Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

Urinary Vitamin-D Binding Protein; Non- Invasive Biomarker for Diabetic Kidney Disease: A Cross- Sectional Study

Bhuneshwar Yadav[1], Shashidhar K N[2], Sai Deepika R[3], Munilakshmi U[3]

1. Assistant Professor, Department of Biochemistry, Shri Rawatpura Sarkar Institute of Medical Sciences & Research, Pacheda-2, Chhattisgarh, India.

2. Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Tamaka, Kolar Karnataka, India.

3. Assistant Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Tamaka, Kolar Karnataka, India.

Corresponding Author: Dr. Bhuneshwar Yadav

(Received: 25 August 2025 Revised: 27 September 2025 Accepted: 14 October 2025)

KEYWORDS

Diabetic Kidney Disease, Biomarker, Type 2 Diabetes Mellitus, Urinary Vitamin D Binding Protein

ABSTRACT:

Objective: For detecting early Diabetic Kidney Disease in patients with type 2 diabetes and to prevent its progression. To evaluate urinary VDBP as an early sensitive non-invasive biomarker for diagnosing of nephropathy in type 2 diabetic patients.

Methods: This Hospital based cross-sectional analytical study carried out on 180 subjects categorized into different groups based on value of microalbumin. Laboratory parameters were analyzed using standard methodology. Urinary VDBP estimation was carried out by using quantitative sandwich ELISA technique. IBM-SPSS version 20 was used for statistical analysis considering p value <0.05 statistically significant.

Results: Mean urinary VDBP levels were significantly higher in diabetic patients than in control. In addition, urinary VDBP levels were observed to be significantly elevated in diabetic subgroups i.e. normo, micro & macroalbuminuric group (955.2±325.7, 1202.2±430.8 & 1550.5±309.2 µg/mL) when compared to control subjects (80.7±43.38 µg/mL). A significant positive correlation with cystatin C (r=0.746) & ACR (r=0.525) while a negative correlation with eGFR (r=-0.77) observed. ROC analysis of uVDBP showed an area under curve of 0.966, sensitivity of 95% & a specificity of 80% at a cut-off point of 1058 µg/mL.

Conclusion: Findings of our study suggested that uVDBP levels were increased in early stages of nephropathy in type 2 diabetic patients & correlated significantly with severity of DKD. Thus, uVDBP could be considered as an early non-invasive biomarker for detection of Diabetic Kidney Disease & may help in prevention of end stage renal disease delaying its progression.

INTRODUCTION:

Diabetic Kidney Disease (DKD), is the most prevalent microvascular complication of type 2 Diabetes Mellitus (T2DM). DKD is an important cause of increased morbidity and mortality in diabetic patients.[1] DKD causes structural (glomerular and tubular) and functional alterations which is induced by disturbance in glucose homeostasis that leading to end stage renal disease (ESRD).[2] The prevalence of DKD increased in direct proportion to the prevalence of diabetes and on an average, it is estimated that DKD develops in 30-40% of patients with diabetes worldwide.[3] In India, DKD accounts for about 46% of chronic kidney disease (CKD) in elderly people and is associated with increased cardiovascular mortality and morbidity. A dramatic increase in prevalence of Diabetic Kidney Disease has been seen in Indian diabetic patients, is the most common cause of ESRD.[1] Microalbuminuria (30-300 mg/g) despite having adequate sensitivity and specificity, is considered the best available, non-invasive diagnostic marker for DKD. Around 20-30% of patients with type 2 diabetes are accompanied by renal insufficiency who has normoalbuminuria. [4]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

Early diagnosis of DKD in patients with T2DM and aiming to delay its progression to improve outcome, it is necessary to implement different strategies. Increased levels of urinary biomarkers can be detected in type 2 diabetic patients before onset of microalbuminuria. That may be used as an early non-invasive biomarker for DKD which plays a significant role for the effective management and treatment approaches in diabetic care.

