DOI: https://doi.org/10.54393/pjhs.v6i10.3159

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 6, Issue 10 (October 2025)

Original Article

OPEN ACCESS

Urinary Vitamin D-Binding Protein as a Diagnostic Marker for Diabetic Nephropathy in Type 2 Diabetic Patients

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ARTICLE INFO

Keywords:
Normoalbuminuria, Microalbuminuria, Albumin to Creatinine Ratio, Vitamin D Binding Protein

How to Cite:
Rasheed, N., Baghdadi, M. H., Sadiq, F., Hasan, S. U., & Mehmood, H. M. K. (2025). Urinary Vitamin D-Binding Protein as a Diagnostic Marker for Diabetic Nephropathy in Type 2 Diabetic Patients: Urinary VDBP in Diabetic Nephropathy. Pakistan Journal of Health Sciences, 6(10), 102–107. https://doi.org/10.54393/pjhs.v6i10.3159

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Received Date: 12th May, 2025
Revised Date: 23rd October, 2025
Acceptance Date: 29th October, 2025
Published Date: 31st October, 2025

ABSTRACT

In T2DM (Type 2 Diabetes Mellitus), the most common microvascular complication is DN (Diabetic Nephropathy). **Objectives:** to explore uVDBP (urinary vitamin D-binding protein) for the detection of DN. **Methods:** This cross-sectional comparative study was conducted in the Chemical Pathology Department, University of Health Sciences, Lahore. The study individuals were mainly categorized into three groups. Group 1 included normoalbuminuric non diabetic control subjects with normal ACR <30mg/g (n=25). Group 2 included normoalbuminuric T2DM patients with normal ACR <30mg/g (n=25). Group 3 comprised microalbuminuric T2DM patients with raised ACR 30 to 299 mg/g (n=25). Spot urine specimens were collected from T2DM patients. The patients were recruited from the Sheik Zayed Hospital Diabetic Clinic, Lahore. Collected data was analyzed by using SPSS version 24.0. Kruskal-Wallis test, Dunn-Bonferroni post-hoc analysis, and Spearman's correlation were applied. **Results:** The findings indicated the Urinary Vitamin D-Binding Protein (uVDBP) level was high in patients with microalbuminuria and normoalbuminuria compared to the control group. Group 3 had the highest median Urinary Vitamin D-Binding Protein (uVDBP) concentration, which was higher than in group 2 and group 1. Among all three groups, there was a statistically significant difference in levels of ACR (Albumin-to-Creatinine Ratio) and urinary Vitamin D-Binding Protein (p value = <0.001). **Conclusions:** In conclusion, the levels of urinary vitamin-D binding protein are significantly increased in T2DM patients having normoalbuminuria and microalbuminuria compared to non-diabetic control subjects. A significant positive correlation was observed between Urinary Vitamin D-Binding Protein (uVDBP) and albumin creatinine ratio.

INTRODUCTION

Diabetes mellitus encompasses a range of metabolic disorders marked by chronic high blood glucose, which arises due to insufficient insulin secretion, impaired insulin action, or both mechanisms combined [1]. T2DM is more prevalent compared to T1DM [2]. Type 2 Diabetes Mellitus (T2DM) is a chronic and progressive metabolic disorder, often asymptomatic in early stages [3]. The resultant chronic hyperglycemia leads to macrovascular and microvascular diabetic complications [4]. Diabetic nephropathy, the most prevalent and severe micro-vascular problem of diabetes mellitus, plays a vital role in enhancing disease and mortality rates seen among patients with diabetes [5]. The prevalence of diabetic nephropathy among diabetics in Pakistan is stated to stand at 27.1 percent [6]. In individuals with diabetes, diabetic nephropathy is an important contributor to end-stage kidney illness [7]. Studies indicate that approximately 30% of diabetic patients develop this condition, placing a significant strain on public healthcare systems [8]. The earliest clinical indicator of diabetic nephropathy is

