https://doi.org/10.46389/rjd-2026-2090
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2026; volume 33, issue 1, pages 64–70

Original Article

Evaluation of pentosidine as an Advanced Glycation End product (AGE) and its association with microalbumin in Diabetic Nephropathy (DN)

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Received: 12 November 2025 / Accepted: 3 February 2026

Abstract

Diabetic nephropathy is a major end stage kidney disorder, and its timely diagnosis enables in preventing the morbidity and mortality related to it, thereby enhancing the quality of the patients suffering from the condition. Advanced glycated end-products (AGEs) which include pentosidine, are produced by covalent binding of amino groups with glucose or different saccharide derivatives during the non-enzymatic Millard reaction and might make a contribution to Cardiovascular Disease and other long-time complications in Chronic Kidney Disease. Microalbumin is a marker of diabetic nephropathy. To estimate levels of AGE serum pentosidine and microalbumin in diabetic nephropathy cases, diabetic controls and healthy controls. To study the association between serum pentosidine and urinary microalbumin in diabetic nephropathy. A case control study was conducted among 30 diabetic nephropathy patients and 30 diabetic patients and 30 healthy controls. Serum Pentosidine was analyzed by ELISA, microalbumin by turbidimetric method. ANOVA and Pearson's correlation and multiple regression analysis was done. Serum pentosidine and urine microalbumin were elevated in cases compared to other two groups. Statistically significant positive association was found between Pentosidine and microalbumin in diabetic nephropathy cases. Multiple regression analysis of Pentosidine and microalbumin with different independent variables was statistically not significant. Uncontrolled blood glucose increases the formation of advanced glycation end products, and it is positively correlated with the urine microalbumin level. Hence evaluation of plasma pentosidine, urine microalbumin may play a vital role in predicting the diagnosis and prognosis of prediabetic nephropathy and severe forms of microvascular and macro vascular complications in diabetes mellitus.

Keywords: microalbumin, pentosidine, diabetic nephropathy, advanced glycation end-product

Introduction

Diabetes mellitus (DM) incidence is increasing in Asian population. The micro and macro vascular complications of uncontrolled diabetes mellitus are the major causes suffering and increase in death rate [1]. Hyper-glycaemia induced oxidative stress causes vascular damage by linking four major metabolic pathways, advanced glycation end products (AGEs) formation, sorbitol pathway, the protein kinase C (PKC)-diacylglycerol (DAG) and the hexosamine pathways. The AGEs are formed by the reaction between reducing sugar and amino group of proteins, nucleic acids and lipids in a series of reactions called Millard reaction [2].

Diabetic nephropathy (DN) is characterized by glomerular sclerosis and fibrosis caused by the metabolic and thermodynamic changes of diabetes mellitus [3]. Renal complications are the one of the most common

long-term complications that can develop in patients with diabetes mellitus.

Uncontrolled diabetes mellitus leads to deposition of AGEs in the glomerular basement membrane, mesangial cells and renal tubules. The oxidative stress, vascular inflammation, macrophage and platelet activation, thrombosis develops due to interaction between AGEs and soluble receptor for advanced glycation end products (sRAGEs), thereby progressing to vascular complications in diabetes [4]. The intense blood glucose monitoring reduces microvascular complications, especially in diabetic kidney disease patients in whom increasing proteinuria increases cardiovascular risk. Hence, a biomarker which could predict deranged renal function in patients with uncontrolled diabetes and microalbuminuria would help to manage DN effectively. Therefore, we evaluated pentosidine levels in diabetic nephropathy which is a sensitive and specific marker for tissue levels of AGEs.

Microalbumin is an early marker and screening test for Diabetic Nephropathy. Microalbuminuria is caused by damage to podocytes of the glomerulus and represents an indicator for diffuse endothelial dysfunction. Vascular dysfunction and leakage of macromolecules like LDL into vessel wall causes inflammation and initiates atherosclerotic process in albuminuria [5].