Vitamin D binding protein (VDBP), is a group-specific component (GC) globulin which is a 58 kDa glycoprotein which acts as main carrier protein for vitamin D metabolites.[5] VDBP is constitutively synthesized by the liver and circulate in great excess (about 400 mg/L), with less than 5% of vitamin D binding sites normally occupied. Besides vitamin D transport, VDBP is also responsible for actin transport, fatty acid transport, macrophage activation, chemotaxis, and osteoclast activation.[6] In normal kidney, VDBP is reabsorbed by megalin mediated endocytosis and catabolized by proximal tubular epithelial cells reducing urinary excretion of VDBP to trace amount.[7] In Diabetic Kidney Disease, hyperglycemia induces Reactive Oxygen Species and Tumour Growth Factor-β production inturn triggers inflammatory cytokines secretion (IL-18) from podocytes; causing a direct or indirect renal damage with destruction of megalin/cubilin receptors in proximal tubular epithelial cells. This mechanism is responsible for VDBP uptake and resultant excretion of VDBP in urine. [8,9] It has been demonstrated clinically that elevated excretion of urinary VDBP is associated with tubular dysfunction.[10]. The increased excretion of VDBP may be linked to disease severity in DKD because of renal tubular cell damage which is involved in reabsorption and catabolism of VDBP. Presence of vitamin D deficiency or insufficiency in patient with diabetes have an important role in the progressive loss of renal function and is independently associated with the development of DKD. This deficiency of vitamin D in DKD may be contributed mechanistically because of excess loss of VDBP through urine.[11] Recent studies have demonstrated increased levels of VDBP excretion through urine in patient with DM.[10, 12] However, the potential role of uVDBP as a non-invasive marker for early detection of DKD has not been well established. Therefore, aim of this study is to evaluate urinary VDBP (uVDBP) as an early sensitive biomarker for assessing tubular damage in patients with Diabetic Kidney Disease.

MATERIALS AND METHODS:

This Hospital based cross-sectional study was carried out at a tertiary care hospital in Karnataka, India. The study protocol was approved by the Institutional Ethics Committee (SDUMC/KLR/IEC/17/2019-20) and was conducted in accordance with Declaration of Helsinki.

Study Subjects: 120 Type 2 Diabetes Mellitus fulfilling American Diabetes Association (ADA) criteria for diabetes and 60 age-sex matched healthy adults within age group between 35-70 years were included in this study. The study participants were informed with the purpose of the study and a written informed consent was obtained from all the participants. The control subjects were randomly selected from the general population without having any signs or clinical symptoms of chronic diseases and were not taking any regular medication. Recruited T2DM study participants were categorized into three different groups based on value of microalbumin into normoalbuminuric (<30mg/L), microalbuminuric (30-300 mg/L) and macroalbuminuric (>300 mg/L) respectively. Patients with liver disease, active urinary tract infections, malignancies, cardiovascular diseases, acute Kidney injury and known renal diseases other than DKD as well as patients on dialysis were excluded from the study.

Laboratory Evaluation: patient's clinical data and anthropometric measurements were obtained from either hospital medical record or from one-to-one interview. Venus blood samples (5 mL) were withdrawn after an overnight fast (8-10 hours) from all the study participants under aseptic condition and their comfortable position and are divided into parts as per the requirement of serum, plasma (blood sugar) and whole blood (for HbA1C). A post-prandial blood sample was also collected from the recruited participates for post-prandial blood sugar measurement.

Routine laboratory parameters including fasting and post-prandial blood sugar, blood urea, serum creatinine, uric acid, serum calcium, total protein, albumin and lipid profile (Total cholesterol (TC), Triglyceride and High-density lipoprotein cholesterol

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

(HDL-C) were done using commercially available kit on Vitros 5.1 FS auto analyzer (Ortho Clinical Diagnosis, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedwald equation. Glycated hemoglobin (HbA1C) was measured by high performance liquid chromatography technique on Bio Rad D10 (Bio-Rad Laboratories, USA). 25-Hydroxy Vitamin D (25-(OH) Vitamin D) levels were measured using chemiluminescence technique on while serum cystatin C was determined using Agappe MISPA i2 based on nephelometry methodology.

A clean-catch midstream urine samples (10-20 mL) were collected into a sterile plastic screw capped urine container and separated into 2 parts, one for microalbumin estimation and another for urinary VDBP measurement. Participants were given clear verbal instructions on how to collect the required midstream urine sample into the container. Urine sample for VDBP measurement was immediately centrifuged at 2000-3000 revolutions per minute for approximately 20 minutes to remove cell debris and particulate matter. The supernatant was stored at -80 ^{0}C until further analysis. Microalbumin levels were measured on Vitros 5.1 FS autoanalyzer by quantitative immune turbidimetric method. The eGFR was calculated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[13] Urinary VDBP estimation was carried out by using quantitative sandwich ELISA technique (ImmunoTag Human VDBP ELISA kit, Geno Technology Inc., USA).

Statistical analysis: Data were statistically described in terms of mean ± standard deviation. For comparing more than 2 groups one way analysis of variance (ANOVA) test was performed along with Bonferroni post hoc analysis. To compare between different variables, The Pearson's correlation coefficient (r) was calculated to assess the correlation between urinary VDBP and other parameters of the study. The diagnostic performance of urinary VDBP were analyzed using Receiver operating curve (ROC). For statistical analysis, IBM, SPSS statistical package 20 program was used and a p value <0.05 was considered statistically significant.

