Clinical Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2016, Vol. 44(3) 673–684
© The Author(s) 2016
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/0300060515593765
imr.sagepub.com
SAGE

Vitamin D deficiency and related risk factors in patients with diabetic nephropathy

Xiaoyan Xiao[1,*], Yajuan Wang[2,3,*], Yanlian Hou[3], Feng Han[4], Jianmin Ren[3,*] and Zhao Hu[1,*]

Abstract

Objectives: To investigate serum 25-hydroxyvitamin D (25[OH]D) levels in patients with diabetic nephropathy, analyse the relationship between 25(OH)D and clinical indexes, and identify risk factors for vitamin D deficiency in diabetic nephropathy.

Methods: Patients with diabetic nephropathy were sequentially enrolled and grouped according to diabetic nephropathy stage. Serum 25(OH)D levels were measured. A control group of healthy subjects was used for comparison.

Results: Out of 240 patients with diabetic nephropathy and 60 healthy controls, 25(OH)D levels were lower in diabetic nephropathy patients than in controls, and showed a gradually decreasing trend with diabetic nephropathy stage. Serum 25(OH)D levels were significantly correlated with age, sex, diabetes history, body mass index, systolic blood pressure, albumin excretion rate (AER), estimated glomerular filtration rate, fasting blood glucose, glycosylated haemoglobin (HbA_{1c}), haemoglobin, serum albumin, creatinine clearance rate, blood urea nitrogen and complicated diabetic retinopathy. Moreover, age, body mass index, AER, haemoglobin, and HbA_{1c} were independent risk factors of 25(OH)D deficiency in diabetic nephropathy.

Conclusions: Vitamin D deficiency is prevalent in patients with diabetic nephropathy and increases in severity with diabetic nephropathy progression. Age, obesity, glucose level and renal function largely affect 25(OH)D deficiency in diabetic nephropathy.

[1]Department of Nephrology, Qilu Hospital of Shandong University, Jinan, China
[2]Department of Endocrinology, Jinan Central Hospital, Jinan, China
[3]Department of Endocrinology, Qilu Hospital of Shandong University, Jinan, China
[4]Department of Endocrinology, Zhangqiu City People's Hospital, Jinan, China

*These authors contributed equally to this work.

Corresponding author:
Zhao Hu, Department of Nephrology, Qilu Hospital, Shandong University, 107 Wenhua Xi Road, Jinan 250012, Shandong Province, China.
Email: hz12040813@163.com

Creative Commons CC-BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 3.0 License (http://www.creativecommons.org/licenses/by-nc/3.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Keywords
25(OH)D, type 2 diabetes, diabetic kidney disease, albumin excretion rate

Date received: 7 August 2014; accepted: 8 June 2015

Introduction

Vitamin D is an essential hormone obtained from the diet (10–20%) or skin synthesis. It has a classical function in maintaining calcium and phosphate homeostasis, but also an extensive role in maintaining immunity, vascular function,[1] cardiomyocyte health, inflammation,[2] insulin resistance[3] and in lowering the urinary albumin concentration in patients with chronic kidney disease.[4] Vitamin D can be hydroxylated into 25-hydroxyvitamin D (25[OH]D) in the liver, then converted to its active form by 1-hydroxylation in the renal proximal tubules. Renal dysfunction can influence serum 25(OH)D levels, and studies have confirmed the occurrence of low serum 25(OH)D levels in patients with chronic kidney disease.[5,6] Low vitamin D levels are suggested to be associated with different impaired glucose metabolism states including diabetes mellitus.[7] Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the leading cause of end-stage renal disease worldwide.[8] Oral paricalcitol has been shown to reduce albuminuria and inflammation in chronic kidney disease,[9] and animal studies have shown that calcitriol supplement can reduce albuminuria in mice with diabetic nephropathy.[10]

Vitamin D deficiency has been reported mainly in patients with chronic kidney disease or in patients with diabetes mellitus who have normal renal function.[7,11] Few studies have analysed vitamin D metabolic levels in patients with diabetic nephropathy. Furthermore, risk factors for vitamin D deficiency in patients with diabetic nephropathy remain unclear. The present study evaluated the prevalence of vitamin D deficiency in patients with diabetic nephropathy and determined their correlation with clinicopathological features and possible risk factors for vitamin D deficiency.