Material and methods

This research project, a case control study was carried out at a tertiary care hospital and Institute Human Ethics Committee approval was obtained. Study participants gave informed consent to participate in the project. Diabetic nephropathy cases and newly diagnosed diabetes mellitus patients in the age group between 30–60 years of both gender were enrolled in the study from general medicine OP/IP and healthy controls both age and gender matched were also enrolled in the study. The study population was divided into three groups. Thirty clinically diagnosed diabetic nephropathy patients were included as cases (Group I), Thirty type 2 diabetes mellitus patients without any complications were included as diabetic controls (Group II) and thirty subjects attending for general health checkup were included as healthy controls (Group III). Patients with type 2 DM with other complications, diabetic ketoacidosis, chronic kidney disease, chronic liver disease, myocardial infarction, urinary tract infection, ACE inhibitors, morbid obesity, and dialysis patients were excluded from the study. Six ml of fasting venous blood was collected after taking all aseptic precautions. Two ml of venous blood was collected in a serum tube, two ml in a fluoride tube and two ml in an EDTA tube. Samples were centrifuged and Serum was separated and stored in deep freezer at -20°C until the required sample size was achieved for further analysis. Ten ml of early morning random urine sample was collected in a sterile container for microalbumin estimation. Microalbumin was estimated by spectrophotometric method. Fasting blood glucose, Postprandial blood glucose was estimated by GOD POD method within an hour of separating plasma. HbA1c was estimated by HPLC method in an EDTA sample. Serum Pentosidine was estimated by ELISA kit.

Statistical analysis

One-way ANOVA test was applied to evaluate the levels of serum pentosidine between cases, diabetic controls and healthy controls. Pearson's correlation analysis was done to check the association between serum pentosidine and urine microalbumin. Multiple regression analyses were applied to determine the influence of age, gender, HbA1c, FBS, PPBS, S. Creatinine and S. Urea on serum pentosidine and urine microalbumin. $P \leq 0.05$ was considered statistically significant. SPSS 17 (SPSS Inc, Chicago) statistical software was used for analysis. Between three groups continuous data was represented as mean±standard deviation.

Results

Totally, 90 study participants were included in this study. The study participants were divided into three groups – diabetic nephropathy, type 2 diabetic mellitus (DM) without complications and healthy controls. The average age of patients in this groups were 56.16±9.95, 53.26±8.83 and 44±10.3 years, respectively. In our study, the number of males study subjects were more compared to females (Table 1 and Figure 1).

Serum pentosidine and urine microalbumin levels were statistically significantly increased in diabetic nephropathy cases (group I) when compared to another groups II&III ($P<0.05$). Fasting and postprandial blood glucose, glycated haemoglobin, urea, and creatinine values were elevated in cases when compared to other groups (Table 2).

Pearson correlation analysis was done between serum pentosidine and urinary microalbumin. A positive correlation was found between pentosidine and

Table 1: Demographic data of the study groups.

Variables		Diabetic nephropathy	DM without complications	Healthy controls
Participants, n		30	30	30
Age (years)		56.16±9.95	53.26±8.83	44±10.3
Gender	**Male, n**	22	18	23
	Female, n	8	12	7

microalbumin which implies as pentosidine levels increase in plasma the excretion of microalbumin in urine increases (Table 3, Figure 2).

Multiple regression analysis was used to determine the influence of age, gender, Hemoglobin A1c, fasting blood sugar, postprandial blood sugar, serum creatinine, serum urea levels as considered the independent variables. Microalbuminuria and serum pentosidine were considered the dependent variables. All independent variables were not significant with the dependent variables ($P<0.05$) (Tables 4 and 5).

Discussion

Diabetic kidney disease (DKD) is a major cause for morbidity and mortality in diabetes patients progressing from mild to severe forms of proteinuria and non-proteinuric forms of end-stage renal disease (ESRD) [6]. Pentosidine an AGE formed by covalent biding of amino groups of aminoacids with reducing sugars in Millard reaction is responsible for chronic complications like cardiovascular diseases and CKD. In ESRD patients there will be a marked increase in pentosidine which is formed by glycosylation and oxidation, and it has been considered that AGE and carbonyl stress leads to long-term complications such as CVD in ESRD patients [7]. The accumulation of the structurally defined pentosidine on tissue and circulating proteins has been associated with the severity of diabetic complications. Metabolism of excess glucose produces glycolytic intermediates and reactive aldehydes leading to accumulation of AGEs. Accumulation of AGE in kidney is an independent risk factor for CKD [8].

