Urinary Vitamin-D Binding Protein as an Early Predictor of Diabetic Nephropathyin Type 1 and Type 2 Diabetes

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Abstract

Diabetic nephropathy (DN) is the most common microvascular complication that may lead to chronic renal failure in diabetic patients. Till now microalbuminuria, with its restrictions, is the early marker of DN, appeared after the disease exacerbation. Thus, new biomarkers are required to predict the early onset of DN before the appearance of microalbuminuria. The aim of this study is to investigate the possible use of uVDBP in the early prediction of DN. Fifty diabetic patients with DN and 40 diabetic patients without DN for both types of diabetes were enrolled in this study. All patients were tested for uACR, uVDBP (measured by ELISA), and blood HbA1c. The results demonstrated a highly significant elevation of uACR, HbA1c and uVDBP in diabetic patients with DN compared to those without DN. uVDBP exhibited a strong positive correlation with HbA1c and uACR in DN patients. ROC curve analysis showed a greater AUC (0.93), and cutoff value was >152 ng/ml with 94% sensitivity and 82% specificity for early detection of DN. These findings suggesting the sensitive and potential role of uVDBP in the early prediction and diagnosis of DN in type 1 and type 2 diabetes.

Keywords: Diabetic nephropathy, vitamin D binding protein, urinary albumin/creatinine ratio

Introduction

Diabetic nephropathy (DN) is the commonest microvascular complication of diabetes mellitus (DM), which is the prime reason of renal failure globally, and it is associated with increased cardiovascular mortality [1]. The prevalence of mortality in diabetic patients with DN is nearly 20-40 times higher than those patients without DN [2]. DN is a progressive disease or damage to the kidneys due to hyperglycemia-induced metabolic and hemodynamic changes resulted from DM [3]. Microalbuminuria (30-300 mg/g), despite its restrictions, still the early marker of DN, which appears after the disease exacerbation [4]. Now, new biomarkers have been found to be early predictors of DN even in normoalbuminuric stage which precedes MAU onset, and these biomarkers can also assess renal functions [5].

Vitamin-D binding protein (VDBP), also known as group-specific component (GC) globulin, is a multifunctional circulating α-globulin protein with 58 kDa of molecular weight [6]. It has various functions such as transports Vitamin-D, fatty acids and actin in the body, increases complement 5 (C5) chemotactic activity and promotes macrophage activation [7]. It is produced mainly by hepatocytes and found in plasma, ascitic fluid, cerebrospinal fluid and on the surface of many cell types [8].

Vitamin-D circulate bound to VDBP (85–90%) and albumin (10–15%), with <1% exists as free form [9]. There are three main roles of VDBP in vitamin D physiology; protecting Vitamin-D from biodegradation, limiting its access to target tissues, and reabsorbing vitamin-D in the kidneys [10]. The VDBP/25(OH)D (25-hydroxyvitamin D) complex is filtered in the glomerulus and then reabsorbed by megalin-cubilin receptors of the proximal tubular (PT) epithelial cells. The carrier VDBP is degraded in lysosomes, while 25(OH)D is converted into biologically active 1,25(OH)2D (calcitriol) via 1α-hydroxylase inside these cells, and re-secreted into the circulation [11].

Therefore, in DN, hyperglycemia induces ROS (reactive oxygen species) and TGF-β (Transforming growth factor-beta) production, as well as, induces proinflammatory cytokines secretion (such as interleukin-18) from podocytes [12]. These directly and indirectly causing renal tubular damage with destruction of megalin/cubilin receptors in PT epithelial cells that responsible for VDBP uptake leading to excretion of VDBP in urine [13]. The aim of this study was to find out the possible use of urinary VDBP (uVDBP) as a sensitive marker for early prediction of DN in diabetic Iraqi patients.

Materials and Methods

Patient: Ninety type 1 (T1D) and type 2 (T2D) diabetic Iraqi patients, with (50 patients) and without (40 patients) nephropathy, were enrolled in this study with diabetic duration ≥5 years and age range between (6-25) for T1D and (35-60) for T2D, selected from the Specialized Center for Endocrinology and Diabetes/Baghdad and the National Center for the Treatment of Diabetes and Research/AL-Mustansiriyah University during the period from October 2016 to March 2017. The presence or absence of DN was defined by the detection of microalbuminuria (30-300 mg/g) using urinary albumin/creatinine ration (uACR). All subjects were screened for their urine microalbumin, creatinine and VDBP, and for their blood HbA1c (glycated hemoglobin). Also, all subjects were excluded from other chronic liver, kidney or heart diseases, urinary tract infections, smoking, and pregnancy.