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microalbuminuria [9]. It is characterized by the emission of albumin 30-300 mg/day in urine, and 30-300 mg/gram in urine creatinine. The gold standard test for the detection of DN in early phases is the detection of micro-albuminuria in 24-hour urine or spot urine. [10]. To enhance the clinical management of diabetes, there is a need for alternative urinary biomarkers capable of detecting diabetic nephropathy at a much earlier stage, ideally, before microalbuminuria becomes evident [11]. Numerous research studies have been carried out to find new biomarkers for DN, which include alpha-1-microglobulin, beta-2-microglobulin, uromodulin, IL-18 (interleukin-18), NGAL (neutrophil gelatinase-associated lipocalin), KIM-1(kidney injury molecule-1), and MCP-1(monocyte chemoattractant protein-1) [12]. The uVDBP biomarker is under investigation for its diagnosis in diabetic nephropathy. VDBP alpha-globulin is synthesized mainly in the liver, with an approximate molecular weight of 58 kDa. It has a serum concentration of 300 to 600 mg/ml. Originally identified as the group-specific element for serum (Gc-globulin), VDBP is recognized for its role in binding roughly 85 percent of 25-hydroxyvitamin-D(25(OH)D) during circulation. It's named VDBP (Vitamin D-binding Protein). Transportation of vitamin D metabolites in the circulation is its primary function. VDBP is filtered through the proximal tubule cells and glomeruli, after which it's reabsorbed via receptor-mediated mechanisms. If this protein is not reabsorbed due to impaired function of the proximal tubules of the glomerulus, it starts appearing in urine [13]. Excretion of VDBP in the urine of T2DM patients has not been evaluated, especially when albuminuria is not present.

Although microalbuminuria remains the gold standard for early detection of diabetic nephropathy, it primarily reflects glomerular damage and may fail to identify earlier tubular injury. Emerging evidence suggests that urinary Vitamin D-Binding Protein (uVDBP) may serve as a marker of proximal tubular dysfunction; however, data evaluating its diagnostic utility in normoalbuminuric T2DM patients are limited and inconsistent. Furthermore, region-specific studies from Pakistan exploring uVDBP across different stages of albuminuria are scarce. This gap underscores the need to assess whether uVDBP can detect renal involvement prior to overt microalbuminuria in local diabetic populations. This study aimed to evaluate and compare the VDBP level in urine of normoalbuminuric non diabetic control subjects, normoalbuminuric T2DM patients, and microalbuminuric T2DM patients to explore the role of uVDBP in the detection of DN at an early stage.

METHODS

This cross-sectional comparative observational study was conducted in the Chemical Pathology Department, University of Health Sciences, Lahore, and approved by the Ethical Review Committee (Ref. No. UHS/EAPC-22/ERC/18). The study was conducted from February 2023 to July 2023. The sample size was determined using G Power version 3.1 from a one-way ANOVA model because in this study, there was a comparison of urinary Vitamin D-Binding Protein (uVDBP) levels between three independent groups. The main outcome variable for which the sample size was estimated was urinary VDBP concentration. A level of significance (α) of 0.05, power (1-β) of 80%, and an estimated large effect size (Cohen's f=0.40) were used, consistent with differences in uVDBP levels documented in a recent meta-analysis by Chen et al. (2023) [14]. Based on these, a minimum of 25 participants in each group was needed, totaling 75 subjects, which was attained in the current study. For each study participant, informed consent was obtained in written form for participation. Among total of 50 T2DM patients and twenty-five (25) normal controls of either gender were recruited for this study, with age ranges between 40-50 years. Only those patients were recruited whose duration of Diabetes was at least five years. The study individuals were divided into three subgroups. Group 1 comprised of twenty-five (n=25) normoalbuminuric non diabetic control subjects with normal ACR <30mg/g. Group 2 comprised of twenty-five (n=25) normoalbuminuric T2DM patients with normal ACR <30mg/g. Group 3 comprised of twenty-five (n=25) microalbuminuric T2DM patients with raised ACR 30 to 299 mg/g. The main outcome variable of the study was the concentration of urinary VDBP. The secondary outcome measures were albumin-to-creatinine ratio (ACR), age, body mass index (BMI), and disease duration. The individuals suffering from infections, liver diseases, or any chronic disease other than diabetes were excluded from the study. Patients taking hormones and drugs such as vitamin D supplements, hypercalcemic drugs at the time of study, were also excluded from the study. Samples were collected from Sheikh Zayed Hospital Diabetic Clinic, Lahore. Data were collected daily from the Outpatient Department (OPD) of Shaikh Zayed Diabetic Clinic, where the researcher obtained the patients' demographic information through taking patient consent. A convenient sampling technique was used among the diagnosed cases of T2DM from Sheik Zayed Hospital Diabetic Clinic, Lahore. Midstream random urine samples of study individuals were collected in a sterile container from patients. Urine creatinine was measured by the Jaffe kinetic method on the Microlab 300 spectrophotometer (Merck, Germany). Urinary albumin was quantified using the immunoturbidimetric method on the same analyzer (Microlab 300, Merck, Germany). The Albumin-to-Creatinine Ratio (ACR) was calculated by dividing urine