Patients and methods

Study population

Patients with different stages of diabetic nephropathy from the Department of Endocrinology, Nephrology, and Geriatrics of Qilu Hospital, Shandong University, Jinan, China and Zhangqiu City People's Hospital, Jinan, China were sequentially enrolled between May 2012 and July 2012, to eliminate the effect of season and geographical area. Data were reviewed retrospectively.

All patients with diabetic nephropathy who met the following inclusion and exclusion criteria were enrolled. Inclusion criteria: patients diagnosed with type 2 diabetes mellitus (including patients with intermittent microalbuminuria (i.e. very mild kidney injury), persistent urinary microalbumin albumin excretion rate [AER] of 30–300 mg/24 h, or macroalbuminuria [AER > 300 mg/24 h].[12] Exclusion criteria: patients <18 years of age; patients with end-stage renal disease not caused by diabetes mellitus and who required renal replacement therapy; patients with an active infection, severe cardiovascular disease, cancer, liver disease, current pregnancy, gallbladder problem or gastrointestinal disorder;[13] patients who had used mineral oil products and daily multivitamins (i.e., vitamin D) for ≥4 weeks prior to the screening visit, or regularly used antacids, used cortisone or other steroids, weight-loss drugs, phenobarbital or acyeterion;[14] patients whose estimated glomerular filtration rate (eGFR) was<10 ml/min/1.73 m^2 and who required dialysis; patients using

renin-angiotensin system inhibitors (as these can reduce albuminuria).[14] Healthy controls were randomly enrolled from people living in the same area as the patients with kidney injury, attending the Physical Examination Centre of Qilu Hospital for health examination; control subjects were age- and sex-matched to the patients, and also matched the exclusion criteria. Diabetic nephropathy stage was based on the 2011 American Diabetes Association guidelines:[12] Stage I, GFR is significantly increased; Stage II, urinary albumin excretion rate is mainly normal, but may increase intermittently (such as following exercise, or stress), and GFR may be slightly increased; Stage III, urinary albumin excretion rate is 20–200 μg/min, GFR remains normal or increased; Stage IV, urinary albumin excretion rate is >200 μg/min, and GFR is decreased; Stage V, urinary albumin excretion rate is decreased and serum creatinine is increased.

The study was approved by the Ethics Committee of Qilu and Zhangqiu City People's Hospitals, and written informed consent was obtained from each participant.

Clinical examination

Body mass index (BMI), waist hip ratio (WHR), creatinine clearance rate (Ccr), and eGFR were measured and calculated for each participant. Blood pressure was measured in a sitting position following 10 min rest using a mercury sphygmomanometer. Standard ophthalmological evaluation was performed in each patient to identify non-proliferative or proliferative diabetic retinopathy. Peripheral neuropathy was identified using an electromyogram.

Laboratory parameters

To measure the standard range of biochemical parameters that would be tested for in patients with diabetes mellitus, using standard methods, fasting blood specimens (~5 ml) were collected into tubes containing separation gel coagulant or ethylenediaminetetraacetic acid (for glycosylated haemoglobin [HbA_{1c}] analysis), then centrifuged at 2 390 ***g*** at room temperature for 5 min and stored at −20°C prior to use. All participants underwent a 24 h urinary albumin excretion test by measuring urinary albumin (N antiserum to human albumin kit; BN prospec, Siemens, Germany) and urinary creatinine (Creatinine Plus version 2 kit; Cobas c701, Roche, Germany) according to the manufacturer's instructions. Serum 25(OH)D levels were measured using high-pressure liquid chromatography, as described previously.[15] A serum 25(OH)D level<15 ng/ml was defined as deficient.

Sample size

The sample size of this cohort study was selected according to the following four aspects:[16] First, morbidity or mortality of the nonexposure population (the required sample size is larger when it is closer to 0.5); secondly, morbidity or mortality of the exposure population; thirdly, the required significance level; fourthly, the required study power. The prevalence of vitamin D deficiency and insufficiency is ~69.0% and ~24.4% of the population in Beijing and Shanghai, China, respectively.[17] The present desired significance level was $P < 0.05$, and the study power was >0.75, thus, the overall required sample size was calculated to be ~210 cases to be able to detect statistical significance.

