Comparative utility of urinary Megalin and Cystatin-c in identifying early renal damage in Type-2 Diabetes mellitus – A cross sectional study

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Abstract-Kidney disease is a major complication of type-2 diabetes mellitus (T2DM) and a leading cause of end-stage renal disease. Early detection is crucial to prevent the progression of kidney damage. However, current markers such as microalbuminuria have low specificity, necessitating the exploration of novel biomarkers. Urinary megalin and cystatin-C have been proposed as potential early indicators of renal damage. The aim of the study is to evaluate urinary megalin and cystatin-C as biomarkers for early detection of renal damage in T2DM patients with different levels of albuminuria.

This diagnostic evaluation study included 120 T2DM patients from a tertiary care hospital in South India, recruited between January and June 2023. Patients were grouped based on their urinary albumin-creatinine ratio (ACR): normoalbuminuria (ACR <30 mg/g) and microalbuminuria (ACR 30-299 mg/g). Urinary megalin and cystatin-c levels were measured using ELISA kits, and their associations with glycemic control, lipid profile, and other parameters were analyzed using Pearson's correlation.

The microalbuminuria group showed significantly lower urinary megalin (15.47 ± 4.99 pg/mL vs. 17.40 ± 5.11 pg/mL, p = 0.04) and cystatin-c levels (14.20 ± 7.87 ng/mL vs. 17.21 ± 7.60 ng/mL, p = 0.035) than the normoalbuminuria group.

Urinary megalin and cystatin-c are promising early biomarkers for detecting renal damage in T2DM patients, potentially improving early diagnosis and outcomes.

Keywords: Diabetic Nephropathy; Type-2 Diabetes Mellitus; Biomarkers; Urinary Megalin; Urinary Cystatin-C; Microalbuminuria; Early Detection; Renal Damage.

Contribution details

concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review.

INTRODUCTION

Kidney disease is a prevalent complication of type-2 diabetes mellitus (T2DM) and a leading cause of end-stage renal disease (ESRD) worldwide (Eckardt et al., 2013). Early detection of renal damage in T2DM patients is crucial to prevent progression to chronic kidney disease (CKD) and its associated complications (Thomas et al., 2015). Currently, microalbuminuria, measured by the urine albumin-creatinine ratio (ACR), is the most widely used biomarker for detecting early diabetic kidney damage. However, its limitations, such as low specificity and the influence of non-renal factors, necessitate the identification of novel biomarkers for more accurate early detection of renal dysfunction (Perkins et al., 2007).

Emerging evidence suggests that urinary megalin, an endocytic receptor expressed in renal proximal tubules, and urinary cystatin-C, a low-molecular-weight protein freely filtered by the glomerulus, may serve as promising early biomarkers for renal damage in diabetes (Christensen et al., 2011; Laterza et al., 2002). Megalin plays a crucial role in the reabsorption of filtered proteins, and its levels may indicate tubular damage before significant albuminuria occurs (Christensen and Birn, 2002). Cystatin-c, a cysteine protease inhibitor, has been reported to correlate with glomerular filtration rate (GFR) and may reflect early changes in kidney function not captured by serum creatinine levels (Shlipak et al., 2006).

Several studies have highlighted the potential advantages of using these biomarkers. Urinary megalin, as a receptor for protein reabsorption in the proximal tubules, decreases in expression in early tubular damage, thereby serving as an indicator before overt proteinuria is detectable (Christensen et al., 2011). Similarly, urinary cystatin-c is less influenced by factors like muscle mass, age, and gender, making it a more stable marker for early kidney function decline than serum creatinine (Stevens et al., 2008). Additionally, cystatin-c has been demonstrated to predict kidney function loss and cardiovascular risk more accurately than traditional measures in T2DM patients (Lamb et al., 2014).

This study aims to evaluate the comparative utility of urinary megalin and cystatin-c as biomarkers for early renal damage in T2DM patients with varying degrees of albuminuria. By analyzing the levels of these biomarkers in patients with normal albuminuria and microalbuminuria, we aim to determine their effectiveness in detecting early kidney damage and their potential correlation with glycemic control and lipid profile. The primary objectives of the study were to estimate urinary megalin and cystatin-c in T2DM patients with normal albuminuria and microalbuminuria and to correlate these urinary markers of tubular injury with glycemic control (HbA1c), duration of diabetes, and lipid profile in type-2 diabetic patients.

MATERIALS AND METHODS

This study was designed as a diagnostic evaluation study and received approval from the Institutional Human Ethics Committee (proposal number 22/195). It was conducted on type-2 diabetes mellitus (T2DM) patients attending the outpatient department of a tertiary care hospital in South India.

