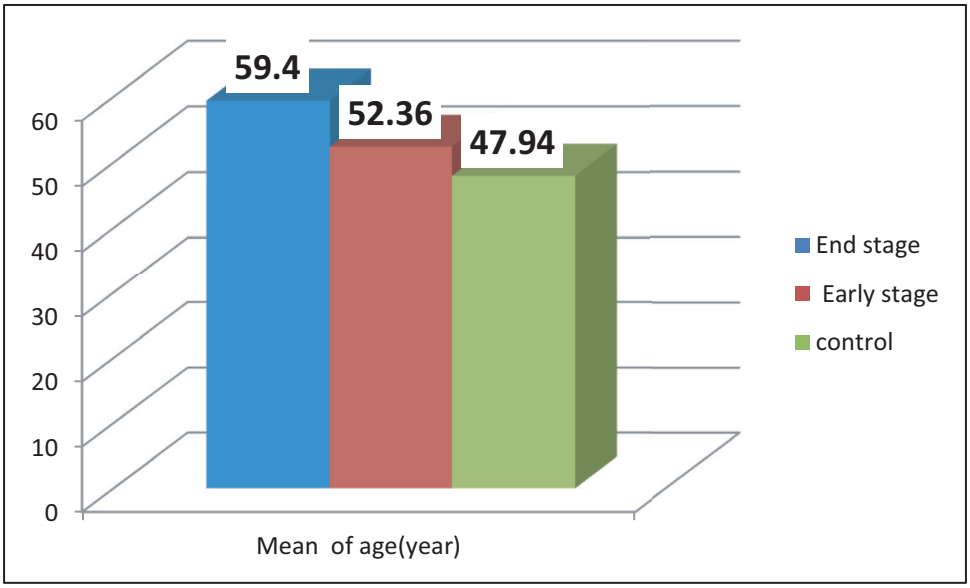

Figure 1: Mean of age among diabetes nephropathies patients group and control groups

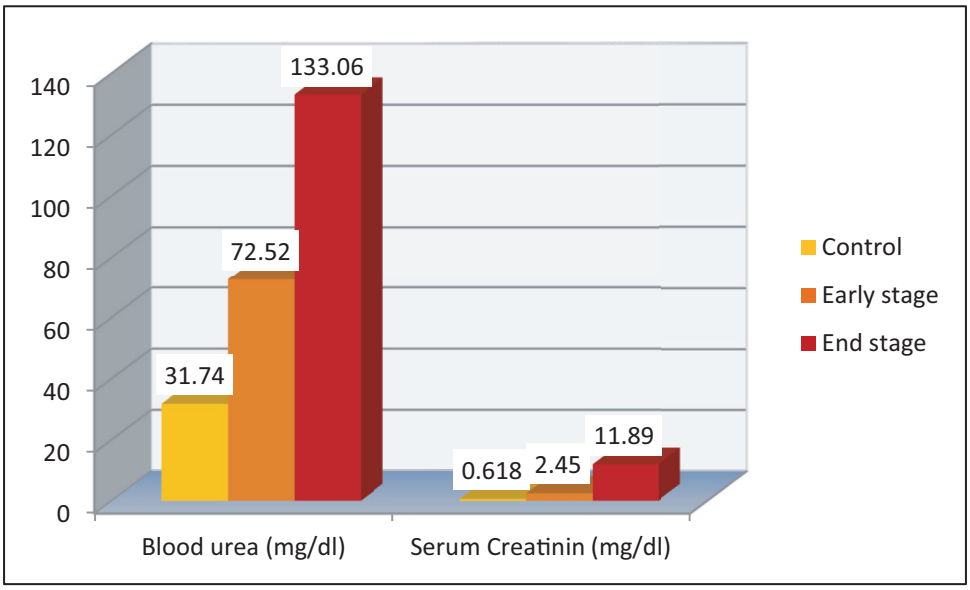

Figure 2: Mean of blood urea and serum creatinine among diabetes nephropathies patients group and control groups