Materials and Methods: Blood and random-spot urine specimens were collected from each subject; uncentrifuged urine was immediately tested for urinary albumin/creatinine ratio (uACR) using microalbuminuria test strips called*Combina 13* (Human, Germany) to detect urine microalbumin and creatinine, then the strips were read using *Combilyzer 13* (Human, Germany) instrument [14]. Whereas centrifuged urine was frozen -20 °C until used for

detection of uVDBP using sandwich ELISA kit (MyBiosource, USA) which based on the reaction of VDBP in patient's urine with anti-VDBP antibody pre-coated onto microwell plate giving a color change proportional directly with VDBP concentration in patient's urine [15]. Whole blood was used to estimate HbA1c using fully automated *Clover A1C*instrument (Infopia, Korea) based on boronate affinity chromatography technique [16]. All results were statistically analyzed using SPSS (version 18.0), a significant of difference was tested by student's *t* test, and the correlation between parameters was obtained by Pearson's correlation (r), the values of >0.05, ≤0.5, and ≤0.01 were considered non-significant, significant and highly significant. ROC (receiver operating characteristic) curve was used to detect the diagnostic predictivity of urinary VDBP using MedCalc software (version 17.8), a value <0.5 of AUC (area under the curve) was considered statistically significant.

Results

Table (1) revealed a significant difference in T1D patients with DN than those without DN for uACR (97.0 vs. 14.0 mg/g), HbA1c (11.0 vs. 9.5%), and uVDBP (347.2 vs. 127.9 ng/ml); also a significant difference in T2D patients with compared to without DN for uACR (74.7 vs. 16.7 mg/g), HbA1c (11.5 vs. 8.6%), and (248.2 vs. 105.3 ng/ml). Additionally, a highly significant difference (P<0.01) in diabetic patients with DN compared to those without DN for uACR (171.7 vs. 30.7 mg/g), HbA1c (22.5 vs.18.1%) and (495.4 vs. 233.2 ng/ml) (data not shown in Table (1)).

Table (1): Comparison of the mean levels of biochemical and uVDBP markers in the diabetic patients with and without nephropathy.

Parame-ters	Statistics	Groups				DPs with DN vs. without DN
		T1D		T2D		
		with DN (n=25)	without DN (n=20)	with DN (n=25)	without DN (n=20)	
uACR (mg/g)	Mean	97.0	14.0	74.7	16.7	0.000 (HS)
	SD	34.0	7.1	29.0	7.3	
	Range	40-150	5-30	35-150	10-30	
	P-value	0.000 (HS)		0.000 (HS)		
HbA1c (%)	Mean	11.0	9.5	11.5	8.6	0.001 (HS)
	SD	1.9	1.9	1.8	1.7	
	Range	8-14	7-13	8-14	6-13	
	P-value	0.020 (S)		0.003 (HS)		
uVDBP (ng/ml)	Mean	347.2	127.9	248.2	105.3	0.000 (HS)
	SD	150.3	61.5	97.8	59.9	
	Range	182-713	85-336	140-454	30-219	
	P-value	0.000 (HS)		0.000 (HS)		

T1D= type 1 diabetes, T2D= type 2 diabetes, DN= diabetic nephropathy, DPs= diabetic patients, uACR= urinary albumin to creatinine ratio, HbA1c= glycated hemoglobin, uVDBP= urinary vitamin D binding protein SD=standard deviation, S= significant, HS= highly significant

Table (2) exhibited a strong positive correlation of uVDBP with uACR in DN patients (r=0.540 with P=0.000) compared to non-significant relation in diabetic patients without DN (r=-0.061 with P=0.708). Whereas a strong positive correlation of uVDBP with HbA1c was seen in both diabetic patients with and without DN (r=0.560 with P=0.000 and r=0.694 with P=0.000, respectively).

Table (2): Correlation of uVDBP with uACR and HbA1c in diabetic patients with and without nephropathy.

Parameters	uVDBP(ng/ml)			
	Diabetic Patients with DN		Diabetic Patients without DN	
uACR (mg/g)	r	0.540	r	-0.061
	P-value	**0.000 (HS)**	*P*-value	0.708 (NS)
HbA1c (%)	r	0.560	r	0.694
	P-value	**0.000 (HS)**	*P*-value	**0.000 (HS)**

r= Pearson's correlation, NS= non-significant

Table (3) and Figure (1) showed ROC curve characteristics of uVDBP with highly significant AUC (0.935). Urinary VDBP showed a diagnostic cutoff value of >152 ng/ml with 94%, 82%, 87%, and 92% of sensitivity, specificity, PPV (positive predictive value), and NPV (negative predictive value) respectively.

Table (3): ROC curve characteristics of uVDBP in diabetic patients with and without nephropathy.

Tests	ROC Curve Characteristics						
	AUC	*P*-valve	Cutoff	Sensitivity	Specificity	PPV	NPV
uVDBP (ng/ml)	0.935	**0.000 (HS)**	>152	94%	82%	87%	92%

ROC= receiver operating characteristic, AUC= area under the curve, PPV= positive predictive value, NPV= negative predictive value.