Statistical analyses

All data were presented as mean ± SD. Statistical analyses were performed using SPSS® software, version 19.0 (SPSS, Inc., Chicago, IL, USA) by an expert statistician. Univariate conditional logistic regression analysis was used to investigate significant factors associated with 25(OH)D deficiency, and to rule out confounding factors.

Multivariate conditional logistic regression was used to determine independent risk factors and prognostic factors) for 25(OH)D deficiency. A P value < 0.05 was considered to be statistically significant, unless specifically explained.

Results

A total of 240 patients with different diabetic nephropathy stages ($n = 60$ per group) and 60 healthy controls were included in the present study.

Clinical characteristics

Basic clinical characteristics of patients with diabetic nephropathy and normal controls are shown in Table 1. Patients with diabetic nephropathy stage V were significantly older than the control group and had a longer history of diabetes than patients with stages III or IV diabetic nephropathy ($P < 0.05$). Patients with diabetic nephropathy stages IV and V had higher BMIs versus controls and patients with diabetic nephropathy stage I/II ($P < 0.05$). All diabetic nephropathy groups had higher systolic blood pressure (SBP) than controls, and SBP showed a trend to increase as diabetic nephropathy progressed (Table 1). AER in patients with diabetic nephropathy stage IV was significantly different from controls and patients with diabetic nephropathy stage I/II or stage III ($P < 0.05$), and AER in patients with diabetic nephropathy stage V was significantly higher than all other groups (all $P < 0.05$). Fasting blood glucose and HbA_{1c} were significantly higher in all diabetic nephropathy groups versus controls and appeared to plateau in patients with diabetic nephropathy stage IV. Haemoglobin levels were significantly lower in patients with diabetic nephropathy stages IV and V versus controls, and significantly lower in patients with stage V versus stage IV diabetic nephropathy (all $P < 0.05$). Serum albumin showed a tendency to decrease with diabetic nephropathy progression, as did Ccr, eGFR,. Blood urea nitrogen (BUN), uric acid and cystatin-C were significantly higher in patients with diabetic nephropathy stage V versus stages I/II and III and controls. Total cholesterol, triglycerides and low-density lipoprotein (LDL) cholesterol in patients with diabetic nephropathy stage V were significantly higher than stages I/II and controls (all $P < 0.05$). Serum calcium levels were significantly lower in patients with diabetic nephropathy stage V versus all other groups (all $P < 0.05$).

Low 25(OH)D levels in patients with diabetic nephropathy

Serum 25(OH)D levels were significantly lower in the four diabetic nephropathy groups than in the healthy controls ($P < 0.05$). Furthermore, serum 25(OH)D levels in diabetic nephropathy groups IV and V were significantly lower than in diabetic nephropathy group I/II ($P < 0.05$; Table 1 and Figure 1).

Association between 25(OH) D levels and clinical parameters

Univariate conditional logistic regression analysis found that serum 25(OH)D levels were significantly associated with age, sex, diabetes history, BMI, SBP, AER, eGFR, fasting blood glucose, HbA_{1c}, haemoglobin, serum albumin, Ccr, BUN and proliferative diabetic retinopathy (Table 2). In addition, serum 25(OH)D levels were lower in older, female and obese participants. Significantly more patients with high AER and high glucose levels had low 25(OH)D levels ($\leq$15 ng/ml), and significantly more patients with low serum albumin, mild anaemia, and decreased eGFR and Ccr had low 25(OH)D levels ($\leq$15 ng/ml; Table 2). Other indices such as diabetes history, SBP, serum creatinine, BUN, smoking, drinking, and

Table 1. Demographic and clinical characteristics of patients grouped according to stage of diabetic nephropathy ($n = 240$) and healthy controls ($n = 60$).