RESULTS:

The anthropometric measures and biochemical parameters of diabetic patients categorized according to the level of albuminuria compared to control group are shown in Table 1. A significant difference was observed with regard to age between control group and diabetic groups whereas BMI does not statistically significant difference among the four groups. We observed a significant increase in SBP and DBP according to level of albuminuria (control<normoalbuminuric<microalbuminuric<macroalbuminuric) in study subjects. The diabetic duration showed a significant difference between normoalbuminuric and macroalbuminuric diabetic subjects.

The biochemical parameters in study subjects showed a significant difference across different groups. FBS, PPBS & HbA1C levels across different groups indicate a significant increase in diabetic group than the control. However, we observed significant decreasing trends in these parameters as diabetic patients progress to macroalbuminuria from normo and microalbuminuria conditions. We observed the HbA1C % of 8.14±2.03 in macroalbuminuric group compared to 10.13±2.36 in microalbuminuric and 11.42±2.64 in normoalbuminuric group respectively which is comparable with the value of FBS and PPBS in these groups. This reduction in micro and macroalbuminuric groups may be because of the awareness of the chemical consequences that diabetic subjects has landed with.

Serum calcium and vitamin D levels were grossly decreased in macroalbuminuric group compared to other diabetic group and control group. An elevated uric acid level was observed in macro (7.71±1.94 mg/dL) and microalbuminuric (6.05±2.6 mg/dL) diabetic subjects than normoalbuminuric diabetics and control subjects. We also observed increased serum phosphate in macro and microalbuminuric group than normoalbuminuric and control group. Renal parameters in the control and normoalbuminuric diabetic groups were in the physiological range compared to diabetic patients with micro and macroalbuminuric group where it is grossly elevated and were statistically significant.

Serum cystatin C, microalbumin and ACR showed a statistically significant difference across groups. The levels of these were higher in diabetic patients with micro and macroalbuminuria when compared with control study subjects and normoalbuminuric diabetic subjects. Cystatin C showed

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

almost 2.5 times higher values in marcoalbuminuric group than control and normoalbuminuric group. We also observed that eGFR levels were lowered in T2DM patients with advanced albuminuria levels and was inversely proportional to the microalbumin as well as cystatin C levels in diabetic patients.

Table 1: Anthropometric Measures and Biochemical parameters represented as Mean ± Standard Deviation

PARAMETERS	**Control Group** (n=60)	**Diabetic Groups** (n=120)			**p value**
		Normo-albuminuria (n=35)	**Micro-albuminuria** (n=39)	**Macro-albuminuria** (n=46)	
Anthropometric Measurements					
AGE(Years)	46.58± 9.7	57.65±8.26[a]	58.43±7.9[a]	57.45±9.6[a]	<0.001
BMI (Kg/m^2)	24.19±4.08	24.21±3.69	25.09±4.43	23.5±3.49	0.334
SBP (mmHg)	121.16±4.8	123.9±13.6	126.4±16.5	135.56±20.1 [a,b,c]	<0.001
DBP (mmHg)	79.36±3.2	78.5±8.2	79.4±10.25	85.2±11.87 [a,b,c]	<0.001
Diabetic Duration (Year)	-	4.62±4.07	8.46±7.3	10.15±8.17[b]	0.002
Biochemical Parameters					
FBS (mg/dL)	95.9±8.93	227.9±66.16[a]	174.9±52.5[a,b]	154.5±62.3[a,b]	<0.001
PPBS (mg/dL)	107.4±21	305.5±93.01[a]	258.1±67.2[a,b]	240.6±71.01[a,b]	<0.001
HbA1C (%)	5.54±0.43	11.42±2.64[a]	10.13±2.36[a,b]	8.14±2.03[a,b,c]	<0.001
Urea (mg/dL)	19.8±5.46	21.6±9.23	63.6±50.7[a,b]	113.9±50.7[a,b,c]	<0.001
SCr (mg/dL)	0.73±0.2	0.62±0.3	2.23±2.08 [a,b]	5.36±3 [a,b,c]	<0.001
UA (mg/dL)	5.04±1.35	3.96±1.47[a]	6.05±2.6[b]	7.71±1.94[a,b,c]	<0.001
Calcium (mg/dL)	9.12±0.36	8.89±0.55	8.44±1[a]	7.8±0.91[a,b,c]	<0.001
Phosphate (mg/dL)	3.75±0.5	3.26±0.65	5.15±4.33[a,b]	5.22±1.9[a,b]	<0.001
Total Protein (g/dL)	7.26±0.45	6.79±0.71[a]	6.28±0.96[a,b]	5.8±0.88[a,b,c]	<0.001
Albumin (g/dL)	4.1±0.27	3.58±0.48[a]	3.15±0.71 [a,b]	2.73±0.56 [a,b,c]	<0.001
Vit D (ng/mL)	24.35±7.6	19.49±7.5[a]	19.13±8.3[a]	14.84±7.58[a,b]	<0.001
Cystatin C (mg/L)	0.81±0.16	0.99±0.28	1.47±0.72[a,b]	2.52±0.59[a,b,c]	<0.001
eGFR (mL/min)	102.1±20.2	81.48±23.9[a]	60.28±30.8[a,b]	25.3±11.14[a,b,c]	<0.001
mALB (mg/L)	9.65±7.54	11.02±7.14	153.1±81.4	1195.2±733.2[a,b,c]	<0.001
ACR (mg/gm)	16.87±16.4	28.45±22.75	468.5±695.7	3111.6±2019.8 [a,b,c]	<0.001
uVDBP (µg/mL)	80.7±43.38	955.2±325.7[a]	1202.2±430.8[a,b]	1550.5±309.2[a,b,c]	<0.001

