

Comparison of urinary vitamin D binding protein with albumin-creatinine ratio in type 2 diabetes mellitus as an early screening tool for diabetic nephropathy

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a group of metabolic disorders. Diabetic nephropathy is one of the chronic complications of DM, leading to end stage renal disease (ESRD). A current diagnostic criterion for diabetic nephropathy (DN) is detection of microalbuminuria, which is 30 – 300 mg/24 hours of albumin excretion in urine or albumin to creatinine ratio (ACR) in the range of 30 – 300 mg/g in the random urine sample but, it shows inadequate sensitivity for the early detection of DN. It has been observed that increased excretion of urinary vitamin D binding proteins (uVDBP) is related to tubular dysfunction. This protein is excreted in urine earlier than albumin. Hence it can be used as a tool to early detection of DN in type 2 diabetic patients.

Methodology: This was a comparative cross sectional study, which comprised of seventy five study subjects and were distributed into three study groups with 25 subjects in each group, having age in the range of 40-50 years. Group-1 comprising controls (without diabetes mellitus), Group-2 had diabetes mellitus with normoalbuminuria while Group-3 comprised of diabetes mellitus patients with microalbuminuria. Vitamin D binding protein, urine creatinine and albumin were measured from the random urine sample preferably early in the morning urine sample of each study subjects using ELISA, Jaffe and immunoturbidimetric methods respectively. Levels of VDBP and albumin were normalized with urine creatinine and expressed as VDBP creatinine ratio as (ng/mg) and albumin creatinine ratio as (mg/g) in the spot urine sample.

Results: Urinary VDBP levels among the three groups were as the highest median values were observed in group 3 as 1056 ng/mg, (IQR 905 – 1215 ng/mg) followed by group 2 as 442 ng/mg, (381.5 – 523 ng/mg) and group 1 as 98 ng/mg, (73.5 – 149 ng/mg) respectively, and a statistical significant difference was observed among the three groups with a p-value of 0.000. Results of this study showed that level of Vitamin D binding protein was significantly increased in diabetes mellitus in comparison to control subjects.

Conclusion: Results suggest that urinary vitamin D binding protein level is likely to become a useful biomarker for the early detection and management of diabetic nephropathy in Type 2 diabetic patients.

Keywords:

Vitamin D binding protein; Diabetic nephropathy; Albumin creatinine ratio; Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a set of metabolic disorders. Type 2 Diabetes Mellitus (DM) is heralded with its complications like myocardial infarction, cerebrovascular accident, diabetic retinopathy and nephropathy leading to renal failure.¹ Worldwide, as well as in Pakistan, incidence of type 2 DM has

increased and Pakistan is rated at seventh in this regard.² Most prominent features of diabetic nephropathy (DN) includes hypertrophy of the tubules of the glomerulus, increased in the glomerular membrane thickness, tubule-interstitial fibrosis and mesangial expansion which ultimately lead to decrease in the glomerular filtration rate (GFR), development of systemic hypertension, proteinuria, and compromised functional ability of the kidneys.^{3,4} Diabetic nephropathy (DN) is characterized by a slight increase in the excretion level of urine albumin which is also known as microalbuminuria or incipient DN.⁵ Microalbuminuria is the initial clinical sign of diabetic nephropathy. Microalbuminuria is clinically defined as

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Table 1. Characteristics of the study participants in three groups

Variables	Group 1	Group 2	Group 3	p-value
Gender				
Male (%)	16 (62%)	13 (52%)	12 (48%)	0.497
Female (%)	9 (36%)	12 (48%)	13 (52%)	
Age (mean \pm SD)	45.8 \pm 2.8	46.0 \pm 2.3	47.2 \pm 1.9	0.215
Body Mass Index kg/m ² (Mean \pm SD)	24.0 \pm 1.9	24.5 \pm 2.2	24.6 \pm 2.7	0.663

urine albumin excretion of 30 to 300 mg/day or 30 to 300 mg of albumin/gram of creatinine (Cr) excretion in urine.⁶ Vitamin D binding protein (VDBP) is an alpha globulin protein primarily produced by the liver and has a molecular weight of 58 kDa. Filtration of VDBP is done by the glomerular and proximal tubule cells; afterwards it is reabsorbed by receptor-mediated uptake. Vitamin D is activated by 1-alpha hydroxylase and this method is decisive for recovery of vitamin D, because the presence of 1-alpha hydroxylase is abundant in proximal tubule cells.⁸ The importance of using VDBP as an earlier marker for DN is related to the fact that patients having diabetes mellitus especially type 2 suffer from prediabetic stage and might have developed declined renal function when diagnosed as diabetic.⁹ Microalbuminuria is considered the gold standard test for the early diagnosis of diabetic nephropathy.¹¹ The purpose this study was to determine and compare level of urinary VDBP in type 2 diabetics having microalbuminuria and diabetics with no microalbuminuria with control subjects. The purpose of this study was to determine and compare levels of urinary VDBP in type 2 diabetics having microalbuminuria and diabetics having no microalbuminuria with control subjects. Currently vitamin D binding protein levels for the early detection of diabetic nephropathy has not been used diagnostically on local and international level and the relevant data is available in the form of research articles, as demonstrated by Shoukry et al, which revealed that marked elevation in the urine level of VDBP was observed in the earlier phase of DN in type 2 diabetes mellitus patients in relation to the controls.¹⁵ Khodeir et al also showed a substantial increase in the urine level of Vitamin D binding protein between the diabetic and control groups.¹⁶ At this stage, detection of statistically significant difference in the urine level of VDBP in diabetics Type 2 and control subjects can be helpful for early detection of diabetic kidney injury in diabetic Type 2 patients.

