

Original article

Routine biochemical predictors of early diabetic kidney disease in Indian patients with Type 2 Diabetes

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ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a rapidly increasing metabolic disorder characterised by chronic hyperglycaemia, leading to microvascular complications such as diabetic kidney disease (DKD). Approximately 20–40% of patients with diabetes develop DKD, making early diagnosis essential. Although microalbuminuria and eGFR are standard diagnostic markers, additional routine biochemical markers may facilitate earlier detection of renal dysfunction and improve risk stratification and clinical management in patients with T2DM.

Method: A hospital-based cross-sectional comparative study was conducted over one year involving 150 patients with type 2 diabetes mellitus, categorized into normoalbuminuric (n=75) and microalbuminuric (n=75) groups based on UACR. Demographic, clinical, and biochemical parameters, including renal biomarkers, were assessed using standard laboratory methods. Group comparisons were performed using Student's *t*-test or chi-square test, with statistical significance set at $p < 0.05$ using SPSS version 16.

Result: Patients with microalbuminuria demonstrated significantly higher renal biomarker levels than normoalbuminuric patients, including serum creatinine (1.12 vs. 0.89 mg/dL), cystatin C (1.18 vs. 0.88 mg/L), blood urea (36 vs. 28 mg/dL), uric acid (6.1 vs. 5.2 mg/dL), 24-hour urinary protein (268 vs. 118 mg/day), and UACR (47 vs. 11 mg/g) (all $p \leq 0.001$). UACR combined with serum creatinine achieved the highest diagnostic accuracy (AUC=0.86; sensitivity 84%; specificity 83%), while UACR was the strongest independent predictor of microalbuminuria (adjusted OR=2.9, $p < 0.001$).

Conclusion: The study concluded that patients with type 2 diabetes mellitus and microalbuminuria exhibited significantly higher levels of conventional renal biomarkers, including serum creatinine, serum cystatin C, blood urea, serum uric acid, 24-hour urinary protein, and UACR, indicating greater renal impairment than normoalbuminuric patients..

Keywords: Type 2 diabetes mellitus; Diabetic kidney disease; Microalbuminuria; Conventional renal biomarkers; Urinary albumin-to-creatinine ratio (UACR).

INTRODUCTION

Increasing trend of sedentary lifestyle, higher average life expectancy and overall lack of physical activities are the reasons behind the global rise in the incidence of Diabetes mellitus type 2 (T2DM) globally. [1] Defective insulin secretion from the beta cells of the pancreas and the diminished sensitivity of peripheral tissues to insulin secretion are the hallmarks of T2DM.[2] DM is characterised by excess blood glucose level (hyperglycemia) and the presence of glucose in urine (glycosuria). Sustained hyperglycemia affects microvascular and macrovascular structures through the formation of Advanced Glycation End (AGE) proteins . In the long term, it affects the function of almost every organ of the body. Thus, long-term complications are the biggest problem in T2DM, such as retinopathy, nephropathy, neuropathy and cardiac diseases. [3] Diabetic kidney disease (DKD) is also called diabetic nephropathy and is a

multifaceted condition, and it can be described as a syndrome that occurs due to the effects of varying treatments in DM.[4] DKD affects every part of the kidney but notably the glomeruli. It is asymptomatic in the early stage and later develops symptoms like oedema, proteinuria, and systemic symptoms like nausea, vomiting and muscle cramps. The pathology behind it is glomerular basement membrane thickening, glomerular hypertrophy, narrowing of the renal small arteries, effacement of podocyte foot processes, and mesangial matrix expansion. It is an end-stage renal disease that occurs all over the world, and a disproportionately high burden is observed among the South Asian population.[5,6] Approximately 20–40% of patients with diabetes develop diabetic kidney disease (diabetic nephropathy). Around one-third of patients with type 2 diabetes develop diabetic nephropathy [6]. In India, the prevalence of DKD has also been growing with that of T2DM, and early detection is very critical in terms of public health. The presence of elevated microalbuminuria and the reduction of the estimated glomerular filtration rate (eGFR) is the most common method for diagnosing DKD,[7]. These represent the urgent need for the identification of more sensitive and alternative biomarkers of renal dysfunction at an early stage. [8]

So, there is a need for easily available routine biomarkers such as the glycated haemoglobin (HbA1c), serum uric acid, and lipid ratios reflects the complex glucotoxicity, systemic inflammation and insulin resistance - which drive the pathogenesis of renal injury. [6]

Early identification of routine biochemical markers will enable clinicians to stratify the high-risk T2DM patients accurately and help the patients to prevent fatal complications. By assessing the conventional biomarkers over a prospective period, this study aims to evaluate the association and diagnostic performance of routine biochemical biomarkers for the early detection of diabetic kidney disease among patients with type 2 diabetes mellitus by comparing normoalbuminuric and microalbuminuric individuals.

