Original Article

Urinary megalin levels in patients with type 2 diabetic nephropathy and its correlation with renal function

Sudharshan Jagennath[1], Vadivelan Mehalingam[1], Prashant S. Adole[2], Gandhipuram Periyasamy Senthilkumar[2]

[1]Department of Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, [2]Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India

Abstract

Purpose: Megalin is a glycoprotein molecule found on proximal renal tubular epithelial cells. The objectives of this study were to determine urinary megalin levels in non-diabetic subjects and in patients with and without type 2 diabetic nephropathy and to assess the correlation between urinary megalin, urinary albumin, and estimated glomerular filtration rate (eGFR) in diabetic patients. **Materials and Methods:** This was a cross-sectional comparative study conducted at a tertiary care teaching hospital in South India for 2 years. Study subjects were divided into three groups: non-diabetic subjects, diabetics with normoalbuminuria, and diabetics with microalbuminuria. Urinary albumin was detected by the dipstick technique in a spot urine sample for all study subjects. Nephelometry was used to quantify urinary albumin levels. The enzyme-linked immunosorbent assay technique estimated urinary megalin. **Results:** Urinary megalin levels were higher in non-diabetic subjects compared to diabetic study subjects. There was a significant difference in urinary megalin levels between non-diabetic subjects and diabetic patients with microalbuminuria. No correlation was found between urinary megalin, urinary albumin, and eGFR in patients with diabetic nephropathy. **Conclusion:** Urinary megalin levels were higher in non-diabetic subjects than in type 2 diabetic patients. There was no correlation between urinary megalin, urinary albumin, and eGFR in patients with diabetic nephropathy.

Keywords: Albuminuria, diabetic nephropathy, urinary megalin

Introduction

Diabetic nephropathy is India's leading cause of chronic kidney disease (CKD).[1] Patients with CKD have a poor quality of life due to its negative effect on multiple dimensions, leading to a reduction in physical performance, poor appetite, impaired nutrition and immune function, bone mineral disease, and fluid retention.[2] The detection of diabetic nephropathy in the early stage can help in slowing down the progression of the disease.

Diabetic nephropathy is a common clinical condition that primary care physicians see in their practice. The early diagnosis of diabetic nephropathy can help in reducing morbidity among patients.

The most commonly used marker of early diabetic nephropathy is microalbuminuria [urine albumin 30–300 mg/day or urine albumin-creatinine ratio (UACR) 30–300 mg/gm creatinine]. The natural history of diabetic nephropathy has been thought to be a progression from normoalbuminuria to microalbuminuria and

Address for correspondence: Dr. Vadivelan Mehalingam, Department of Medicine, 3rd Floor, Institute Block, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry - 605 006, India.
E-mail: mevadivelan@hotmail.com

Received: 25-07-2023 **Revised:** 24-08-2023
Accepted: 01-09-2023 **Published:** 06-03-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_1207_23

How to cite this article: Jagennath S, Mehalingam V, Adole PS, Senthilkumar GP. Urinary megalin levels in patients with type 2 diabetic nephropathy and its correlation with renal function. J Family Med Prim Care 2024;13:635-9.

finally lead to macroalbuminuria.[3] Recent studies have shown that there could be non-albuminuric pathways leading to loss of renal function in patients with diabetic nephropathy.[4,5]

Megalin is a glycoprotein molecule expressed on the proximal renal tubular epithelial cells.[6] It is also known as the low-density lipoprotein receptor-related protein 2 (LRP2) since it displays structural similarities to the low-density lipoprotein receptor. Its full-length form is called C-megalin. This receptor is essential in the endocytosis of several reabsorbed proteins in the renal tubule, including albumin.[7] A study done by Ogasawara *et al.* showed that urinary C-megalin was elevated in patients with type 2 diabetes mellitus having microalbuminuria compared to patients with normoalbuminuria.[8] Hence, there is a possibility that urinary C-megalin could be a marker of diabetic nephropathy.

There are no studies in the Indian population regarding urinary megalin in type 2 diabetic patients. The present study was designed to determine if urinary megalin could be a possible biomarker for detecting early diabetic nephropathy.

The objectives of this study were to estimate urinary megalin levels in non-diabetic subjects and in patients with and without early diabetic nephropathy and to find out the correlation between urinary megalin, urinary albumin levels, and estimated glomerular filtration rate (eGFR) in patients with and without early diabetic nephropathy.