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

[***p<0.05 considered as significant;** BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1C: Glycated Haemoglobin; SCr: Serum Creatinine; UA: Uric Acid; TC: Total Cholesterol; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; nHDL-C: Non High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; VLDL-C: Very Low Density Lipoprotein Cholesterol; Vit D: Vitamin D;eGFR: Estimated Glomerular Filtration Rate; mALB: Micro albumin; ACR: Albumin Creatinine Ratio; uVDBP: Urinary Vitamin D Binding Protein]

[a] Compared to control group; [b] Compared to diabetic normoalbuminuria group; [c] Compared to diabetic microalbuminuria group.

Mean urinary VDBP levels were significantly higher in diabetic patients than in control (Table 1 and Figure 1). In addition, urinary VDBP levels were observed to be significantly elevated in diabetic subgroups i.e. normo, micro and macroalbuminuric group (955.2±325.7, 1202.2±430.8 and 1550.5±309.2 µg/mL) when compared to control subjects (80.7±43.38 µg/mL). These results showed even in normoalbuminuric diabetic patients where other renal markers were within physiological reference limit, the level of uVDBP showed a markedly elevated level.

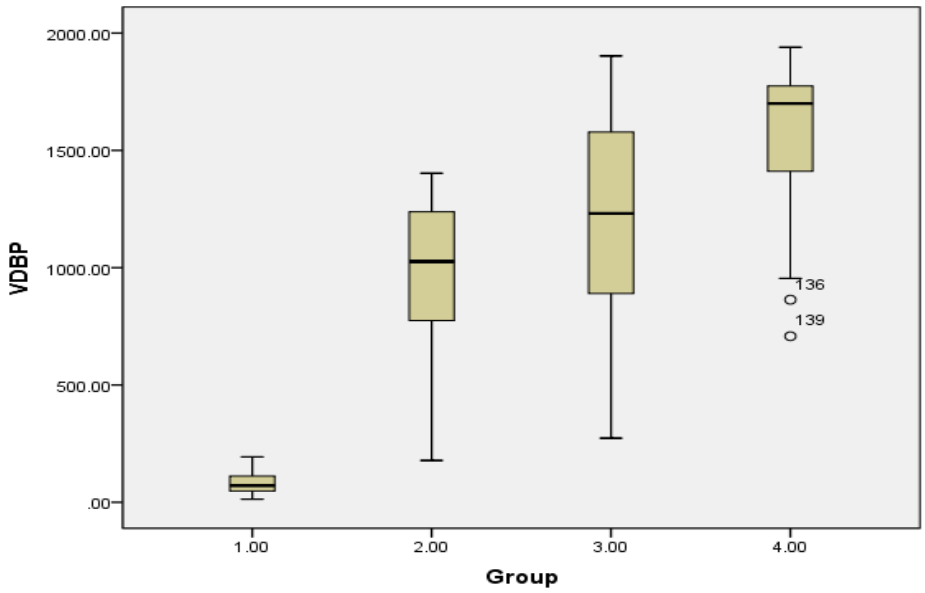

Fig 1: Urinary Vitamin D Binding Protein levels among the study groups.