We found that plasma levels of pentosidine were higher among cases and slightly high among diabetic controls when compared to healthy controls which was an indicative of prediabetic nephropathy among controls (Table 2). Sanaka T *et al.* [9], Machowska A *et al.* [10], reported that in end stage renal failure, pentosidine content was markedly increased in glomerular basement membrane, skin and blood and its increase associated with the severity of complications. In early stages of diabetic nephropathy, pentosidine content

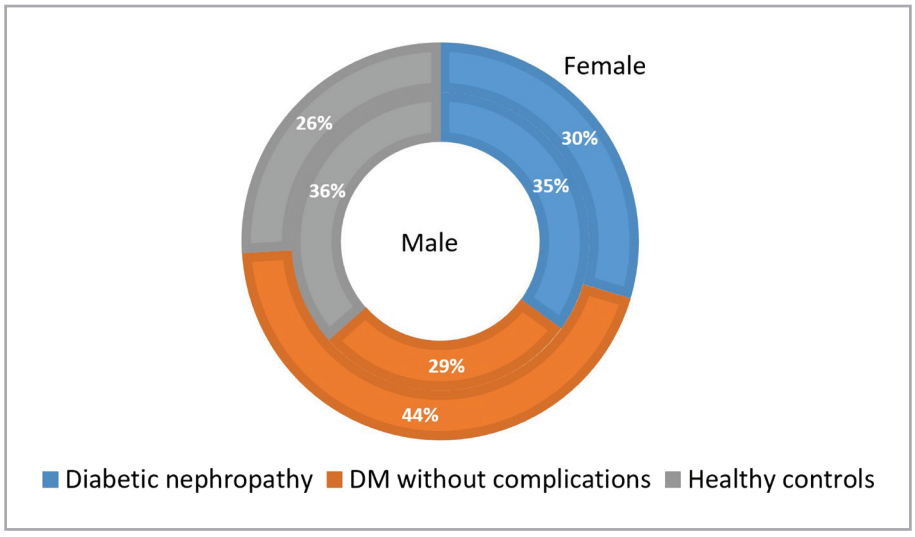

Figure 1: Gender distribution among study subjects.

https://doi.org/10.46389/rjd-2026-2090

Table 2: Statistical comparison between the study parameters (ANOVA).

Parameters	Diabetic nephropathy	Type 2 DM without complications	Healthy controls	P-value
FBS (mg/dl)	162.5±57.7	140.5±47.8	87.6±8.4	0.001**
PPBS (mg/dl)	238.6±74.6	211.5±117	107.7±22.8	0.001**
HbA1c%	8.96±2.36	8.17±1.97	5.05±0.48	0.000**
Creatinine (mg/dl)	1.36±0.82	0.90±0.42	0.91±0.38	0.003**
Urea (mg/dl)	44.2±13.4	22.9±5.55	23.2±5.05	0.000**
Pentosidine (ng/dl)	76±13.3	39.6±12.3	33±7.04	0.000**
Microalbumin (mg/day)	87.7±15.3	52.6±15.04	17.6±4.80	0.000**

Note: * – Significant difference (P≤0.05); HbA1c – Hemoglobin A1c; FBS – Fasting Blood Sugar; PPBS – Postprandial Blood Sugar.

on skin collagen was markedly increased and in renal diseases 16-fold when compared to healthy subjects [11]. Although there is a substantial amount of data to support that AGEs play an important role in diabetic complications, a limited number of clinical studies have been performed to assess the relation between AGEs and diabetic complications.