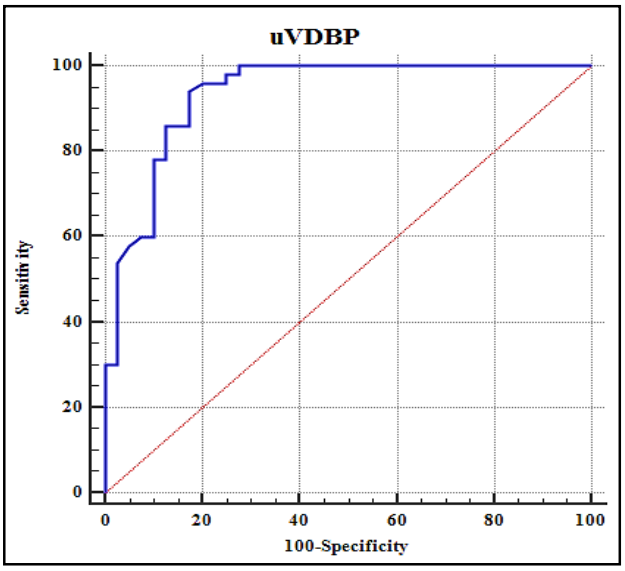

Figure (1): Receiver operating characteristic (ROC) curve analysis of uVDBP in diabetic patients with and without nephropathy

Discussion

In the current study, diabetic patients with nephropathy demonstrated a significantly elevated levels of uACR, HbA1c and uVDBP compared to diabetic patients without nephropathy (Table 1). These results were consistent with a study in Morocco that revealed a higher significant levels of albuminuria were seen in T2D patients with MAU compared to

those with normoalbuminuria [17], also a study in Egypt showed that there was a significant higher percentage of MAU (89%) in T1D patients with poor glycemic control (HbA1c >8.0%) and disease duration >10 years [18]. Ufuoma*et al.* (2016) mentioned that HbA1c levels were significantly higher (P<0.001) in T2D patients with MAU than those without MAU [19], and Al-Eisa*et al.* (2017) also agreed with our results that the mean HbA1c levels were higher significantly in T1D with DN vs. without DN [20]. However, our results were inconsistent with a study which stated that there was no significant difference in HbA1c levels in diabetic patients with micro and macroalbuminuria, and those with normoalbuminuria for both types of diabetes [21]. The possible explanation of increased uACR and HbA1c in DN patients was due to hyperglycemia. Hyperglycemia causes hyperproduction of TGF-β and ROS, which directly damage the glomerular endothelium. Hyperglycemia also stimulates the production of VEGF-A that induce glomerular hyperpermeability [12] and inflammatory cytokines that destruct megalin-cubilin receptors involved in albumin reabsorption in renal tubules, leading to the appearance of MAU [22], indicating that poor glycemic control could be an important risk factor for DN initiation and progression. Concerning to uVDBP which was elevated significantly in DN patients than those patients without DN, these finding were in agreement with studies which reported that uVDBP levels were significantly higher (*P*=0.004) in diabetic patients with micro and macroalbuminuria compared to diabetic patients with normoalbuminuria [23, 24], whereas another study noticed that uVDBP was higher in microalbuminuric patients than normoalbuminuric patients with non-diabetic chronic kidney disease (CKD) [25]. The possible explanation of elevated uVDBP in DN patients was resulted from hyperglycemia-induced ROS, TGF-β and proinflammatory cytokines (IL-18) production from podocytes [12] causing renal tubular damage with destruction of megalin/cubilin receptors in PT epithelial cells that responsible for VDBP reabsorption leading to urinary excretion of VDBP [13]. Furthermore, a significant positive association of uVDBP with uACR and HbA1c was manifested in diabetic patients with nephropathy (Table 2). These results were similar to Thrailkill*et al.* (2011) and Mohamed (2016) studies, who found that uVDBP was strongly positively associated (*P*<0.001) with elevated HbA1c levels in T1D and T2D patients (respectively) with normo and microalbuminuria [23, 26]. Other studies also agreed with our finding, which demonstrated a strong positive correlation (*P*<0.001) between uVDBP and uACR was seen in DN patients, and uVDBP levels were directly proportional with increased uACR levels [27, 28]. However, another study reported that there was no significant relation between uVDBP and uACR in microalbuminuric diabetic patients, but only in diabetic patients with macroalbuminuria [29]. These inconsistent results might be due to different study designs, different stages of DN, and different populations that had been studied. ROC curve analysis exhibited a greater AUC (0.935) for uVDBP with cutoff value (>125 ng/ml), this value has sensitivity (94%), specificity (82%), PPV (87%), and NPV (92%) as shown in Table (3) and Figure (1). These findings were concurred with many studies which revealed a higher AUC (0.96) of uVDBP with 90% sensitivity, 77% specificity, 79% PPV, and 88% NPV [27,29, 30], suggesting a sensitive and potential role of urinary VDBP in the early prediction, diagnosis and assessment of DN in diabetic patients. In conclusion, poor glycemic control is an important risk factor for the initiation and development of DN, uVDBP levels were significantly elevated in DN patients and positively associated with higher HbA1c and uACR, therefore it can be used as an early predictor for the detection and may prevent the early onset of DN in Iraqi diabetic patients.

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