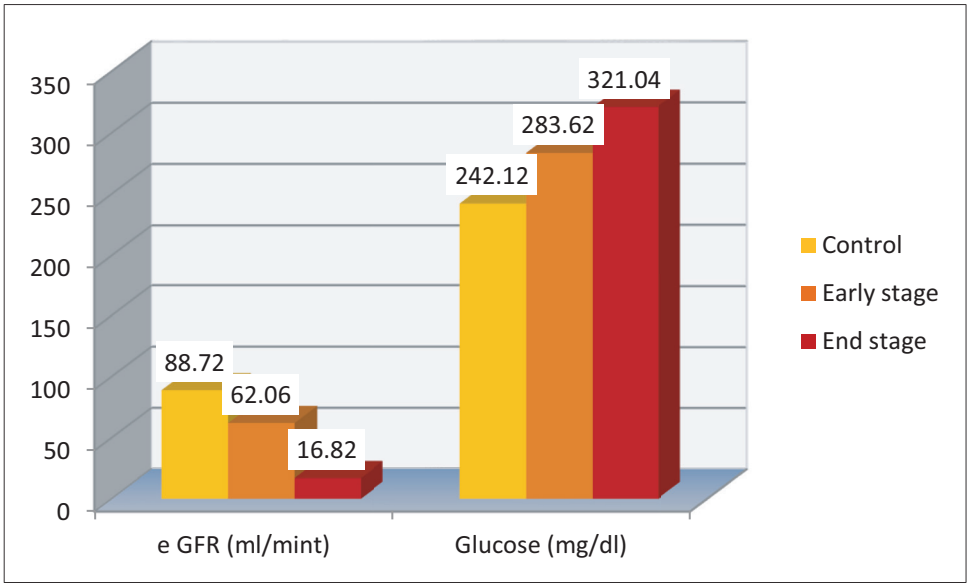

Figure 3: Mean of eGFR and glucose among diabetes nephropathies patients group and control groups

Table 2: Mean of pentosidine among diabetes nephropathies patients group and control groups

Variable	Group	Mean ± SE	*P*-value
Pentosidine (ng/mL)	Early-stage	192.58 ± 6.21	0.0001**
	Control	128.21 ± 2.70	
	End-stage	303.41 ± 6.76	0.0001**
	Control	128.21 ± 2.70	
	Early-stage	192.58 ± 6.21	0.0001**
	End-stage	303.41 ± 6.76	

** $P \leq 0.01$

Table 3: Mean of vitamin D3 among diabetes nephropathies patients group and control groups

Variable	Group	Mean ± SE	*P*-value
Vitamin D3 (ng/mL)	Early-stage	22.44 ± 1.06	0.0001**
	Control	32.63 ± 0.78	
	End-stage	11.58 ± 0.65	0.0001**
	Control	32.63 ± 0.78	
	Early-stage	22.44 ± 1.06	0.0001**
	End-stage	11.58 ± 0.65	

** $P \leq 0.01$.

diabetes mellitus diagnosed as a child or adolescent. Type 1 diabetes mellitus diagnosed at an early age is a risk factor for DN.

Diabetics patients in the early- and late-stages of nephropathy exhibited lower BMIs than diabetics with adequate renal function in the control group. That

lowering in BMI had many causes such as reduction of bone mass as a complication to chronic kidney disease, reduction in body fluid as renal problem, changes in nutrition habits in patients with chronic disease [Table 1]. According to O'Sullivan *et al.*,[13] patients with a relatively mild degree of chronic renal insufficiency are characterized by decreased lean body mass, bone mineral content, and basal energy expenditure. Furthermore, that study agreed with Carrero *et al*.,[14] in that while reduced appetite and dietary limitations lead to insufficient food intake (real under nutrition), other highly prevalent characteristics are necessary for the entire syndrome to occur. Uremia-induced changes include increased energy expenditure, prolonged inflammation, acidosis, and a variety of endocrine abnormalities that cause hyper metabolism and excess catabolism of muscle and fat.