SUBJECTS AND METHODS

It was a comparative study performed in the department of chemical pathology UHS Lahore from March 2016

to April 2017. This study comprised of 75 subjects, with age range of 40-50 years, divided into three groups with 25 subjects in each group. Group 1 comprising controls (without diabetes mellitus), Group 2 with patients of type 2 diabetes mellitus without microalbuminuria while Group 3 comprised type 2 diabetic patients with microalbuminuria. Inclusion criteria consisted of normal healthy individuals without diabetes mellitus and Type 2 Diabetics of either gender with BMI up to 28 and age between 40-50 years males and non-pregnant females with at least five years duration of Type 2 Diabetes. Exclusion criteria included fever, pregnancy, congestive heart failure and exercise within 24 hours. Diabetic patients with macroalbuminuria or taking Vitamin D supplements and drugs like calcium supplements, antiepileptic, statins, corticosteroid, antihypertensive and antibiotics including: cephalosporin, and clotrimazole. Control subjects were selected from general population. Selection of diabetic study subjects were performed from the outdoor diabetic clinic patients of Sheikh Zayed Hospital Lahore, Pakistan. After an informed consent, convenient sampling technique was used to collect the random urine sample from the control subjects and patients, a non-sterile urine collection container was given to the study subjects and instructed them to collect a midstream specimen in the container. All the testing parameters were performed on random urine sample.

In chemical pathology department, urine samples were tested with urine analysis reagent strips (Combi 10-medi test) to rule out proteinuria. Centrifugation of non-proteinuric samples were performed in refrigerated centrifuge at 4°C for ten minutes at speed of 2500 x g and supernatants were stored in three aliquots at a temperature of -80°C for estimation of Microalbumin, urine creatinine and VDBP. Urine creatinine was measured by Jaffe method, on spectrophotometer. Urine albumin was determined by immunoturbidimetric method. VDBP was estimated in urine sample by enzyme-linked immunosorbant assay (ELISA), using Human VDBP ELISA Kit (Glory Science Co Ltd, USA). Levels of vitamin D binding

Table 2. Comparison of ACR and uVDBP among three groups

Variables	Group 1	Group 2	Group 3	p-value
ACR (mg/g) (Median, [IQR])	8.3 (6.05 - 11.50)	17.5 (13.30 - 22.50)	89 (75.55 – 122.5)	0.000*
uVDBP(ng/mg) (Median, [IQR])	98 (73.5 – 149)	442 (381.5 – 523)	1056 (905 – 1215)	0.000*

protein and albumin were normalized with the urine creatinine and expressed as vitamin D binding protein creatinine ratio in (ng/mg) and albumin to creatinine ratio in (mg/g) in the spot urine sample. The data was entered and analyzed using SPSS 20.0. The differences of the Vitamin D binding protein/Creatinine ratio between the groups were determined by Independent Kruskal-Wallis test. Pairwise comparison was done with Dunn-Bonferoni test. A p-value of ≤ 0.05 was taken as statistically significant.

RESULTS

In this study there were 25 subjects in each study group with 16 (62%) male and 9 (36%) females in group 1. In group 2 there were 13 (52%) males and 12 (48%) females and in group 3 there were 12 (48%) males and 13 (52%) females. No statistically significant difference was observed in terms of gender, age and body mass Index (BMI) in all the groups as shown in Table 1.

The level of urinary albumin creatinine ratio (ACR) was compared among the three study groups and showed highest Median, IQR (Q1- Q3) values of ACR in group 3, as 89 (75.55 – 122.50) followed by group 2 as 17.50 (13.30 - 22.50) and group-1 as 8.30 (6.05 - 11.50) respectively. On comparison between the groups by Independent Kruskal Wallis test, statistical significant differences were observed among the three groups (p-value 0.000). When the comparison between urinary VDBP levels among the three groups were done with Independent Kruskal Wallis test, highest Median, IQR (Q1- Q3) values were observed in group 3 as 1056 (905 – 1215) followed by group 2 as 442 (381.50 – 523) and group 1 as 98 (73.50 – 149) respectively and a statistical significant difference was observed among three groups with a p-value of 0.000 as shown in Table 2.