Sell DR *et al.* [12] showed that significant changes in glomerular morphology when an elevation in plasma pentosidine is observed. Sulliman ME *et al.* [13], in their study showed that plasma pentosidine level increases with progression in renal disease. The buildup of AGEs in blood causes damage to tissue by protein crosslinking, changes in structure of proteins and by activating inflammatory and oxidative pathways. For instance, binding of AGEs to RAGE or toll-like receptor 2 and 4 triggers intracellular signaling and stimulates many inflammatory responses, leading to oxidative stress. Renal tubular cells when get exposed to AGEs there will be activation of transforming growth factor beta, plasminogen activator inhibitor-1, tissue transglutaminase, and MCP1 [14]. Increase in AGE levels is directly associated faster progression to diabetic nephropathy. The accumulation of AGEs in advanced stages of diabetic nephropathy contributes to primary endothelial injury and dysfunctional endothelium triggers the further progression in diabetic nephropathy. Microalbumin is one of the earliest markers to appear in urine in diabetic nephropathy. In our study Pentosidine and microalbumin are positively correlated as shown in Table 3 and Figure 2 which indicates as AGE accumulation progresses there is further progression in diabetic nephropathy. Hence evaluation of pentosidine and urine microalbumin can play a vital role in predicting the diagnosis and prognosis of prediabetic nephropathy and severe forms of microvascular and macro vascular complications in diabetes mellitus. Multiple regression analysis of serum pentosidine and urine microalbumin with different independent variables was not statistically significant as shown in Tables 4 and 5. Piarulli *et al.* [15] showed that variables like vitamin E, TRAP, HbA1c and MDA, but not AGE and pentosidine, were significantly associated with microalbuminuria. Beisswenger *et al.* [16] described that three factors namely age, duration of diabetes and gender are statistically not significantly associated with the diabetes-related complications and tissue levels of AGEs or pentosidine. Sugiyama *et al.* [17] showed that variables such as age, duration of diabetes, urinary albumin/creatinine ratio, fructosamine, total cholesterol, LDL-cholesterol, triglyceride with plasma pentosidine and ischemic heart diseases, were statistically not significant but multiple regression analysis identifies at least Hemoglobin A1c, serum creatinine as independent determinants.

There are limited number of studies done in south Indian population on effects of AGEs in diabetes mellitus. Targeting AGEs therapeutically is a major clinical breakthrough to reduce the burden of diabetes related complications globally. It can be a novel area of future research.

Table 3: Pearson correlation analysis of serum pentosidine with urinary microalbumin.

PAIRS	r-value	p-value
Pentosidine *versus* Microalbumin	0.465	0.000**

Note: * – Significant difference (P≤0.05).