Variable	Control group	Diabetic nephropathy stage			
		I/II	III	IV	V
Participants, *n*	60	60	60	60	60
Age, years	50 ± 7	57 ± 8	56 ± 10	59 ± 10	62 ± 9*
Sex, male/female	27/33	29/31	22/38	26/34	30/30
Diabetes history, years	–	14.9 ± 4.8	10.2 ± 4.9#	12.4 ± 5.3	16.0 ± 6.4△&
BMI, kg/m^2	24.1 ± 2.9	24.5 ± 2.5	25.7 ± 1.7	27.1 ± 2.7*#	26.9 ± 2.8*#
WHR	0.93 ± 0.06	0.90 ± 0.14	0.93 ± 0.10	0.96 ± 0.09	0.96 ± 0.09
SBP, mmHg	120.7 ± 12.0	139.4 ± 13.1*	140.1 ± 17.8*	150.1 ± 20.6*#	158.7 ± 18.8*#△
DBP, mmHg	76.9 ± 7.6	78.5 ± 8.8	81.3 ± 7.5	83.0 ± 7.6	79.6 ± 8.3
AER, mg/24 h	10.5 ± 2.5	13.2 ± 3.7	139.1 ± 53.3	1231.2 ± 332.2*#△	1799.2 ± 495.7*#△&
eGFR, ml/min/1.73 m^2	102.5 ± 10.4	78.3 ± 14.5*	43.1 ± 11.8*	17.9 ± 12.6*#△	13.5 ± 3.1*#△&
25(OH)D	29.43 ± 10.15	12.23 ± 4.07*	10.31 ± 3.36*	8.44 ± 2.53*#	7.74 ± 2.90*#
Fasting blood glucose, mmo/l	5.18 ± 0.46	8.47 ± 2.40*	10.40 ± 1.75*#	9.37 ± 3.89*△	7.36 ± 1.23*&
HbA_{1c}, %	5.0 ± 0.3	7.8 ± 1.8*	9.4 ± 2.1*#	9.8 ± 2.2*#	9.0 ± 2.2*#
Haemoglobin, g/l	135 ± 11	130 ± 18	128 ± 11	112 ± 21*#	81 ± 15*#△&
Serum albumin, g/l	46.0 ± 3.6	42.8 ± 3.3*	41.3 ± 5.7*	36.9 ± 8.1*#△	33.2 ± 5.8*#△&
Serum creatinine, μmol/l	77.2 ± 15.7	62.9 ± 11.1	71.5 ± 17.4	114.5 ± 39.0	363.3 ± 47.6*#△&
Ccr, ml/min	92.4 ± 18. 8	107.5 ± 33.0	112.4 ± 32.5	73.3 ± 25.0*#	29.6 ± 9.6*#△&
BUN, mmol/l	4.64 ± 1.31	5.08 ± 1.11	6.00 ± 2.05	7.64 ± 2.21	16.52 ± 4.79*#△&
Uric acid, umol/l	312.4 ± 94.2	236.0 ± 57.9*	291.3 ± 78.5	294.9 ± 81.0#△	381.6 ± 133.0*#△&
Cystatin-C, mg/l	0.78 ± 0.12	0.83 ± 0.19	0.90 ± 0.18	1.15 ± 0.39*#△	2.69 ± 0.64*#△
Total cholesterol, mmol/l	1.47 ± 1.20	1.17 ± 0.62	2.58 ± 0.66*	1.71 ± 0.81*	2.14 ± 0.91*#△&
Total triglyceride, mmol/l	4.74 ± 0.75	4.85 ± 1.35	5.19 ± 1.07*#	4.78 ± 1.17	6.04 ± 1.17*#△&
HDL cholesterol, mmol/l	1.37 ± 0.35	1.35 ± 0.28	1.15 ± 0.22*	1.20 ± 0.53	1.22 ± 0.28
LDL cholesterol, mmol/l	2.64 ± 0.69	3.00 ± 0.90*	3.12 ± 1.00*	2.83 ± 0.97*	3.70 ± 1.56*#&
Serum Ca, mmol/l	2.29 ± 0.10	2.25 ± 0.12	2.23 ± 0.14*	2.22 ± 0.18*	2.10 ± 0.19*#△&
Serum P, mmol/l	1.26 ± 0.18	1.15 ± 0.19	1.38 ± 1.12	1.24 ± 0.20	1.35 ± 0.35
DRP, yes/no	0/60	20/40	32/28	41/19	49/11
DNP, yes/no	0/60	23/37	30/30	29/31	40/20

(continued)

Table 1. Continued.