[VDBP: Urinary Vitamin D Binding Protein; 1 Control group; 2 Normoalbuminuric diabetes group; 3 Microalbuminuric group; 4 Macroalbuminuric diabetes group]

Pearson's correlation analysis between urinary VDBP and other clinic-laboratory parameters among study participants showed a significant positive correlation with age, SBP, DBP, diabetic duration, FBS, PPBS, HbA1C, urea, serum creatinine, uric acid, phosphate, cystatin C, microalbumin and ACR (Table 2, Figure 2 & 3). Whereas a significant negative correlation with serum calcium, total protein, albumin, eGFR and vitamin D levels were observed (Figure 2). However, we did not observe any correlation between uVDBP and BMI of study subjects.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

Table 2: Correlation of Urinary Vitamin D Binding Protein with Anthropometric and Biochemical Indices

Variables	**uVDBP**	
	r-value	**p-value**
Anthropometric Measures		
AGE	0.457	<0.001
BMI	-0.014	0.849
SBP	0.310*	<0.001
DBP	0.196*	0.008
Diabetic Duration	0.305**	0.001
Diabetic Assessment		
FBS	0.335**	<0.001
PPBS	0.526**	<0.001
HbA1C	0.400**	<0.001
Renal Parameters		
Urea	0.688**	<0.001
Serum Creatinine	0.646**	<0.001
Uric Acid	0.432**	<0.001
Calcium	-0.577**	<0.001
Phosphate	0.308**	<0.001
Total Protein	-0.553**	<0.001
Albumin	-0.698**	<0.001
Cystatin C	0.746**	<0.001
eGFR	-0.770**	<0.001
mALB	0.551**	<0.001
ACR	0.525**	<0.001
Vitamin D Status		
Vitamin D	-0.358**	<0.001

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed)

[BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1C: Glycated Haemoglobin; eGFR: Estimated Glomerular Filtration Rate; mALB: Micro albumin; ACR: Albumin Creatinine Ratio; uVDBP: Urinary Vitamin D Binding Protein]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

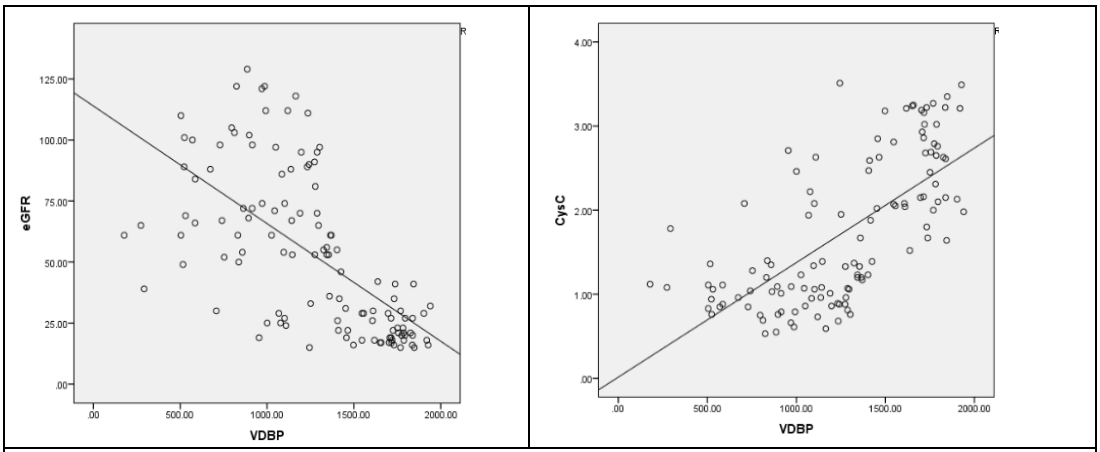

Figure 2: Correlation of uVDBP with eGFR and Cystatin C in T2DM

[eGFR: Estimated Glomerular Filtration Rate; CysC: Cystatin C; VDBP: Urinary Vitamin D Binding Protein]

Receiver operating characteristic analysis was performed for evaluating urinary VDBP as a biomarker for Diabetic Kidney Disease. Urinary VDBP showed an area under curve (AUC) of 0.966 (95%CI, 0.943-0.990). Urinary VDBP showed a sensitivity of 95% and a specificity of 80% at a cut-off point of 1058 µg/mL.

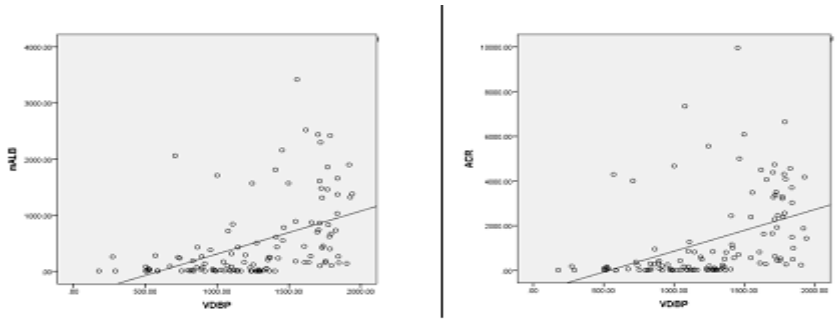

Figure 3: Correlation of uVDBP with mALB and ACR in T2DM

[mALB: Micro albumin; ACR: Albumin Creatinine Ratio; VDBP: Urinary Vitamin D Binding Protein]