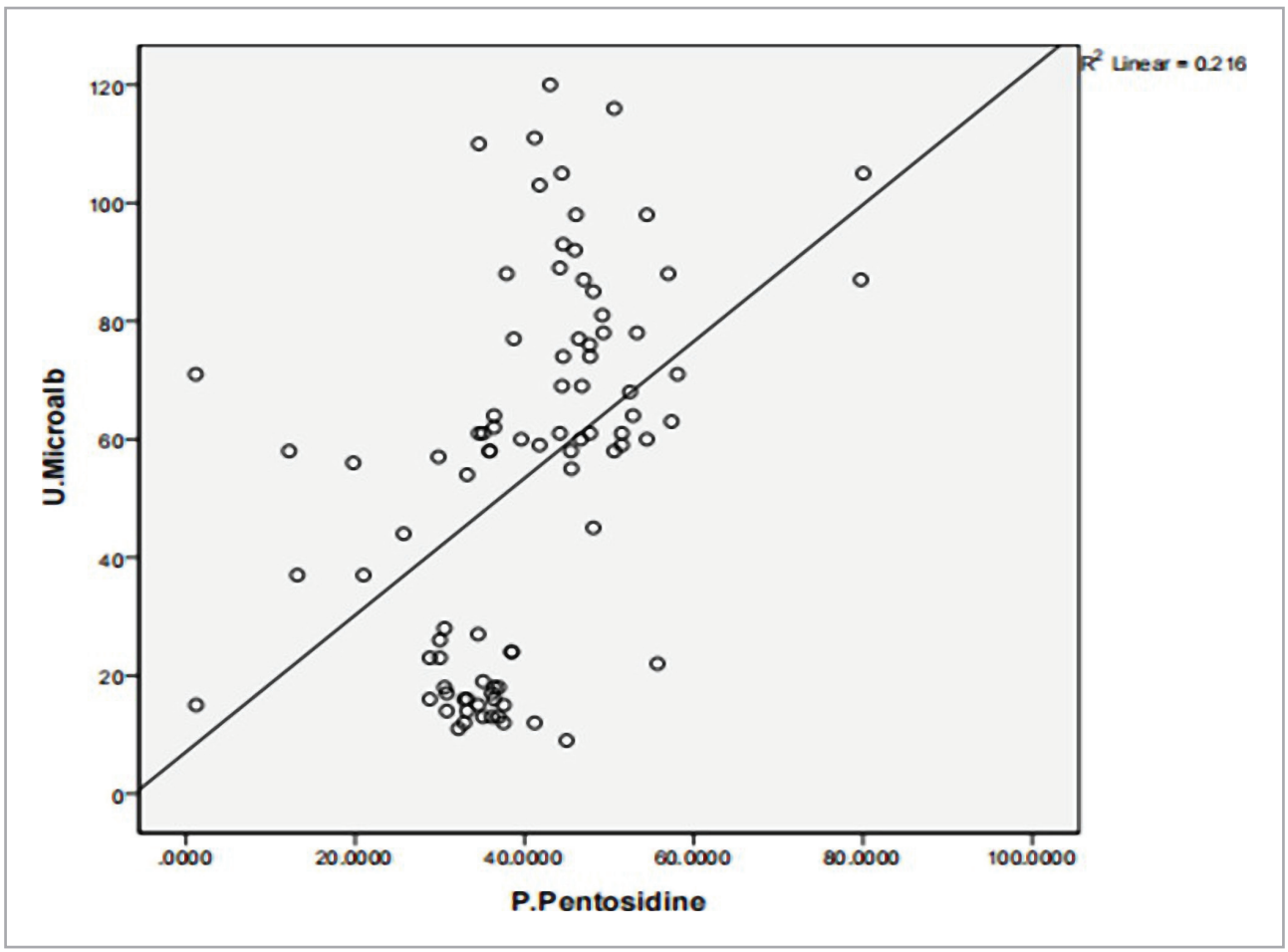

Figure 2: Pearson's correlation analysis of pentosidine with urine microalbumin.

Table 4: Regression analysis of U. Microalbumin.

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
Constant	7.341	9.518		0.771	0.443
Age	0.060	0.146	0.021	0.411	0.682
Gender	3.376	3.232	0.049	1.045	0.299
HbA1c	0.539	0.898	0.042	0.600	0.550
FBS	0.014	0.041	0.024	0.340	0.735
PPBS	-0.034	0.027	-0.089	-1.243	0.217
Creatinine	3.957	2.822	0.078	1.402	0.165
Urea	0.164	0.150	0.071	1.095	0.277
R2					0.848
F					55.005
P					0.05
a. Dependent Variable: U. Microalbumin					

Note: HbA1c – Hemoglobin A1c; FBS – Fasting Blood Sugar; PPBS – Postprandial Blood Sugar; U. Microalbumin – Microalbuminuria.

https://doi.org/10.46389/rjd-2026-2090

Table 5: Regression analysis of S. Pentosidine.

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
Constant	22.731	8.546		2.660	0.009
Age	0.115	0.131	0.101	0.877	0.383
Gender	2.787	2.902	0.102	0.960	0.340
HbA1c	-0.210	0.806	-0.041	-0.260	0.795
FBS	0.052	0.037	0.224	1.429	0.157
PPBS	-0.020	0.024	-0.129	-0.805	0.423
Creatinine	2.209	2.534	0.108	0.872	0.386
Urea	0.053	0.135	0.057	0.395	0.694
R2					0.238
F					3.092
P					0.05
a. Dependent Variable: S. Pentosidine					

Note: HbA1c – Hemoglobin A1c; FBS – Fasting Blood Sugar; PPBS – Postprandial Blood Sugar; S. Pentosidine – Serum Pentosidine.

Conclusion

In conclusion, diabetes mellitus is rising at an alarming rate in Asian populations and is strongly associated with the development of both macrovascular and microvascular complications, particularly diabetic nephropathy. Chronic hyperglycemia promotes the formation of advanced glycation end products such as pentosidine, which play a critical role in the pathogenesis of these complications. Our findings demonstrate significantly elevated plasma pentosidine levels in patients with diabetic nephropathy compared to individuals with type 2 diabetes and healthy controls. Additionally, microalbuminuria—an early marker of diabetic nephropathy—was markedly increased and showed a positive correlation with pentosidine levels, supporting the involvement of advanced glycation end products in podocyte injury. These results highlight the potential translational value of therapeutically targeting advanced glycation end product formation to delay or prevent the progression of diabetes-related complications.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures in the study follow the guidelines laid down in Declaration of Helsinki (2020).

Informed consent

Written informed consent was obtained from all the participants.

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 https://doi.org/10.46389/rjd-2026-2090