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albumin concentration (mg) by urine creatinine concentration (g), and expressed in mg/g. Urinary Vitamin D-Binding Protein (uVDBP) was determined using a commercial ELISA kit on the Bio-Rad ELISA Reader Model 410 (Bio-Rad Laboratories, USA), following the manufacturer's instructions. Results were expressed as uVDBP/creatinine ratio to account for variations in urine concentration. Urine analysis reagent strips (Combi10-Medi Test) were used to screen samples for proteinuria, and only those without detectable protein levels were selected for inclusion in the study. By the use of SPSS version 24.0 collected data were analyzed. Qualitative variable like gender distribution, was expressed in the form of a percentage, while quantitative variables, such as age, VDBP/Creatinine ratio, and urine creatinine, were presented as Median and IQR. Data normality was checked with the help of the Shapiro-Wilk test. By using an independent Kruskal–Wallis test to compare uVDBP and ACR values among the three groups. Post-hoc pairwise comparisons were performed using the Dunn–Bonferroni test. Spearman's rank correlation coefficient was used to evaluate the association between uVDBP and ACR within each group (p-value ≤0.050 for significant outcomes)

RESULTS

Among all participants, 50 T2DM patients and 25 normal non diabetic controls of either gender were recruited for this study, with an age range between 40-50 years. Only those patients were recruited whose duration of Diabetes was at least five years. The age, gender, and BMI (Body Mass Index) of the patients were noted. In Group 1, the number of male participants was high (62%), trailed by Group 2(58%) and Group 3(48%), also showing statistically non-significant findings after comparison among the three groups (p=0.497). In group 3, the values of interquartile range (IQR) and Median age were detected as high, trailed by groups 2 and 1. The median and interquartile range for BMI (Body Mass Index) were higher in group 3 than in group 1. Conversely, the median and interquartile range of diabetes duration were greater in group 3 than in group 2, and the difference was significant, p=<0.001 (Table 1).

Table 1: Demographics of the Study Participants

Demographics		Group 1 (n=25)	Group 2 (N=25)	Group 3 (N=25)	p-Value
Gender	M (%)	16 (62%)	13 (52%)	12 (48%)	0.497a
	F (%)	9 (36%)	12 (48%)	13 (52%)	
Age (years)	Median	42	46	48	<0.001*a 0.215b
	IQR	41-46	44-48	46.00-48.50	
BMI kg/m^2	Median	23.70	25.10	25.50	0.663a
	IQR	22.90-25.40	22.70-26.50	23.75-26.60	
Disease Duration (years)	Median	–	6.0	7.00	<0.001*a
	IQR	–	5.0-6.5	6.50-8.00	

(a) all groups comparison, (b) group 2 and comparison, (*) showed significancy

Group 3 had the greatest median and interquartile range (Q1–Q3) of ACR at 89 mg/g (75.55–122.50 mg/g), followed by group 2 at 17.50 mg/g (13.30–22.50 mg/g), and group 1 at 8.30 mg/g (6.05–11.50 mg/g) (Table 2).

Table 2: UVDBP (Urinary Vitamin D Binding Protein) and ACR (Albumin Creatinine Ratio) Comparison

Variables	Group1		Group 2		Group 3		p-Value
	Median	IQR (Q1-Q3)	Median	IQR (Q1-Q3)	Median	IQR (Q1-Q3I)	
ACR (mg/g)	8.30	(6.05-11.50)	17.50	(13.30-22.50)	89	(75.55-122.50)	<0.001*
uVDBP (ng/mg)	98	(73.50-149)	442	(381.50-523)	1056	(905-1215)	<0.001*

(*) Denotes statistically significant ≤0.050, IQR= Interquartile range

The comparison by the Independent Kruskal-Wallis test revealed that there was a significant difference among all groups on ACR values, p=<0.001 (Figure 1).