Variable	Control group	Diabetic nephropathy stage			
		I/II	III	IV	V
Smoking, male, yes/no	13/14	12/17	15/7	18/8	19/11
Drinking, male, yes/no	15/12	19/10	8/14	14/12	16/14

Data presented as mean ± SD or *n* prevalence.

BMI, body mass index; WHR, waist hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; AER, albumin excretion rate; eGFR, estimate glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D; HbA_{1c}, glycosylated haemoglobin; Ccr, creatinine clearance rate; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DRP, diabetic retinopathy; DNP, diabetic neuropathy.

*$P < 0.05$ versus controls; #$P < 0.05$ versus diabetic nephropathy I/II; △$P < 0.05$ versus diabetic nephropathy III; &$P < 0.05$ versus diabetic nephropathy IV (all one-way analysis of variance).

complicated diabetic neuropathy were considered to be confounding factors (Table 2).

Multivariate conditional logistic regression analysis showed that age, BMI, AER, haemoglobin, and HbA_{1c} remained significant as independent prognostic factors for 25(OH)D deficiency, and (Table 3).

Discussion

The present study showed that 25(OH)D levels were lower in patients with diabetic nephropathy than in healthy controls, and tended to decrease with diabetic nephropathy stage. Calcitriol or 1, 25(OH) vitamin D is a steroid hormone that has genomic and nongenomic effects. Calcitriol expresses these functions by the ubiquitous existence of a vitamin D receptor in the body, such as in endothelial cells and vascular smooth muscle cells.[18] In the present study, 25(OH)D was used as a sensitive marker of total body vitamin D storage. Extra-renal 1α-hydroxylase is known to be expressed outside the kidney,[19] and the half-life of 25(OH)D is ~3 weeks,[20] which is longer than that of calcitriol. Thus, 25(OH)D is a convenient and sensitive molecule for testing vitamin D levels.

In the present study, AER was determined to be an independent risk factor of 25(OH)D deficiency. Serum 25(OH)D levels were negatively associated with AER, which is not only the primary sign of kidney disease severity but also a marker of diabetic nephropathy progression. Vitamin D deficiency was associated with an increased risk of albuminuria and renal failure in the Third Health and Nutrition Examination Survey (NHANES III) cohort.[21] In patients with chronic kidney disease and vitamin D deficiency, urine protein levels can be decreased, and renal function can be improved to some extent, with regular vitamin D supplementation.[5] The present study found that the 25(OH)D level was positively associated with the serum albumin level. Serum 25(OH)D is known to bind proteins such

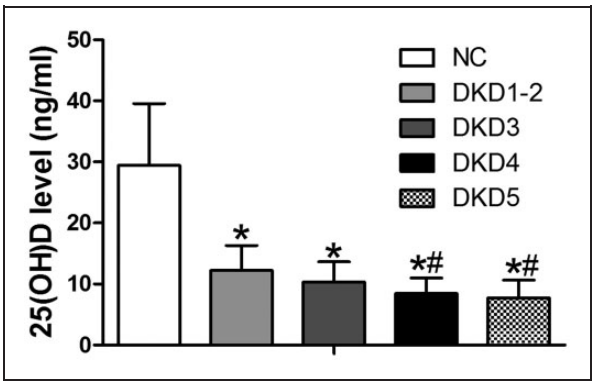

Figure 1. Serum 25-hydroxyvitamin D (25[OH]D) levels in patients grouped into different diabetic nephropathy stages. Data presented as mean ± SD; $^{*}P < 0.05$ versus healthy controls; $^{\#}P < 0.05$ versus diabetic nephropathy 1–2; NC, healthy controls; CKD, diabetic nephropathy groups (all one-way analysis of variance).

as the D-binding protein and albumin in blood to perform its function.[22] Lost urinary microalbumin can decrease the serum albumin level, which explains the decrease in protein synthesis and the large loss in urinary albumin that affects serum 25(OH)D concentration.