The diabetics nephropathies patient early- and late-stages had higher glucose levels than non-nephropathy diabetic individuals, whereas eGFR diabetics nephropathies patients in both early and end stages were lower than other non-nephropathy diabetic individual group, as well as urea and creatinine levels were higher in DN patients than DM control, as shown in Figures 1–3. This finding is similar to the findings of Furuichi *et al*.[15] That is what they assumed. In addition to urinary albumin increase, nodular lesion and mesangiolysis were pathological characteristics of DN patients with rapidly declining kidney function represented by a decrease in eGFR. The research findings also agree with Samsu,[16] who claims that the definition of DN is based on existing standards and four major criteria: reduction in renal function, diabetic retinopathy, proteinuria, and a decrease in e GFR.

Another study found that an elevated amount of AGEs in serum is a sign of decreased renal function, which explains the involvement of AGEs in DN. Pentosidine is a well-known AGE intermolecular cross-link that is used as an indirect biomarker of AGEs. When the kidney serves as the primary pentosidine removal site, some studies found increases in serum pentosidine levels in diabetics with overt nephropathy. Renal failure may also lead to pentosidine buildup in the blood, as renal function is the major predictor of serum pentosidine. The current study, as shown in Table 2, shows elevated levels of pentosidine in both DN groups when compared to the DM control group, that is coherent with Hamid *et al.*,[17] who assume that the level of pentosidine was significantly higher in DN patients than in the DM control group. Furthermore, the findings demonstrated a significant positive correlation between pentosidine and creatinine and blood urea levels, as well as a negative correlation with eGFR as showed in Figures 4 and 5. Also our study agrees well with previous studies of Mamilly *et al*.[18] and Vodošek Hojs *et al.*[19]

Many animal and human researches suggest that vitamin D plays an important role in the development of DN. Nonetheless, the possibility of vitamin D's cytoprotective action and its influence on the reversal of preexisting renal damage remains unproven. There are now a few ideas on the underlying biochemical and genetic pathways, including the association of vitamin D and inflammation, oxidative stress, and extracellular matrix aggregation.[20] Vitamin D has been suggested to harbor multiple biological activities,