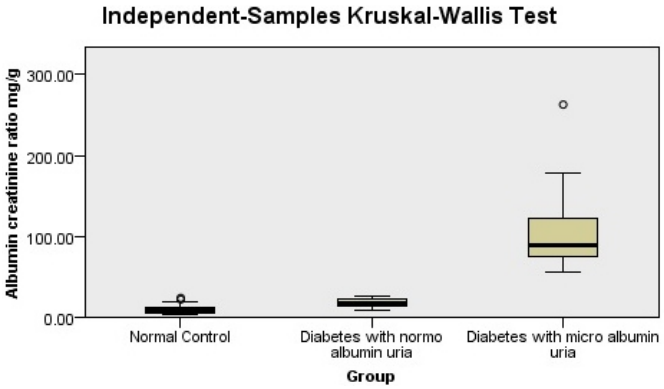

Figure 1: Comparison of ACR Among All Three Groups

Similarly, the comparison of uVDBP levels across the groups using the Independent Kruskal-Wallis test revealed group 3 with the highest median and IQR (Q1-Q3) at 1056ng/mg (905-1215ng/mg), followed by group 2 at 442ng/mg (381.50-523ng/mg), and last group 1 at 98ng/mg (73.50-149ng/mg). This comparison also revealed significant differences among all groups, p=<0.001 (Figure 2).

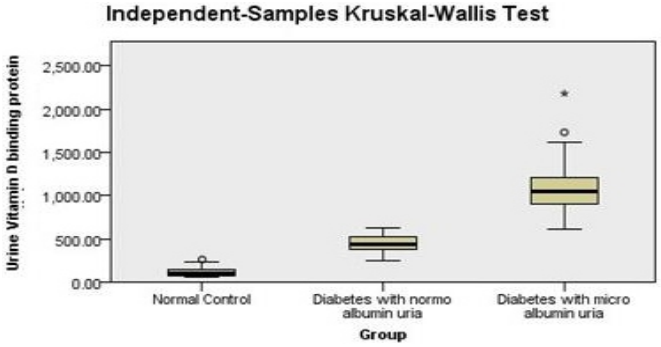

Figure 2: Comparison of uVDBP Levels Among All Three Groups

By using the Dunn-Bonferroni post-hoc test, multiple ACR

DOI: https://doi.org/10.54393/pjhs.v6i10.3159

comparisons within the groups showed that group 1 and 3 had a significant difference with p=<0.001, also group 2 and 3 with p=<0.001, while there was no statistically significant difference between group 1 and group 2, p=0.567. Similarly, the multiple comparisons within the groups using uVDBP showed a significant difference between groups 1 and 3, and groups 2 and 3 also showed the same results (p =<0.001). Additionally, groups 1 and 2 showed significant differences, with a p=<0.001(Table 3).

Table 3: ACR and uVDBP Multiple Comparison (n=25 Each Group)

Variables	Groups	Median, IQR(Q1- Q3)	p-Value
ACR (mg/g)	1	8.30 (6.05-11.50)	<0.001*[a] 0.567 [b] <0.001*[c] <0.001*[d]
	2	17.50 (13.30-22.50)	
	3	89 (75.55-122.50)	
uVDBP (ng/mg)	1	98 (73.50-149)	0.001*[a] 0.001*[b] <0.001*[c] <0.001*[d]
	2	442 (381.50-523)	
	3	1056 (905-1215)	

(*) significance of results ≤0.050, (a) all groups comparison, (b) group 1 and 2 comparison, (c) group 1 and 3 comparison, (d) group 2 and 3 comparison

Spearman's rank correlation between urinary VDBP (uVDBP) & albumin-to-creatinine ratio (ACR) levels in group 1 was positive and very strong, with a correlation coefficient (rs) of 0.732, which was statistically significantly high (p<0.001). In group 2, there was a positive moderate correlation with a rs value of 0.427, which was significant, p=0.011. Group 3 showed a highly positive association between two variables, with a rs of 0.879, and this finding was significant, p<0.001(Table 4).