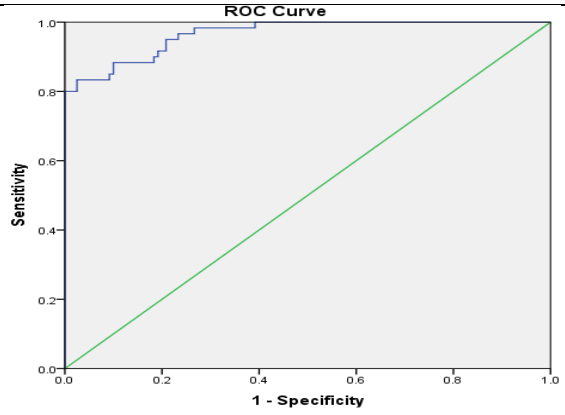

Figure 4: ROC plots to assess the diagnostic efficiency of urinary vitamin D Binding Protein to predict Nephropathy in T2DM patients

DISCUSSION:

D iabetic Kidney Disease

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

is a serious complication of diabetes and leads to ESRD if effective managements of the disease not started at the earliest. To reduce the burden of chronic kidney diseases in diabetic patients and facilitate the monitoring of the condition identification of novel biomarkers of early stages of DKD is mandatory.[14] Therefore, early prediction and detection of DKD would play a significant role in the effective management and treatment approaches. To explore whether uVDBP levels could be a novel non-invasive early biomarker for DKD, the result of this study demonstrated that uVDBP levels were grossly elevated in DKD patients and were correlating with the degree of albuminuria.

The result of our study demonstrated a significant increase in SBP and DBP in diabetic macroalbuminuric group compared to control group and normo & microalbuminuric diabetic groups. The average diabetic duration in macro and microalbuminuric group were 2.5 and 2 times greater than normoalbuminuric diabetic group (4.62±4.07 years). We also observed higher levels of basic diabetic profile and general renal parameters in all diabetic groups than the control group and were like results of earlier studies conducted by Patel et al [15] and Tarik M et al [16] in the year 2019 and 2016 respectively. Hyperglycemia being a most important factor in the development of Diabetic Kidney Disease causes glomerular hyperfiltration, glomerular and tubular epithelial hypertrophy and the development of microalbuminuria, which is followed by thickening of glomerular basement membrane, accumulation of mesangial matrix and overt proteinuria, eventually leading to glomerulosclerosis and end stage renal disease.[17] On correlation with basic diabetic profile uVDBP showed a significant positive correlation suggesting hyperglycemia as an important factor for renal damage in diabetic patients and a resultant excess excretion of uVDBP in such patients. The elevated general renal parameters in diabetics with micro and macroalbuminuria are due to the progressive loss of renal function as consequences of hyperglycemia.

In the present study we observed a significant reduction in serum calcium and vitamin D levels in diabetic group with micro and macroalbuminuria compared to control subjects and are alike findings of study conducted by Peng Y et al. [18] With decline renal function in patients with DKD, serum level of vitamin D decreases progressively leading to active vitamin D deficiency. Also, a significant negative correlation between uVDBP and serum calcium and vitamin D was observed. As VDBP is the main carrier protein for the transport of vitamin D metabolites, loss of excess VDBP through urine directly affects the level of vitamin D as well as serum calcium levels in Diabetic Kidney Disease patients. The low level of serum total protein and albumin in diabetic group with micro and macroalbuminuria in the current study indicates the defective glomerular and tubular functions with decline in eGFR. It has been documented that angiotensin II increases efferent arteriolar pressure and play an important role in the autoregulation of renal blood flow and GFR. Prolonged inappropriate increase in angiotensin II leads to decrease on renal blood flow.[19] Serum cystatin C is a promising marker of decline renal function and were significantly elevated in diabetics with micro and macroalbuminuric group with almost 2-3 times higher than healthy control. Our result was in accordance with the findings of Assal HS et al. [20] A strong positive correlation (r=0.746) between uVDBP and serum cystatin C was also observed in the present study (Fig 2).