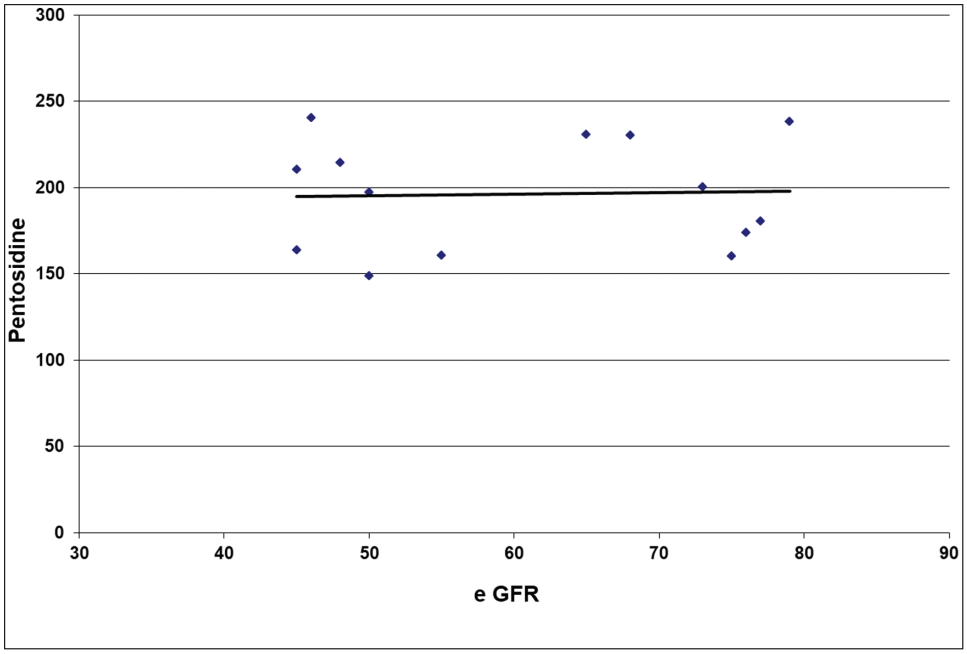

Figure 4: Correlation between eGFR and pentosidine in early-stage DN

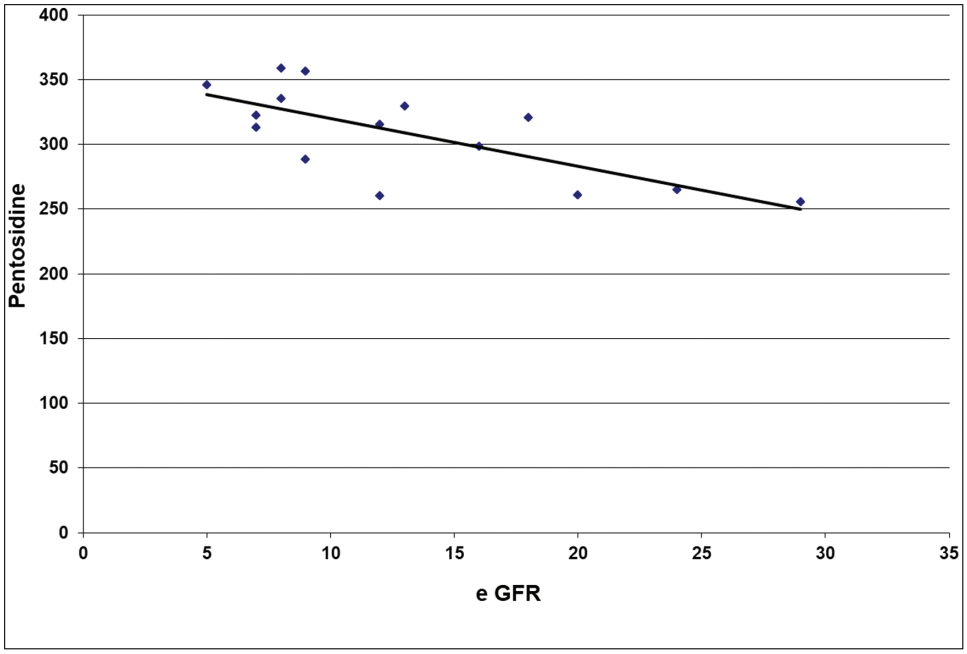

Figure 5: Correlation between eGFR and pentosidine in end-stage DN

among them the potential of vitamin D in the protection of diabetic nephropathy (DN) has attracted special attention. Both animal studies and clinical trials have documented an inverse correlation between low vitamin D levels and DN risk and supplementation with vitamin D or its active derivatives has been demonstrated to improve endothelial cell injury, reduce proteinuria, attenuate renal fibrosis, and resultantly retard DN progression. Vitamin D exerts its pharmacological effects primarily via vitamin D receptor. Vitamin D may reduce diabetic nephropathy not only by improving blood glucose and insulin levels, but also by modulating hexosamine pathways in kidney .Calcitriol & vitamin D receptor (VDR) signaling attenuated diabetic nephropathy and podocytes injury by restoring podocytes autophagy.

Table 3 shows that vitamin D3 levels in DN groups are lower than in control groups. Those findings are in agreement with Hong *et al.*[21] and Esfandiari *et al.*[22]

Conclusion

The BMI of DN patients was considerably lower compared to the DM control group, and it declines with increasing stages of DN due to malnutrition in chronic illness patients. Renal function parameters (urea, creatinine, and eGFR) revealed a decline that was influenced by the severity and duration of the disease. Pentosidine levels were higher in diabetic nephropathies patients when compared to control group, whereas vitamin D3 levels were lower. Pentosidine had a negative correlation with eGFR and might be used as a biomarker for DN.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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