Materials and Methods

Study design

This cross-sectional comparative study recruited patients attending the Medicine out-patient department (OPD) of a tertiary care teaching hospital in south India from December 2019 to December 2021.

Inclusion and exclusion criteria

Adult patients (>18 years) with type 2 diabetes mellitus were included as the study subjects. Healthy individuals with a random blood sugar below 200 mg/dL without any symptoms of diabetes were included as the control subjects. Patients with urinary tract infection, those with systemic arterial hypertension, those on angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), and those having a serum creatinine value more than 1.5 mg/dL (males) and more than 1.4 mg/dL (females) were excluded from the study.

Data collection

Three groups were formed for this study. Group 1 included control subjects (non-diabetic without any renal disease), group 2 included patients with normoalbuminuria (UACR < 30 mg/g of creatinine), and group 3 included patients with microalbuminuria (UACR 30–300 mg/g of creatinine).

A consecutive sampling technique was used for this study. Since there were no previous Indian studies on urinary megalin in diabetic subjects, the sample size was arbitrarily taken as 50 in each group. Three subjects were excluded from group 1 since they had elevated urine albumin (>30 mg/L). Ten diabetic patients were excluded from the study since they had macroalbuminuria (one in group 2 and nine patients in group 3). The final sample size reached was 137 subjects (47 subjects in group 1, 49 patients in group 2, and 41 patients in group 3).

Study procedure

Data were obtained from the study subjects and their health records regarding the duration of diabetes. Height, weight, and body mass index were recorded for all the subjects. A spot random urine sample was tested for albuminuria for all study participants using the dipstick method. Patients and controls were enrolled in the study if the test for urinary albumin was negative or trace. Subjects with dipstick-positive albuminuria were excluded from the study. All urine samples were centrifuged, and 2 mL of each supernatant fluid was collected in two separate Eppendorf tubes for quantitative estimation of albumin and megalin. All urine samples were stored at -80°C in a deep freezer.

Urinary albumin level was estimated using the nephelometry technique for controls and subjects. Subjects with spot urinary albumin levels below 30 mg/g of creatinine were included in the normoalbuminuria group (group 2), and those with urinary albumin between 30 and 300 mg/g of creatinine were included in the microalbuminuria group (group 3). This was based on the correlation of spot urinary albumin value in mg/L to urinary albumin excretion in mg/g of creatinine.

Urinary megalin estimation was done using a quantitative human LRP-2 ELISA kit (supplied by Abbkine Ltd.) with a reported sensitivity of 5 pg/mL and a detection range of 5–160 pg/mL.

eGFR was calculated for all study subjects using the 2009 CKD-EPI equation.

Statistical analysis

Data analysis was done using STATA V14, a general-purpose statistical software package developed by StataCorp for data visualization, statistics, and automated reporting. Continuous data were expressed as mean with standard deviation or median with inter-quartile range (IQR) based on the normality of data. The normality of data was assessed using the Shapiro–Wilk test.

Urinary albumin and megalin data were expressed as median with IQR. A comparison of urinary megalin levels between cases and controls was made using the Kruskal–Wallis test. Spearman's correlation test determined the correlation between urinary albumin and urinary megalin.

All statistical analyses were carried out at a 5% significance level, and a *P*-value of less than 0.05 was considered significant.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of the study participants.

eGFR

89% of study participants had an eGFR value above 90 mL/min/1.73 m^2. Twelve participants in the diabetic sub-groups had an eGFR value <90 mL/min/1.73 m^2.

Urinary albumin

Table 2 shows the urinary albumin values of study participants.

Urinary megalin

Urinary megalin values in non-diabetic subjects and diabetic subjects are shown in Table 3.

Among the diabetic study subjects, the median urinary megalin level was 42.63 pg/mL, with an IQR of 34.33–42.64. Urinary megalin was found to be non-normally distributed. There was a significant difference in the median values of urinary megalin between diabetic subjects and non-diabetic subjects ($P = 0.018$).

In sub-group analysis, there was no significant difference in urinary megalin levels between diabetic patients with normoalbuminuria and non-diabetic subjects ($P = 0.09$) and between individual diabetic sub-groups ($P = 0.056$). There was a significant difference in urinary megalin levels between diabetic patients with microalbuminuria and non-diabetic subjects ($P = 0.002$).