In the present study, a significantly higher level of urinary VDBP was identified in diabetic group than the control group. The result also demonstrated that uVDBP levels were significantly higher in diabetic patients with micro and macroalbuminuria compared to normoalbuminuric diabetic patients. In consistence with the finding of our study, recent studies have demonstrated a marked increase in the urinary excretion of VDBP in patient with normo, micro and macroalbuminuria in type 2 diabetes, compared with the control study subjects. [10,21] Mirkovic K et.al in their study has shown uVDBP was increased with increasing severity of renal damage and responded well to renoprotective therapy.[22] These facts suggest that tubulointerstitial damage is considered as final common pathway for end stage renal disease (ESRD) and is present at an early asymptomatic stage of chronic kidney disease.[23] Our study showed a positive correlation between uVDBP with HbA1C and ACR which is in accordance with study conducted by Madha Mohammed Sheet Saleh et al[24] who demonstrated that uVDBP can be used as an early predictor for the detection of DKD which may help in prevention of the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

early onset of DKD.[24] Study conducted by Shoukry A et al[25] showed that a strong positive correlation ($p<0.001$) between uVDBP and uACR where uVDBP levels were directly proportional with increased uACR levels comparable to finding of our study. Increased urinary excretion of VDBP in the diabetic patients is probably due to hyperglycemia induced ROS and TGF-β production and induces inflammatory cytokines secretion (IL-18) from the podocytes; causing a direct or indirect renal damage with destruction of megalin/cubilin receptors in the proximal tubular epithelial cells responsible for VDBP uptake and resultant excretion of VDBP in urine. [9, 26]

We also observed urinary VDBP levels were closely associated with renal dysfunction which is indicated by elevated levels of renal parameters. uVDBP showed a positive significant correlation with urea, serum creatinine, uric acid, cystatin C, microalbumin and ACR and in accordance with earlier study[12]. However, a negative correlation with albumin and eGFR (Fig 2) was observed in the present study. Receiver operating characteristics analysis for the evaluation of diagnostic accuracy of uVDBP showed an area under curve (AUC) of 0.966 and a sensitivity of 95% and a specificity of 80% at a cut-off point of 1058 µg/mL. Study conducted by Shoukry et al demonstrated the potential use of elevated uVDBP as a novel biomarker for predicting DKD with 96% sensitivity and 84% specificity[12]. In the present study, to the best of our knowledge, it was demonstrated for the first time that elevated uVDBP levels in diabetic patients in India and there was a strong positive association between uVDBP levels and nephropathy development in diabetic patients.

Some major limitations of the present study include small sample size with single centric nature of study. Another limitation was that we do not have direct measure of GFR to precisely reflect kidney function. We included only Diabetic Kidney Disease patient only in our study, no other patients with additional nephropathies were included which might have overestimated the uVDBP as a biomarker for detection of DKD. Therefore, with larger sample from with various non- Diabetic Kidney Disease patients are required to spell out this issue.

CONCLUSION:

The findings of present study suggested that uVDBP levels were increased in the early stages of nephropathy in type 2 diabetic patients and correlated significantly with the severity (degree of albuminuria) of DKD. Thus, uVDBP could be considered as an early non-invasive biomarker for detection of Diabetic Kidney Disease and may help in prevention of end stage renal disease delaying its progression.

Acknowledgements: We would like to thank the Laboratory technicians and phlebotomist for their constant support in sample analysis and processing. We thank the academy for the logistic support.

Funding Sources: Nil

Conflicts of Interests: Nil

REFERENCES

1. Sharma RK. API Textbook of Medicine. Diabetes and kidney disease. 10th edition. Jaypee Brothers Medical Publisher, Vol 1, 2015; p. 528-533.
2. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J. Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care. 2014; 37:2864-2883.
3. American Diabetes Association. Standard of medical care in diabetes. Diabetes care. 2013; 36: S11-S66.
4. Satirapoj B. Tubulointerstitial Biomarkers for Diabetic Kidney Disease. Hindawi Journal of Diabetes Research 2018; Article ID 2852398: 1-6.
5. Song W, Wang X, Tian Y, ZhangX, Lu R, Meng H. GC Gene Polymorphisms and Vitamin D Binding Protein Levels Are Related to the Risk of Generalized Aggressive Periodontitis. International journal of endocrinology, 2016; 1(1): 1-8.
6. Wookey AF, Chollangi T, Yong HE, Kalionis B, Brennecke SP, Murthi P. Placental Vitamin D-Binding Protein expression in human idiopathic fetal growth restriction. Journal of Pregnancy. 2017:1-5.
7. Mahadevappa R, Nielsen R, Christensen EI, Birn H. Megalin in acute kidney injury-foe and friend. American Journal of Physiology Renal Physiology, 2014; 306(2): 147-154.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2649-2658 | ISSN:2251-6727