Patients included were aged 18-80 years, known cases of T2DM with a minimum 5-year history of T2DM and had undergone an extended diabetic profile as part of routine care. The extended diabetic profile parameters included fasting blood sugar (FBS), post-prandial blood sugar (PPBS), urea, creatinine, glycated hemoglobin (HbA1c), serum cholesterol, triglycerides (TGL), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and urine albumin creatinine ratio (ACR). Patients with recent infections, hypertension, use of nephrotoxic medications, chronic kidney diseases of other etiologies, thyroid disorders, or pregnancy were excluded. The cases will be divided into 2 groups based on their urinary albumin/creatinine ratio (ACR) (KDIGO guidelines) - normal albuminuria group with ACR >30 mg/g creatinine & moderately increased albuminuria group with ACR 30-299 mg/g creatinine.

A consecutive sampling technique was used for this study. Patients who met the inclusion and exclusion criteria were consecutively recruited from January to June 2023. Leftover urine samples, collected for ACR estimation, were centrifuged at 4,000 rpm for 10 minutes and stored at -80°C until biomarker assays were performed.

Demographic data and laboratory parameters were obtained from the hospital information system, while routine biochemical investigations were retrieved from the laboratory information system. All parameters listed in the extended diabetic profile were measured in the clinical biochemistry laboratory using a Roche Cobas 6000 auto-analyzer with dedicated kits and reagents.

Urinary megalin levels were measured using a quantitative human LRP-2 ELISA kit (Abbkine, Inc.) with a detection range of 5–80 pg/mL. Urinary cystatin-C levels were measured using a quantitative human cystatin-C ELISA kit (Elabscience

Biotechnology Inc.), with a sensitivity of 0.19 ng/mL and a detection range of 0.31–20 ng/mL.

Data were analyzed using SPSS software. Continuous variables were presented as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Differences in biomarker mean values between the two groups were assessed using the Student's t-test. Pearson's correlational analysis was done to establish correlation between the biomarkers and baseline biochemical investigations. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 120 T2DM patients were included in the study. The study population was divided into two groups based on the ACR values. A total of 60 participants were included in group-1 (normoalbuminuria) and 60 were included in group-2 (microalbuminuria). Table-1 lists the baseline demographics and biochemical investigations in the study population.

Table-1: Descriptive statistics of the study population

Parameter	Group 1 Normoalbuminuria	Group 2 Microalbuminuria
Males n (%)	40 (66.7%)	44 (73.3%)
Age (years)	60.65 ± 10.55	58.08 ± 10.07
Duration of T2DM (years)	7.91 ± 5.12	8.54 ± 7.09
FBS (mg/dL)	134 ± 49	152 ± 75
PPBS (mg/dL)	200 ± 74	219 ± 109
HbA1c	7.29 ± 1.50	7.86 ± 2.06
Serum urea (mg/dL)	24.91 ± 7.90	25.58 ± 11.30
Serum creatinine (mg/dL)	0.91 ± 0.25	1.01 ± 0.35
Serum cholesterol (mg/dL)	145.75 ± 33.91	157.92 ± 38.80
Serum triglycerides (mg/dL)	134.23 ± 58.99	158.06 ± 79.36
Serum HDL-c (mg/dL)	39 ± 8.43	41.39 ± 15.39
Serum LDL-c (mg/dL)	91.47 ± 32.43	98.10 ± 39.70
Urine albumin creatinine ratio (mg/g creatinine)	10.84 ± 7.25	110.27 ± 73.06

The mean urine megalin in the 2 groups were as follows (Group-1 vs Group-2) 17.40 ± 5.11 vs 15.47 ± 4.99 pg/mL (p-value 0.04). The mean urine cystatin-c among the 2 groups were (Group-1 vs Group-2) 17.21 ± 7.60 vs 14.20 ± 7.87 ng/mL (p-value 0.035). (Figure-1)