Correlation analysis of urinary megalin with urinary albumin and eGFR

There was a non-significant moderate negative correlation between urinary megalin and urinary albumin values among diabetic subjects (r = -0.129, $P = 0.22$). The scatter plot depicting the same is shown in Figure 1. There was no significant correlation between eGFR and urinary megalin (r = 0.07, $P = 0.44$).

Receiver operator characteristic curve for urinary megalin

The receiver operating characteristic (ROC) curve was plotted to assess the utility of urinary megalin as a marker for early diabetic nephropathy. We found a cut-off value of 40.92 pg/mL for urinary megalin to have a sensitivity of 76.6% and a specificity of 51.2% (AUC = 0.679, 95% CI 0.567–0.791). The ROC curve is shown in Figure 2.

Summary of results

1. Urinary megalin levels were higher in non-diabetic subjects than in diabetic study subjects.
2. There was a significant difference in urinary megalin levels between non-diabetic subjects and diabetic patients with microalbuminuria.
3. There was no significant difference in urinary megalin levels between non-diabetic subjects and diabetic patients with normoalbuminuria or between diabetic patients with normoalbuminuria and diabetic patients with microalbuminuria.
4. There was no significant correlation between urinary megalin and urinary albumin or between urinary megalin and eGFR in patients with diabetic nephropathy.
5. Urinary megalin was found to have moderately good sensitivity but poor specificity for predicting the risk of

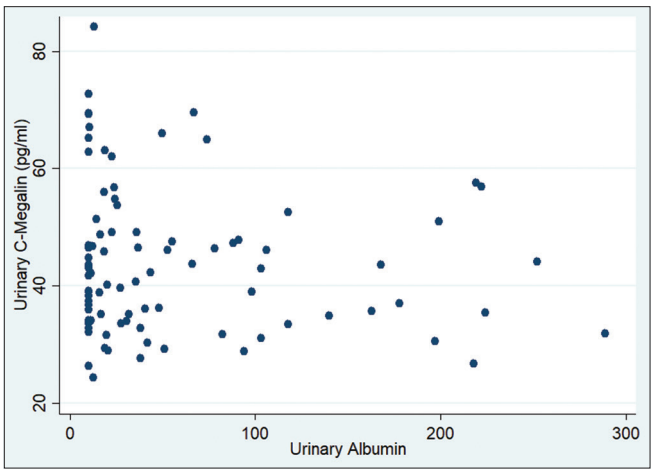

Figure 1: Scatter plot showing a correlation between urinary albumin and urinary megalin

Table 1: Baseline characteristics of study participants

Parameter	Non-diabetic subjects (*n*=47)	Diabetic patients with normoalbuminuria (*n*=49)	Diabetic patients with microalbuminuria (*n*=41)
Median age in years (IQR)	37 (27.5-51.5)	49 (45-55)	50 (40-60)
Male gender (%)	34 (72%)	30 (61%)	25 (61%)
BMI in kg/m^2 (IQR)	20.9 (18.6-23.4)	25 (21.8-29.3)	23.9 (20.8-26)
The median duration of diabetes in years (IQR)	-	2 (0.5-4)	3 (1-6)
Median serum creatinine in mg/dL (IQR)	0.7 (0.6-0.82)	0.7 (0.6-0.76)	0.72 (0.58-0.85)
Median eGFR in mL/min/1.73 m^2 (IQR)	113 (103-129.5)	108 (98-116)	107 (90-119)
Duration of diabetes			
<1 year	-	22 (44.9%)	11 (26.8%)
1-5 years	-	20 (40.8%)	18 (43.9%)
>5 years	-	7 (14.3%)	12 (29.3%)

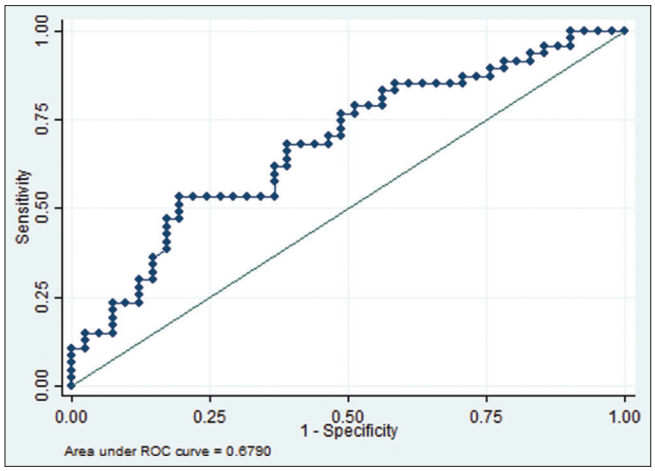

Figure 2: ROC curve for urinary megalin

early diabetic nephropathy in patients with type 2 diabetes mellitus.