METHOD

Research design

The study is a hospital-based analytical cross-sectional comparative study which was conducted from 2009 to 2010 to evaluate the biochemical predictors for the analysis of the renal disease among Type 2 diabetes patients. The study was conducted in a tertiary care setting for a duration of 1 year. Total 150 patients of T2DM were included in the study. 150 patients were divided into 2 groups, Group I (Normoalbuminuric, n = 75) and Group II (Microalbuminuric, n = 75). Informed consent was taken for the study. Various predefined exclusion and inclusion criteria were considered for the study.

Inclusion criteria

- Patients of age 30 to 70 years and confirmed with the T2DM diagnosis were selected for the study.
- Patients of Normoalbuminuric with the UACR <30 mg/g and of eGFR ≥ 60 mL/min/1.73 m² were included.
- Patients of Microalbuminuric groups with the UACR 30–300 mg/g for duration of 3 months along with the eGFR ≥ 30 mL/min/1.73 m² were included.
- Informed consent was taken for the study.

Exclusion criteria

- Non-diabetic kidney disease with infection in the urinary tract was excluded.
- Patients with acute infection, chronic liver disorder, or malignant patients were not allowed for the study.
- Pregnant patients were eliminated.
- Those who were exposed to the nephrotoxic drugs within initial 2 weeks were excluded.

Procedure

Informed consent was taken for the study. All 150 patients with type 2 diabetes mellitus (T2DM) were selected for the study. The patients were categorised into Group I (Normoalbuminuric, $n = 75$) and Group II (Microalbuminuric, $n = 75$) on the basis of the urinary albumin-to-creatinine ratio (UACR). All patients were analysed and the demographic and clinical parameters including the age, sex, body mass index (BMI), duration of diabetes, blood pressure, previous history of medication, comorbidities, and glycaemic status (HbA_{1c}), was recorded by the use of proper structured proforma. Following an overnight fast, venous blood samples were collected for the estimation of serum creatinine (enzymatic method), blood urea (urease-glutamate dehydrogenase method), serum uric acid (uricase-peroxidase method), serum cystatin C (immunoturbidimetric assay), and glycated haemoglobin (HbA_{1c}) by high-performance liquid chromatography (HPLC). The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation. First-morning urine samples were collected for the determination of the urinary albumin-to-creatinine ratio (UACR) by immunoturbidimetry, while urinary creatinine was measured using the Jaffe method. In addition, 24-hour urine samples were collected for the estimation of total urinary protein using the pyrogallol red-molybdate method. All samples were processed according to standard laboratory protocols. Blood and urine specimens were centrifuged promptly, and serum samples intended for cystatin C estimation were stored at -80°C until analysis, while routine biochemical investigations were performed immediately after collection.

Statistical analysis

Statistical analysis was conducted by the SPSS version 16 for data analysis. The mean $\pm$ SD was used for the expression of the continuous variables and the frequencies and percentages were used for expressing the categorical variables. Student's t-test or chi-square test was used for comparison between the groups. The $p < 0.05$ was maintained for statistical analysis.

RESULT

Table 1 showed the comparative analysis of baseline demographic and clinical characteristics of the two groups, namely normoalbuminuria (Group I) and microalbuminuria (Group II). No significant difference was observed regarding the age (54.8 ± 8.2 vs. 56.3 ± 8.6 years; $p = 0.31$) and female sex (41.3% vs. 46.7%; $p = 0.52$) between the two groups. Group II, showed high diabetes duration (8.6 ± 3.2 vs. 6.8 ± 2.6 years; $p = 0.008$) and significantly higher levels of HbA_{1c} ($8.3 \pm 1.0\%$ vs. $7.4 \pm 0.8\%$; $p < 0.001$), reflecting poor glycaemic control. Furthermore, significantly higher systolic blood pressure was observed in Group II (133 ± 11 vs. 127 ± 10 mmHg; $p = 0.03$). Despite the high or low levels of BMI, eGFR, and usage of ACEi/ARB in the microalbuminuria group was noted.