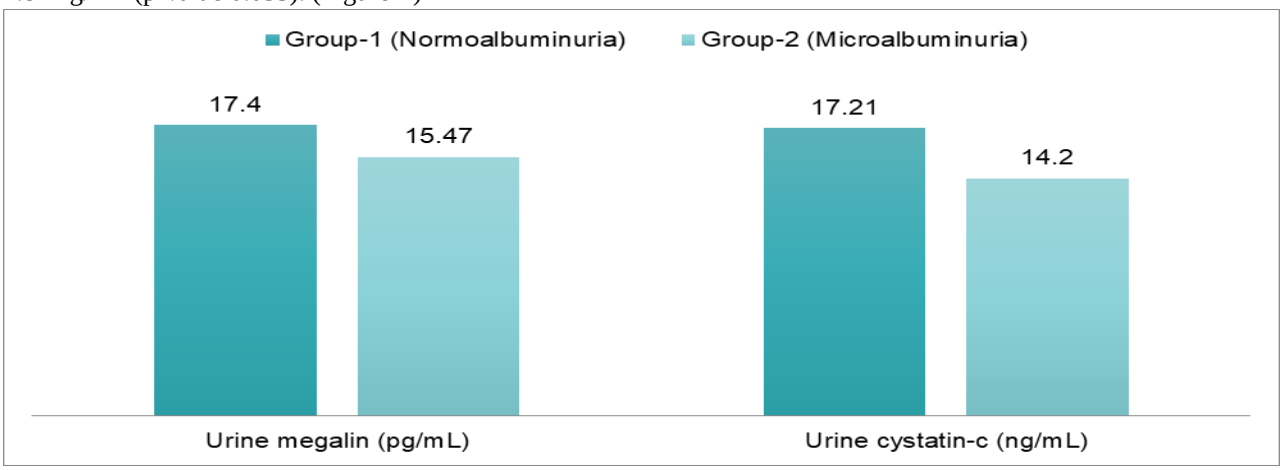

Figure 1: Graph representing mean urine megalin & cystatin-c levels in the study groups

Correlational analysis was done to establish an association of the urinary markers megalin and cystatin-c with glycemic control (HbA1c), duration of DM, lipid profile. The results are tabulated in Table-2.

Table-2: Correlational analysis betwen urinary megalin with urine cystatin-c, HbA1c, T2DM duration and lipid profile

Variables	R value	p value
Urine Cystatin-C	0.09	
Urine Microalbumin	0.08	0.99
Serum LDL-c	0.09	0.33
Serum HDL-c	-0.02	0.45
Serum cholesterol	0.09	0.39
Serum triglycerides	0.08	0.46
HbA1c	0.23	0.11
Duration of T2DM	-0.09	0.33

There was statistically significant difference in the levels of urine megalin and cystatin-c among the 2 groups of study participants.

DISCUSSION

The present study evaluated urinary megalin and cystatin-C levels as potential early biomarkers for renal damage in T2DM patients. Our findings demonstrated a statistically significant reduction in urinary megalin and cystatin-C levels in patients with microalbuminuria compared to those with normal albuminuria, suggesting that both megalin and cystatin-C could serve as sensitive biomarkers for detecting early diabetic nephropathy (Thomas et al., 2015; Chen et al., 2018).

The decrease in urinary megalin levels observed in this study aligns with prior research indicating its potential role as an early indicator of tubular dysfunction in diabetic patients (Moestrup and Verroust, 2001; Christensen and Birn, 2002). Megalin, a multiligand endocytic receptor, is critical for the reabsorption of proteins and other substances in the renal proximal tubules. Reduced megalin expression or function has been associated with increased urinary excretion of proteins and other biomolecules, indicating tubular damage that precedes significant glomerular involvement (Saito et al., 2010; Nielsen et al., 2016).

Similarly, the observed decrease in urinary cystatin-C levels in patients with microalbuminuria is consistent with previous studies that highlight its utility as a marker for early kidney dysfunction (Perkins et al., 2005; Laterza et al., 2002). Cystatin-C is freely filtered by the glomeruli and completely reabsorbed by the proximal tubules without tubular secretion, making it a potentially valuable marker for early renal function decline in diabetic patients. Unlike serum creatinine, cystatin-C is less influenced by muscle mass, age, and other non-renal factors, providing a more reliable estimate of GFR in the early stages of diabetic kidney disease (Randers and Erlandsen, 1999; Knight et al., 2004).

Moreover, the lack of significant correlation between urinary megalin and cystatin-C levels with HbA1c and lipid profile suggests that these biomarkers may reflect early renal damage independently of glycemic control and lipid metabolism (Fukuda et al., 2011; Van Deventer et al., 2014). This finding supports the hypothesis that tubular damage in diabetes may occur before or independent of overt changes in glomerular filtration (Lamb et al., 2004).

In conclusion, our study provides evidence that urinary megalin and cystatin-C are promising biomarkers for detecting early renal damage in T2DM patients, particularly in those with microalbuminuria. Further longitudinal studies with larger cohorts are needed to validate these findings and determine their clinical utility in the routine monitoring of kidney disease in T2DM patients.

CONCLUSION

This study demonstrates that urinary megalin and cystatin-C are promising biomarkers for the early detection of diabetic nephropathy in patients with type-2 diabetes mellitus (T2DM). The significant reduction in these biomarkers in the microalbuminuria group compared to the normoalbuminuria group suggests that they can detect renal damage before the onset of overt proteinuria.

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