Discussion

Megalin is an important endocytic receptor in the proximal renal tubule. Mouse models have shown that megalin and cubilin in the proximal renal tubule play an essential role in the reabsorption of albumin. Megalin is a crucial receptor for this process. The knockout of megalin from the proximal renal tubule has been shown to play a role in causing albuminuria.[6]

Megalin consists of three domains: a sizeable extracellular domain, a transmembrane segment, and a cytoplasmic domain.[9] The entire glycoprotein molecule is called C-megalin, while the ectodomain alone is called A-megalin. The primary function of megalin is the reuptake of several proteins in the proximal renal tubule. These proteins are albumin, vitamin D, retinol-binding protein, and lactoferrin.[10] Studies in mice demonstrated the utility of megalin in the reabsorption of albumin, but the evidence needed to be more consistent.[11,12]

Human studies have shown that C-megalin is excreted in the urine in increasing quantities before the onset of albuminuria.[13-15] These studies have also demonstrated an inverse correlation between eGFR and urinary C-megalin levels.[14,15]

In our study, the median urinary megalin concentration was 42.63 pg/mL in diabetic patients. In another study done by Kurita *et al.*, the median urinary C-megalin concentration in diabetic patients was 155 pmol/L, which is equivalent to 93.3 pg/mL. The reason for the difference in urinary megalin levels in the two studies could be due to a difference in the inclusion criteria. The study by Kurita *et al.* included patients with type 1 and type 2 diabetes and on anti-hypertensive drug therapy (like ACE inhibitors and ARBs), which can affect proteinuria. Also, there could be a difference in urinary megalin concentration between the Japanese and Indian populations.

Table 2: Urinary albumin levels in various groups

Group	Median urinary albumin level (in mg/L)	IQR
Non-diabetic subjects	10.3	10.3-17.2
Diabetic patients with normoalbuminuria	10.3	10.3-18.4
Diabetic patients with microalbuminuria	88	48-163

Table 3: Urinary megalin levels in various groups

Group	Median urinary megalin level (pg/mL)	IQR
Non-diabetic subjects	48.12	41.2-54
Diabetic patients with normoalbuminuria	43.62	35.9-54.8
Diabetic patients with microalbuminuria	40.68	33.5-47.2

Our study found a significant difference in urinary megalin levels between healthy individuals and diabetic patients. This finding is consistent with previous human studies on urinary C-megalin.[16] However, non-diabetic subjects had a higher concentration of urinary megalin when compared with diabetic patients. The reason for this finding needs to be clarified. The skewed distribution of age and gender in the groups may have contributed to this finding since urinary megalin is known to be elevated in males and elderly individuals.[15]

Previous studies have shown a significant inverse linear association between urinary C-megalin levels and eGFR. This finding is a possible indication of its use in identifying early diabetic nephropathy.[8,15,16] Our study found no correlation between urinary megalin values and eGFR. In contrast, Ogasawara *et al.*, in their research, found an inverse correlation between eGFR and urinary C-megalin in subjects with eGFR below 60 mL/min/1.73 m^2. In our study, there were no subjects with eGFR less than 60 mL/min/1.73 m^2. Another study by Kurita *et al.* found a significant inverse linear association between urinary C-megalin and eGFR in subjects with preserved eGFR. This finding was, however, not seen in subjects with reduced eGFR.[15] Their study population was more heterogeneous than ours, and they used a Japanese 3-variable equation to determine eGFR and not the CKD-EPI equation.

Our study found no significant correlation between urinary megalin levels and albuminuria. This finding contrasts with other studies, where urinary C-megalin positively correlated with albuminuria.[15,16] Megalin is known to be excreted in the urine in increasing quantities with a decreasing eGFR. The absence of a correlation between urinary megalin and albuminuria may be because our study did not include subjects with reduced eGFR.