Table 4: Spearman's Rank Correlation Coefficient Analysis of ACR and uVDBP Levels

Groups	Independent Variables	Correlation Coefficients (r_s)	Dependent variables (uVDBP)
Group 1	ACR	r_s	0.732
		p-value	<0.001**
Group 2	ACR	r_s	0.427
		p-value	0.011*
Group 3	ACR	r_s	0.879
		p-value	<0.001**

(rs) Correlation coefficient, (+) correlation, 0.7 to 0.9 correlation, strong, 0.4 to 0.6 intermediate correlation, and 0 to 0.3 weak correlation, (**)<0.010 significant correlation, (*)<0.05 significant correlation

DISCUSSION

An important finding of the study was that the urinary levels of VDBP were meaningfully amplified in patients with microalbuminuria and normoalbuminuria compared to the control group. As this protein is increased in patients who have a normal ACR ratio, it indicates that proximal tubular damage starts before the glomerular membrane damage in T2DM patients, even before the appearance of microalbumin in urine. This finding defines the function of uVDBP for DN diagnosis at a very early stage. Recently, a meta-analysis has evaluated the role of uVDBP in the diagnosis and monitoring of DN and has concluded that a novel biomarker, uVDBP, is likely to be used for DN diagnosis in the early stage and also could be used for the assessment of the DN severity [15]. Another review study highlighted the role by mean of a potential biomarker for kidney disease early diagnosis. (16) One previous study by Fawzy *et al.* (2018 on the Saudi population also observed similar findings that uVDBP used as an early biomarker to detect DN [16]. Among the previous, Khodeir *et al.* revealed a significant increase in urinary Vitamin D–D-binding protein (VDBP) content in comparison between diabetic patients and healthy controls [17]. Likewise, another topical study on patients with T2DM, grouped according to their albumin-to-creatinine ratio as normoalbuminuric, microalbuminuric, and macroalbuminuric, found a significant increase in the VDBP-to-creatinine ratio in both microalbuminuric and macroalbuminuric patients when compared to non-albuminuric patients [18]. The exact mechanism of increased urinary excretion of VDBP in diabetic nephropathy is not defined. Yet, it is established that proximal tubular epithelial cells possess a multiligand endocytic receptor called megalin, which performs a crucial function of reabsorbing filtered proteins, such as low molecular weight proteins like VDBP, from the glomerular filtrate. Another integral part, cubilin, co-functions with megalin to mediate renal uptake of multiple ligands, including vitamin D-carrying proteins. Renal disease-associated impairment or shedding of the megalin-cubilin complex underlies albuminuria and can partially account for the increased urinary VDBP excretion reported in such patients [19]. Another significant observation made in the contemporary study, the presence of (+) correlation amongst levels of urinary VDBP and ACR in the study groups. A particularly strong relationship was observed in both the normoalbuminuric (group 1) and microalbuminuric (group 3) groups. In support of this finding, a six-year longitudinal study detected a persistent and strong correlation between urinary ACR and VDBP levels at baseline and during the follow-up [20]. The study's limitations include convenience sampling, small sample size, and the cross-sectional design, which prevents assessment of DN progression. Further longitudinal studies with larger sample sizes are recommended.

This study has certain limitations, including its cross-sectional design and relatively small sample size, which limit causal inference and broader generalizability. The use of convenience sampling and the absence of longitudinal follow-up prevent evaluation of the predictive value of uVDBP for progression to overt nephropathy. Additionally, serum vitamin D levels and other tubular injury markers

DOI: https://doi.org/10.54393/pjhs.v6i10.3159

were not assessed for comparative analysis. Future large-scale, multicenter prospective studies incorporating longitudinal monitoring and combined biomarker panels are warranted to validate uVDBP as an early diagnostic and prognostic tool in diabetic nephropathy.

CONCLUSIONS

In conclusion, the present study indicates that modestly higher urinary VDBP concentrations exist in T2DM patients who have normoalbuminuria or microalbuminuria, compared to non-diabetic controls. A positive, significant correlation between uVDBP and ACR was also found, suggesting the role of uVDBP as an early indicator in diabetic nephropathy.

Authors' Contribution

Conceptualization: NR
Methodology: MHB, SH, HMKM
Formal analysis: MHB, SH
Writing and Drafting: NR, FS, HMKM
Review and Editing: NR, FS, HMKM, MHB, SH

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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