8. Satirapoj B, Adler SG. Comprehensive approach to Diabetic Kidney Disease. Kidney research and clinical practice 2014; 33(3): 121-131.
9. Fowlkes JL, Bunn RC, Cockrell GE, Clark LM, Wahl EC, Lumpkin CK. Dysregulation of the intrarenal vitamin D endocytic pathway in a nephropathy-prone mouse model of type 1 diabetes. Experimental diabetes research 2011: Article ID 269378: 1-7.
10. Thrailkill KM, Jo CH, Cockrell GE, Moreau CS, Fowlkes JL: Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency? J Clin Endocrinol Metab. 2011; 96:142–149.
11. Svoren BM, Volkening LK, Wood JR, Laffel LM. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. The Journal of Pediatrics. 2009; 154(1): 132–134.
12. Shoukry A, Bdeer SE, El-Sokkary RH. Urinary monocyte chemoattractant protein-1 and vitamin D-binding protein as biomarkers for early detection of Diabetic Kidney Disease in type 2 diabetes mellitus. Molecular and Cellular Biochemistry. 2015; 408(1-2): 25–35.
13. Keddis MT, Amer H, Voskoboev N, Kremers WK, Rule AD, Lieske JC. Creatinine–Based and Cystatin C–Based GFR Estimating Equations and Their Non-GFR Determinants in Kidney Transplant Recipients. Clin J Am Soc Nephrol. 2016; 11(9): 1640-1649.
14. Campion CG, Sanchez-Ferras O, Batchu SN. Potential role of serum and urinary biomarkers in diagnosis and prognosis of Diabetic Kidney Disease. Canadian Journal of Kidney Health and Disease. 2017; 4: 1-8.
15. Patel DKD, Kaila K. Characterization of low molecular weight urinary proteins at varying time intervals in type 2 diabetes mellitus and Diabetic Kidney Disease patients. Diabetol Metab Syndr. 2019; 11(39).
16. Takir M, Unal AD, Kostek O, Bayraktar N, Demirag NG. Cystatin-C and TGF-β levels in patients with Diabetic Kidney Disease. Nefrología. 2016.
17. Nazar CMJ. Diabetic Kidney Disease; principles of diagnosis and treatment of diabetic kidney disease. J Nephropharmacol 2014; 3(1): 15-20.
18. Peng Y, Li L. Serum 25-hydroxyvitamin D level and Diabetic Kidney Disease in patients with type 2 diabetes mellitus. Int Urol Nephrol. 2015; 47(6): 983-989.
19. Navarro JF, Milena FJ, Mora C, Leon C, Garcia J. Renal pro-inflammatory cytokine gene expression in Diabetic Kidney Disease: Effects of angiotensin-converting enzyme inhibition and pentoxifylline administration. Am J Nephrol. 2006; 26(6):562-70.
20. Assal HS, Tawfeek S, Rasheed E, Dalia E, Thabet E. Serum Cystatin C and Tubular Urinary Enzymes as Biomarkers of Renal Dysfunction in Type 2 Diabetes Mellitus. Clinical Medicine Insights. Endocrinology and Diabetes. 2013; 6:7–13.
21. Tian XQ, Zhao LM, Ge JP, Zhang Y, Xu YC. Elevated urinary level of vitamin D-binding protein as a novel biomarker for Diabetic Kidney Disease. Exp Ther Med. 2014 Feb; 7(2): 411-416.
22. Mirkovic K, Doorenbos CR, Dam WA, Lambers Heerspink HJ, Slagman MC, Nauta FL. Urinary vitamin D binding protein: a potential novel marker of renal interstitial inflammation and fibrosis. PLoS One 2013; 8(2): e55887.
23. Chaykovska L, Heunisch F, Einem G, Alter ML, Hocher CF, Tsuprykov O. Urinary Vitamin D Binding Protein and KIM-1 Are Potent New Biomarkers of Major Adverse Renal Events in Patients Undergoing Coronary Angiography. PLoS One 2016; 11(1): e0145723.
24. Saleh Sheet MM, AL-karawi INS, Alkhafajy HSA. Urinary Vitamin D Binding Protein as an Early Predictor of Diabetic Kidney Disease in Type 1 and Type 2 Diabetes. Ibn Al-Haitham Jour. For Pure and Appl.Sci 2018; 31(1):15-22.
25. Shoukry A, Bdeer SEA, El-Sokkary RH. Urinary monocyte chemoattractant protein-1 and vitamin D-binding protein as biomarkers for early detection of diabeticnephropathy in type 2 diabetes mellitus. Molecular and cellular biochemistry 2015; 408(1-2):25-35.
26. Satirapoj B, Adler SG. Comprehensive approach to Diabetic Kidney Disease. Kidney research and clinical practice 2014; 33(3): 121-131.