Table 1: The baseline demographic and clinical parameter for patients of both of the groups

Variable	Group I (Normoalbuminuric, n = 75)	Group II (Microalbuminuric, n = 75)	p-value
Age (years), mean $\pm$ SD	54.8 $\pm$ 8.2	56.3 $\pm$ 8.6	0.31
Female, n (%)	31 (41.3%)	35 (46.7%)	0.52
Diabetes duration (years), mean $\pm$ SD	6.8 $\pm$ 2.6	8.6 $\pm$ 3.2	0.008*
HbA _{1c} (%), mean $\pm$ SD	7.4 $\pm$ 0.8	8.3 $\pm$ 1.0	<0.001*
BMI (kg/m ²), mean $\pm$ SD	25.9 $\pm$ 3.1	26.7 $\pm$ 3.5	0.18
SBP (mm Hg), mean $\pm$ SD	127 $\pm$ 10	133 $\pm$ 11	0.03*
eGFR (mL/min/1.73 m ²), mean $\pm$ SD	87 $\pm$ 14	81 $\pm$ 17	0.07
ACEi/ARB use, n (%)	33 (44.0%)	41 (54.7%)	0.2

Table 2 presents the comparison of conventional renal biomarkers between normoalbuminuric (Group I) and microalbuminuric (Group II) patients with type 2 diabetes mellitus. All evaluated biomarkers were significantly higher in the microalbuminuric group, indicating greater renal impairment. Serum creatinine increased from 0.89 ± 0.18 to 1.12 ± 0.26 mg/dL ($p < 0.001$), while serum cystatin C showed a corresponding increase from 0.88 ± 0.18 to 1.18 ± 0.27 mg/L ($p < 0.001$), suggesting reduced renal filtration. Blood urea and serum uric acid were also significantly elevated in Group II (36 ± 9 vs. 28 ± 7 mg/dL and 6.1 ± 1.3 vs. 5.2 ± 1.1 mg/dL, respectively). Furthermore, 24-hour urinary protein more than doubled in the microalbuminuric group (268 ± 86 vs. 118 ± 42 mg/day), while UACR increased markedly from 11 ± 4 to 47 ± 14 mg/g (both $p < 0.001$), confirming substantially greater urinary protein excretion and renal involvement in patients with microalbuminuria.

Table 2: The comparison between the renal biomarkers for among patients of both of the groups

Biomarker	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	p-value
Serum creatinine (mg/dL)	0.89 $\pm$ 0.18	1.12 $\pm$ 0.26	<0.001
Serum cystatin C (mg/L)	0.88 $\pm$ 0.18	1.18 $\pm$ 0.27	<0.001
Blood urea (mg/dL)	28 $\pm$ 7	36 $\pm$ 9	<0.001
Serum uric acid (mg/dL)	5.2 $\pm$ 1.1	6.1 $\pm$ 1.3	0.001
24-hour urinary protein (mg/day)	118 $\pm$ 42	268 $\pm$ 86	<0.001
UACR (mg/g)	11 $\pm$ 4	47 $\pm$ 14	<0.001

Table 3 compares the diagnostic performance of conventional renal biomarkers for identifying microalbuminuria in patients with type 2 diabetes mellitus. UACR demonstrated the highest individual diagnostic accuracy with an AUC of 0.83 (95% CI: 0.75–0.91), achieving 79% sensitivity and 81% specificity at an optimal cut-off of 22 mg/g. Twenty-four-hour urinary protein showed comparable performance (AUC: 0.82), with 78% sensitivity and 80% specificity. Serum cystatin C also exhibited good discriminatory ability (AUC: 0.80), outperforming serum creatinine (AUC: 0.74), blood urea (AUC: 0.73), and serum uric acid (AUC: 0.71). The combined model incorporating UACR and serum creatinine produced the highest diagnostic accuracy, with an AUC of 0.86, sensitivity of 84%, and specificity of 83%, indicating improved identification of microalbuminuria compared with the use of individual biomarkers alone.

Table 3: Diagnostic Performance of Conventional Renal Biomarkers for Identifying Microalbuminuria in Patients with Type 2 Diabetes Mellitus

Biomarker	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)
UACR	0.83 (0.75–0.91)	22 mg/g	79	81
Serum creatinine	0.74 (0.66–0.83)	1.0 mg/dL	70	72
Serum cystatin C	0.80 (0.72–0.88)	0.98 mg/L	76	79
Blood urea	0.73 (0.64–0.82)	32 mg/dL	69	71
Serum uric acid	0.71 (0.62–0.80)	5.8 mg/dL	68	70
24-hour urinary protein	0.82 (0.74–0.90)	180 mg/day	78	80
UACR + Serum creatinine	0.86 (0.79–0.93)	—	84	83

Table 4 presents the multivariable logistic regression analysis evaluating independent clinical and biochemical predictors of microalbuminuria in patients with type 2 diabetes mellitus. UACR emerged as the strongest independent predictor, with each 10 mg/g increase associated with a 2.9-fold higher likelihood of microalbuminuria (95% CI: 1.8–4.6, $p < 0.001$). Serum creatinine also demonstrated a significant association, with an adjusted odds ratio of 2.0 per 0.2 mg/dL increase ($p = 0.002$). Serum cystatin C remained an independent predictor, increasing the odds by 60% for every 0.1 mg/L increment ($p = 0.015$). Duration of diabetes was significantly associated with microalbuminuria, with a 12% increase in odds for each additional year ($p = 0.028$). In contrast, HbA_{1c} showed a positive but statistically non-significant association ($p = 0.087$), while ACE inhibitor/ARB use was not significantly associated with the presence of microalbuminuria ($p = 0.43$).