After controlling for seasonal affects on vitamin D (i.e., the present study was conducted during the summer), serum 25(OH)D levels were found to be significantly associated with age, sex, and BMI. In addition, a higher proportion of older, female and obese patients with low 25(OH)D levels was observed. Progression of diabetic nephropathy, enzyme activity reduction, gut dysfunction, low vitamin D absorption from the diet, oestrogen decrease in older female patients, and lack of UVB radiation are all thought to contribute to low 25(OH)D levels.[23] Many studies conducted in the general population have shown a high prevalence of vitamin D deficiency in obese subjects.[24]

In the present study, the serum 25(OH)D level was negatively correlated with with fasting blood glucose and HbA_{1c}; in addition, HbA_{1c} was shown to be an independent risk factor of 25(OH)D deficiency. Vitamin D is thought to be involved in the pathophysiology of insulin resistance, insulin sensibility, and β-cell function.[25] The direct effect of vitamin D can be mediated through binding of its circulating active form (i.e., 1, 25-$[OH]_2$D) to β-cell receptors. Indirect effects of vitamin D are mediated by its important and classical role in regulating extracellular calcium and calcium channels through β-cells, because insulin secretion is a calcium-dependent process.[18]

The present study showed that vitamin D deficiency was associated with proliferative diabetic retinopathy. Diabetic retinopathy is a microvascular complication of diabetes mellitus that shares similar pathophysiological features with diabetic nephropathy. Retinal involvement exists in most cases of diabetic nephropathy.[26] Microalbuminuria has been suggested to significantly increase the risk of development and progression of diabetic retinopathy in patients with type 2 diabetes, even after adjustment for diabetes duration.[27] Vitamin D receptors are present in the human retina, and vitamin D deficiency is related to retinopathy risk in diabetes.[28] Molecular research has shown that the Fok-1 single nucleotide polymorphism of the vitamin D (1,25- dihydroxyvitamin D3) receptor (*VDR*) gene is associated with

Table 2. Univariate conditional logistic regression analysis of significant factors for 25-hydroxyvitamin D (25[OH]D) deficiency in patients with diabetic nephropathy ($n = 240$) and healthy controls ($n = 60$).

	25(OH)D level		
Factor	> 15 ng/ml $n = 84$; 28%	≤ 15 ng/ml $n = 216$; 72%	Statistical significance
Age, years			
<40	29	78	ref
40–60	41	71	$P = 0.003$
>60	14	64	$P < 0.001$
Sex, male/female			
Male	50	84	
Female	34	132	$P = 0.009$
Diabetes history, years			
<10[a]	37	71	
≥10	47	145	$P = 0.020$
BMI, kg/m^2			
<20	9	12	ref
20–25	52	50	NS
26–30	13	134	$P = 0.002$
>30	10	20	$P < 0.001$
WHR			
<0.9	39	85	
≥0.9	45	131	NS
SBP, mmHg			
<140	67	99	ref
140–160	17	67	$P = 0.030$
>160	0	60	$P < 0.001$
AER, mg/24 h			
<30	73	47	ref
30–300	11	49	$P < 0.001$
>300	0	120	$P < 0.001$
eGFR, ml/min/1.73 m^2			
<15	0	60	NS
15–30	0	60	$P < 0.001$
31–60	11	49	$P < 0.001$
>60	73	47	ref
Fasting blood glucose, mmol/l			
<7.8	34	41	ref
7.8–11.1	47	124	$P < 0.001$
>11.1	3	51	$P < 0.001$
HbA_{1c}, %			
<6.0	60	3	ref
6.0–8.0	21	69	$P < 0.001$
>8.0	3	144	$P < 0.001$
Haemoglobin, g/l			
<60	0	0	

(continued)

Table 2. Continued.

Factor	25(OH)D level		Statistical significance
	> 15 ng/ml $n = 84$; 28%	≤ 15 ng/ml $n = 216$; 72%	
60 – 90	1	58	NS
91 – 120	14	90	$P = 0.015$
>120	69	68	ref
Serum albumin, g/l			
<35	0	120	
≥35	84	96	$P < 0.001$
Serum creatinine, μmol/l			
<97	84	156	
≥97	0	60	NS
Ccr, ml/min			
<80	0	120	
≥80	84	96	$P = 0.010$
BUN, mmol/l			
<20	84	156	
≥20	0	60	$P = 0.026$
Uric acid, μmol/l			
<488	82	216	
≥488	2	0	NS
Cystatin-C, mg/l			
<1.09	79	117	
≥1.09	5	99	NS
Total cholesterol, mmol/l			
<5.98	66	134	
>5.98	18	82	NS
Total triglyceride, mmol/l			
<1.7	69	137	
≥1.7	15	79	NS
HDL cholesterol, mmol/l			
<0.94	6	63	
≥0.94	78	153	NS
LDL cholesterol, mmol/l			
<3.12	64	95	
≥3.12	20	121	NS
Serum Ca, mmol/l			
<2.25	4	157	
≥2.25	80	59	NS
Serum P, mmol/l			
<1.61	78	113	
≥1.61	6	103	NS
DRP, yes/no			
Yes	10	122	
No	74	94	$P < 0.001$
DNP, yes/no			
Yes	5	83	
No	79	123	NS