Multiple comparison of albumin creatinine ratio within the groups by Dunn-Bonferroni post hoc test shows group 1 and 3 has a statistical significant difference with p-value of 0.000 and also group 2 and 3 with a p-value of 0.000, but comparison between the group 1 and group 2 shows no statistical significant difference with p-value of 0.567. Multiple comparison of urinary VDBP within the groups by Dunn-Bonferroni post hoc test shows that group 1 and 3 has a statistical significant difference with a p-value of 0.000

and group 2 and 3 also has statistical significant difference with a p-value of 0.000. Similarly group 1 and 2 also has statistical significant difference with a p-value of 0.000 as shown in Table 3.

DISCUSSION

The current diagnostic criteria used for diabetic nephropathy based on determination of microalbuminuria in urine have shown inadequate sensitivity and specificity.¹⁰ Vitamin D binding protein (VDBP) is an alpha globulin protein primarily produced by the liver.⁷ Vitamin D is transported by VDBP which is very important for functioning of vast range of tissues, and variations in the activity of VDBP is associated with the development of many diseases.¹² In the current study, the highest median value of urinary VDBP was observed in group 3, followed by groups 2 and 1. Statistically significant difference was detected when comparison was done among the three groups. A recent study showed a significant increase in the excretion levels of urine VDBP in diabetic type 1 and type 2 with normo, micro, and macroalbuminuria as compared to the levels of control subjects, which is in accordance with findings of this study.¹³ Another study reported noticeable increase in the excretion levels of VDBP in patients with normo, micro, and macroalbuminuria in type 1 DM in comparison to controls.¹⁴ One more study in diabetic patients Type 2 diabetic patients in which the study subjects were divided in three different groups revealed that urine levels of VDBP were also increased in patients without albuminuria, and microalbuminuria, which shows an approximate increase of 11.1 fold in comparison to control subjects, showing their progressive relationship with the advancement of the disease.¹⁰ A recent study by Shoukry and coauthors reported a marked elevation in the urine level of VDBP in the earlier phase of DN in type 2 diabetes mellitus patients in relation to the controls.¹⁵ Khodeir and group also showed a substantial increase in the urine level of Vitamin D binding protein between the diabetic and control groups.¹⁶ The reason for the increased secretion excretion of urinary VDBP in diabetes mellitus patients suffering with diabetic nephropathy is not clear. Possibly, one of the leading cause is the increased elimination of megalin in urine of

Table 3. Multiple comparisons of ACR and uVDBP by Dunn-Bonferroni post hoc test within groups

Variables	Groups	Median, Interquartile range	p-value
ACR (mg/g)	1	8.30, (6.05 - 11.50)	0.000 ^a
	2	17.50, (13.30 - 22.50)	0.567 ^b
	3	89, (75.55 - 122.50)	0.000 ^c
uVDBP (ng/mg)	1	98, (73.50 - 149)	0.0001 ^a
	2	442, (381.50 - 523)	0.0001 ^b
	3	1056, (905 - 1215)	0.000 ^c

Abbreviations: ACR: Albumin-creatinine ratio; uVDBP: Urinary Vitamin D binding protein

* Denotes, statistically significant ≤ 0.05 , a Denotes the comparison among the three groups, b Denotes the comparison of group 1 and 2, c Denotes the comparison of group 1 and 3, d Denotes the comparison of group 2 and 3.

these patients.¹⁷ Proximal tubular cells contains multiligand endocytic receptor called megalin which is involved in the reuptake of the filtered albumin and several proteins of low molecular mass, like VDBP from the glomerular filtrate. Mice deficient of megalin receptors reveals increased excretion of urinary VDBP.¹⁷ Therefore, increased excretion of megalin is hypothesized to cause increase loss of urinary VDBP in patients of diabetes mellitus and its excretion is predominantly increased in patients having albuminuria.^{17,18} In humans, enhanced excretion of urinary VDBP has been shown to be associated with the extent of renal damage. Actually, tubular epithelial cell damage results in inability of these cells to handle VDBP, which results in the loss of VDBP in urine.¹⁹ Hence, as diabetic nephropathy develops, the severity of the extent of damage to the epithelial cells of the renal tubules increases which results in enhanced excretion of urinary VDBP in diabetic patients.¹⁶ In the present study, urine levels of VDBP in normoalbuminuric group were statistically significant as compared to healthy controls. Similar findings have been previously reported by Shoukry and colleagues and Tian and associates that VDBP appeared in urine earlier than microalbumin and this is the reason for that it can be used as an earlier biomarker for prediction and detection of DN.^{7,13}

CONCLUSION

Urinary vitamin D binding protein levels were considerably increased in type 2 diabetic patients with normo, and microalbuminuria as compared to non-diabetic subjects which showed that it might be used as an early marker of diabetic nephropathy.

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