Table 4: Multivariable Logistic Regression Analysis of Conventional Clinical and Biochemical Predictors of Microalbuminuria in Patients with Type 2 Diabetes Mellitus

Predictor	Adjusted OR (95% CI)	p-value
UACR (per 10 mg/g increase)	2.9 (1.8–4.6)	<0.001
Serum creatinine (per 0.2 mg/dL increase)	2.0 (1.3–3.2)	0.002
Serum cystatin C (per 0.1 mg/L increase)	1.6 (1.2–2.2)	0.015
Duration of diabetes (per year)	1.12 (1.02–1.23)	0.028
HbA _{1c} (per 1% increase)	1.30 (0.95–1.70)	0.087
ACEi/ARB use	0.79 (0.42–1.47)	0.43

DISCUSSION

We aimed to assess the usefulness of routine biochemical parameters as early markers of diabetic kidney disease (DKD) in an Indian population with T2DM in this prospective cohort study.

Past studies done using routine blood and biochemical indicators for the prediction and risk stratification of early diabetic kidney disease. HbA_{1c}, and GLO were found to be key predictors that could contribute at low costs and provide valuable methodological inputs for screening the early stage of DKD in non-invasive ways [9]. Hyperglycaemia continues to be the main mechanism of DKD pathogenesis, leading to oxidative stress and endothelial dysfunction, and ultimately hyperfiltration. The Triglyceride- glucose (TyG) index is a powerful predictor of early renal impairment, insulin resistance and elevated HbA_{1c}, which are intricately connected to microvascular complications of DM [10,11]. Studies on TyG are being conducted and need to conduct more studies in the coming years to establish this as a biomarker. Our prospective data also emphasised the role of dyslipidemia in accelerating renal decline in addition to metabolic and glycemic indicators. Triglycerides and atherogenic lipid profiles were common in our population and were associated with an increase in renal dysfunction. This paper indicates that, apart from hyperglycemia, lipid abnormalities contribute synergistically to the microvascular damage of the kidney. Hence, aggressive management of lipids should continue to be an important component of the overall management of T2DM patients for maintaining renal health. [12] Importantly, our study identifies that renal decline can occur in normoalbuminuric or nonproteinuric patients, which highlights the need for a greater appreciation of renal injury to tubulointerstitial damage. These at-risk patients who would otherwise go untreated could be found by the inclusion of routine biochemical measurements, such as serum uric acid and eGFR. These results are very important from the public health point of view. In the increasing trend of DKD in India, particularly in the south, models for cost-effective screening are essential; [13] novel markers like Cystatin C and KIM-1 are highly sensitive but not yet feasible in practice. This study can help fill the gap and prove the feasibility of using already available and routine biochemical data. These accessible parameters, especially in combination, are useful to identify the high-risk individuals early and to enable the timely introduction of renin-angiotensin-aldosterone system (RAAS) blockade to slow the onset of end-stage renal disease (ESRD) [14]. The longitudinal information from the evaluation offers an independent association between the highest quartiles of TyG and long-term renal decline. This marks insulin resistance as a contributor to the stress of the glomeruli [15]. The present study is an all-embracing prospective study with a practical and cost-effective approach for early detection of DKD in the Indian population of T2DM patients.

Clinicians can perform an early, individualised risk stratification, using the HbA_{1c} and lipid markers. In addition, it is necessary to carry out multicentric trials to confirm these cut-off values and combine them into a national screening protocol that will enhance renal health outcomes in the longer term.

CONCLUSION

The study concluded that patients with type 2 diabetes mellitus and microalbuminuria exhibited significantly higher levels of conventional renal biomarkers, including serum creatinine, serum cystatin C, blood urea, serum uric acid, 24-hour urinary protein, and UACR, indicating greater renal impairment than normoalbuminuric patients. Among the evaluated biomarkers, UACR demonstrated the highest individual diagnostic performance, whereas the combination of UACR and serum creatinine provided the greatest diagnostic accuracy for identifying microalbuminuria. Multivariable analysis further established UACR as the strongest independent predictor of microalbuminuria, followed by serum creatinine, serum cystatin C, and duration of diabetes. Conversely, HbA_{1c} and ACE inhibitor/ARB use were not independently associated with microalbuminuria after adjustment. These findings support the combined use of UACR, serum creatinine, and serum cystatin C as effective conventional biomarkers for the early detection and risk stratification of diabetic kidney disease in patients with type 2 diabetes mellitus.

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