(continued)

Table 2. Continued.

Factor	25(OH)D level		Statistical significance
	> 15 ng/ml $n = 84$; 28%	≤ 15 ng/ml $n = 216$; 72%	
Smoking, male, yes/no			
Yes	21	59	
No	18	36	NS
Drinking, male, yes/no			
Yes	19	50	
No	20	45	NS

Data presented as *n* prevalence.
[a]Healthy control data were included in this subgroup.
Ref, reference group; BMI, body mass index; WHR, waist hip ratio; SBP, systolic blood pressure; AER, albumin excretion rate; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycosylated haemoglobin; Ccr, creatinine clearance rate; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DRP, diabetic retinopathy; DNP, diabetic neuropathy.
NS, no statistically significant difference ($P > 0.05$; univariate conditional logistic regression).

Table 3. Multivariable conditional logistic regression of significant factors for 25-hydroxyvitamin D (25[OH]D) deficiency in study participants.

Characteristic	25(OH)D > 15 ng/ml	25(OH)D ≤ 15 ng/ml	OR	95% CI	Statistical significance
Age, years >60	14	64	0.297	0.228, 0.466	$P = 0.008$
BMI, kg/m^2 >25	23	154	1.109	1.042, 2.176	$P = 0.002$
AER, mg/24 h >30	11	169	1.927	1.780, 3.064	$P = 0.009$
Haemoglobin, g/l<90	3	38	0.289	0.089, 0.811	$P = 0.012$
HbA_{1c}, % >6.0	24	213	1.828	1.358, 3.298	$P = 0.001$

Data presented as *n* prevalence.
Dependent variables, 25(OH)D < 15 ng/ml.
OR, odds ratio; CI, confidence interval; BMI, body mass index; AER, albumin excretion rate; HbA_{1c}, glycosylated haemoglobin.

increased transcriptional activity of *VDR* and less-severe (nonproliferative) diabetic retinopathy.[29]

The results of the present study are limited by several factors: First, the sample size was relatively small; Secondly, residual confounding factors related to vitamin D deficiency, such as lifestyle, were not ruled out during this study, although the statistical analysis was carefully adjusted; Thirdly, the dietary intake of vitamin D was ascertained by a 24 h diet recall (which has a recall bias); finally, the diabetic nephropathy diagnosis in the present study was based on clinical diagnosis, and no renal biopsy, renal ultrasound, or emission computed tomography tests were performed.

In conclusion, 25(OH)D deficiency is prevalent among nondialysed patients with diabetic nephropathy and tends to decrease

with diabetic nephropathy stage. Age, obesity, glucose level, and renal function are independent risk factors for 25(OH)D deficiency, and serum 25(OH)D appears to be a favourable inverse predicator of diabetic nephropathy progression.