We also generated an ROC curve to determine the utility of urinary megalin as a biomarker to detect early diabetic nephropathy compared to microalbuminuria. The area under the curve (AUC) obtained was 0.679, with a sensitivity of 76.6%

and a specificity of 51.1% for a 40.92 pg/mL cut-off value. The utility of urinary megalin is thus uncertain in determining early diabetic nephropathy.

Limitations of the study

1. We could not ascertain the temporal association between elevated urinary megalin levels and the onset of diabetic nephropathy since this was a cross-sectional study.
2. We did not match the controls and cases. Hence, age, gender, and BMI could have played a role in the lack of difference between the groups.
3. A single-spot urinary albumin value was used to determine entry into the study groups instead of a 24-hour urinary albumin value with more validity.

Conclusion

Urinary megalin levels were higher in non-diabetic subjects than in patients with type 2 diabetes mellitus. Urinary megalin levels significantly differed between non-diabetic subjects and diabetic patients with microalbuminuria. However, the levels were not very different between non-diabetic subjects and diabetic patients with normoalbuminuria. There was no correlation of urinary megalin with urinary albumin or eGFR in patients with diabetic nephropathy.

Financial support and sponsorship

Intramural Research Grant, JIPMER, Puducherry.

Conflicts of interest

There are no conflicts of interest.

References

1. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, *et al.* What do we know about chronic kidney disease in India: First report of the Indian CKD registry. BMC Nephrol 2012;13:10.
2. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, *et al.* Diabetic kidney disease. Nat Rev Dis Primers 2015;1:15018.
3. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes 1983;32(Suppl 2):64-78.
4. MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, Smith TJ, McNeil KJ, Jerums G. Nonalbuminuric renal insufficiency in type 2 diabetes. Diabetes Care 2004;27:195-200.
5. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR, UKPDS Study Group. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. Diabetes 2006;55:1832-9.
6. Christensen EI, Birn H, Verroust P, Moestrup SK. Membrane receptors for endocytosis in the renal proximal tubule. Int Rev Cytol 1998;180:237-84.
7. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. Kidney Int 2016;89:58-67.
8. Ogasawara S, Hosojima M, Kaseda R, Kabasawa H, Yamamoto-Kabasawa K, Kurosawa H, *et al.* Significance of urinary full-length and ectodomain forms of megalin in patients with type 2 diabetes. Diabetes Care 2012;35:1112-8.
9. Saito A, Sato H, Iino N, Takeda T. Molecular mechanisms of receptor-mediated endocytosis in the renal proximal tubular epithelium. J Biomed Biotechnol 2010;2010:403272.
10. Christensen EI, Moskaug JO, Vorum H, Jacobsen C, Gundersen TE, Nykjaer A, *et al.* Evidence for an essential role of megalin in transepithelial transport of retinol. J Am Soc Nephrol 1999;10:685-95.
11. Leheste JR, Rolinski B, Vorum H, Hilpert J, Nykjaer A, Jacobsen C, *et al.* Megalin knockout mice as an animal model of low molecular weight proteinuria. Am J Pathol 1999;155:1361-70.
12. Motoyoshi Y, Matsusaka T, Saito A, Pastan I, Willnow TE, Mizutani S, *et al.* Megalin contributes to the early injury of proximal tubule cells during nonselective proteinuria. Kidney Int 2008;74:1262-9.
13. Thrailkill KM, Nimmo T, Bunn RC, Cockrell GE, Moreau CS, Mackintosh S, *et al.* Microalbuminuria in type 1 diabetes is associated with enhanced excretion of the endocytic multiligand receptors megalin and cubilin. Diabetes Care 2009;32:1266-8.
14. Akour A, Kasabri V, Bulatova N, Al-Motassem Y, Fahmawi H, Momani M, *et al.* Urinary megalin in association with progression factors of diabetic nephropathy. Bratisl Lek Listy 2019;120:532-5.
15. Kurita N, Kinoshita M, Fujimura M, Kurosawa K, Sakuramachi Y, Takano K, *et al.* Association of urinary C-megalin with albuminuria and renal function in diabetes: A cross-sectional study (Diabetes Distress and Care Registry at Tenri [DDCRT 21]). J Nephrol 2022;35:201-10.
16. De S, Kuwahara S, Hosojima M, Ishikawa T, Kaseda R, Sarkar P, *et al.* Exocytosis-mediated urinary full-length megalin excretion is linked with the pathogenesis of diabetic nephropathy. Diabetes 2017;66:1391-404.