Acknowledgement

The authors thank the statistician Junrong Wang, School of Public Health, Shandong University, Jinan, China for her analyses.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Singer T. Nutrition: the vitamin D complex. *Nature* 2012; 489: S10–S11.
2. Sanchez-Niño MD, Bozic M, Córdoba-Lanús E, et al. Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy. *Am J Physiol Renal Physiol* 2012; 302: F647–F657.
3. Kabadi SM, Lee BK and Liu L. Joint effects of obesity and vitamin D insufficiency on insulin resistance and type 2 diabetes: results from the NHANES 2001–2006. *Diabetes Care* 2012; 35: 2048–2054.
4. Zhang Z, Sun L, Wang Y, et al. Renoprotective role of the vitamin D receptor in diabetic nephropathy. *Kidney Int* 2008; 73: 163–171.
5. de Zeeuw D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. *Lancet* 2010; 376: 1543–1551.
6. González EA, Sachdeva A, Oliver DA, et al. Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. *Am J Nephrol* 2004; 24: 503–510.
7. Forouhi NG, Ye Z, Rickard AP, et al. Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia* 2012; 55: 2173–2182.
8. Gross JL, de Azevedo MJ, Silveiro SP, et al. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care* 2005; 28: 164–176.
9. Ortiz A, Sanchez Niño MD, Rojas J, et al. Paricalcitol for reduction of albuminuria in diabetes. *Lancet* 2011; 377: 635–636. author reply 636–637.
10. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008; 29: 726–776.
11. Song Y, Wang L, Pittas AG, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 2013; 36: 1422–1428.
12. Basevi V, Di Mario S, Morciano C, et al. Comment on: American Diabetes Association. Standards of medical care in diabetes-2011. *Diabetes Care* 2011; 34(Suppl. 1): S11–S61. e53; author reply e54.
13. Singh DK, Winocour P, Summerhayes B, et al. Are low erythropoietin and 1,25-dihydroxyvitamin D levels indicative of tubulo-interstitial dysfunction in diabetes without persistent microalbuminuria? *Diabetes Res Clin Pract* 2009; 85: 258–264.
14. Echida Y, Mochizuki T, Uchida K, et al. Risk factors for vitamin D deficiency in patients with chronic kidney disease. *Intern Med* 2012; 51: 845–850.
15. Baecher S, Leinenbach A, Wright JA, et al. Simultaneous quantification of four vitamin D metabolites in human serum using high performance liquid chromatography tandem mass spectrometry for vitamin D profiling. *Clin Biochem* 2012; 45: 1491–1496.

16. Carneiro AV. Estimating sample size in clinical studies: basic methodological principles. *Rev Port Cardiol* 2003; 22: 1513–1521. [in English and Portuguese].
17. Yao P, Lu L, Hu Y, et al. A dose-response study of vitamin D3 supplementation in healthy Chinese: a 5-arm randomized, placebo-controlled trial. *Eur J Nutr* 2015; Feb 19: [Epub ahead of print].
18. Talaei A, Mohamadi M and Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. *Diabetol Metab Syndr* 2013; 5: 8.
19. Anker SD and von Haehling S. Vitamin D in chronic kidney disease: more questions than answers. *JAMA* 2012; 307: 722–723.
20. Snellman G, Melhus H, Gedeborg R, et al. Determining vitamin D status: a comparison between commercially available assays. *PLoS One* 2010; 5: e11555.
21. de Boer IH, Ioannou GN, Kestenbaum B, et al. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis* 2007; 50: 69–77.
22. Dusso AS. Renal vitamin D receptor expression and vitamin D renoprotection. *Kidney Int* 2012; 81: 937–939.
23. Spedding S. Vitamin D and human health: celebrating diversity. *Nutrients* 2013; 6: 11–14.
24. vinh quốc Lu'o'ng K and Nguyên LT. The beneficial role of vitamin D in obesity: possible genetic and cell signaling mechanisms. *Nutr J* 2013; 12: 89.
25. Bachali S, Dasu K, Ramalingam K, et al. Vitamin D deficiency and insulin resistance in normal and type 2 diabetes subjects. *Indian J Clin Biochem* 2013; 28: 74–78.
26. Romero-Aroca P, Baget-Bernaldiz M, Reyes-Torres J, et al. Relationship between diabetic retinopathy, microalbuminuria and overt nephropathy, and twenty-year incidence follow-up of a sample of type 1 diabetic patients. *J Diabetes Complications* 2012; 26: 506–512.
27. Chen YH, Chen HS and Tarng DC. More impact of microalbuminuria on retinopathy than moderately reduced GFR among type 2 diabetic patients. *Diabetes Care* 2012; 35: 803–808.
28. Payne JF, Ray R, Watson DG, et al. Vitamin D insufficiency in diabetic retinopathy. *Endocr Pract* 2012; 18: 185–193.
29. Taverna MJ, Sola A, Guyot-Argenton C, et al. Taq I polymorphism of the vitamin D receptor and risk of severe diabetic retinopathy. *Diabetologia* 2002; 45: 436–442.