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Original Article

Calcitriol reduces proteinuria and improves bone mineral density in patients with diabetic nephropathy: a prospective randomized controlled study

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Abstract: Objectives: The progression of diabetic nephropathy (DN) is highly associated with proteinuria and bone mineral density (BMD). This study aimed to evaluate the efficacy of oral calcitriol on proteinuria and BMD in DN patients. Methods: In this 12-week, prospective, randomized controlled study, we randomly assigned 60 patients with nephropathy due to type 2 diabetic mellitus (T2DM) to receive calcitriol (0.25 μg/d) or placebo (control) added to the standard treatment. The outcome was determined by changes of urinary albumin-to-creatinine ratio (ACR) and BMD after 12 weeks of treatment in both groups. Results: The baseline characteristics such as age, sex distribution, and BMI in the two groups were similar. Compared with pre-calcitriol treatment group, levels of fasting plasma glucose, fasting insulin, glycosylated hemoglobin, total cholesterol, triglyceride, low-density lipoprotein cholesterol, homeostasis model of assessment for insulin resistance index, and ACR in the patients of post-calcitriol treatment were significantly reduced, whereas high-density lipoprotein cholesterol was elevated markedly. The difference was statistically significant ($P<0.05$). However, there were no statistical differences between pre-placebo treatment and post-placebo treatment group ($P>0.05$). Besides, serum creatinine did not differ significantly after treatment with placebo or calcitriol ($P>0.05$). Furthermore, oral calcitriol significantly increased serum osteocalcin concentrations and BMD of lumbar spine and femoral shaft ($P<0.05$), whereas the difference of femoral neck BMD had no statistical significance ($P>0.05$). Conclusion: Oral calcitriol treatment may improve glucose and lipid metabolism, ameliorate proteinuria as evaluated by reduced ACR level, and improve BMD in type 2 DN patients.

Keywords: Calcitriol, proteinuria, BMD, ACR, diabetic nephropathy

Introduction

Diabetic nephropathy (DN), generally defined as urinary albumin excretion greater than 300 mg/24 hr [1], is one of the most common complications of both type 1 and type 2 diabetes mellitus (DM). It was caused by damage to the capillaries in the kidneys' glomeruli [2]. DN is the leading cause of chronic progressive kidney disease and death in DM patients [3]. The evolution from DM to DN originally begins from a slight increase of urinary albumin excretion (microalbuminuria) to macroalbuminuria with higher and higher glomerular filtration rate, and ultimately deteriorates into end-stage renal disease (ESRD) which is pathologically featured by glomerulosclerosis and tubulointerstitial fibrosis [4, 5]. DN was reported to be the primary cause for ESRD in diabetic patients worldwide, with rising prevalence in developed countries [2, 6, 7]. Furthermore, the high incidence, the poor prognosis, and the high treatment cost of DN have caused it to be a significant public health burden [8, 9].

Proteinuria, of which albumin is the major constituent, is one of the widely-used hallmarks of DN and usually followed by a progressive decline in renal function [10-12]. Current therapeutic approaches for DN are focusing on strict control of blood glucose and blood pressure as well as inhibition of the renin angiotensin system using angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) [11]. However, DN patients who received ACEIs or ARBs failed to completely avoid proteinuria and had annual renal event rates of 15% or more [13, 14]. Therefore, other

Table 1. Patient characteristics

Characteristics	Control group (n = 30)	Calcitriol group (n = 30)
Age (years)	52.89±10.38	56.13±10.69
Male/Female	12/18	14/16
Duration of DM (years)	6.70±4.90	7.50±3.40
BMI (kg/m^2)	27.42±4.17	27.83±3.41
Systolic blood pressure (mm Hg)	133.7±15.80	134.1±14.47
Diastolic blood pressure (mm Hg)	72.16±9.86	71.10±11.32
Serum total cholesterol (mmol/L)	5.68±0.67	5.69±0.64
Serum calcium (mg/dL)	9.35±0.51	9.27±0.56
Serum phosphorus (mg/dL)	3.99±0.68	4.05±0.75

Baseline characteristics of type 2 DN patients. Results are given as absolute numbers or as means ± SD. There were no significant differences between groups in patient characteristics using independent *t*-test (P>0.05). DM, diabetes mellitus; BMI, body mass index.

treatments which can further ameliorate proteinuria are needed to decrease the burden of DN patients. Previous studies revealed that vitamin D (VD) treatment reduced urinary albumin [15] and had the potential to prevent kidney damage in DN patients [16-18]. Calcitriol (1.25-dihydroxyvitamin D3) [19], an active form of vitamin D3, showed a modest antiproteinuric effect in patients with IgA nephropathy and persistent proteinuria [20]. Thus, we speculated that calcitriol can serve as an adjuvant therapy for DN patients receiving standard treatment and further reduce proteinuria.

Recent studies suggest that DN along with inhibited insulin activity or insulin insufficiency is the important reason for reduction of bone density and higher incidence of osteoporosis in elderly male patients with type 2 DM (T2DM) [21], indicating that bone mineral density (BMD) and alterations in bone metabolism are correlated with DN. Of note, calcitriol has been generally recognized as an effective and commonly used medication for increasing calcium absorption and deterring osteoporosis progress in the elderly population [22, 23]. We, therefore, assumed that calcitriol might recover the loss of BMD in DN patients.

Our study aimed to evaluate the efficacy of oral calcitriol as an adjuvant therapy for diabetic nephropathy patients receiving standard treatment. We monitored the proteinuria and the changes of BMD at the levels of lumbar spine, femoral neck and femoral shaft in DN patients.

Materials and methods

Patients

Type 2 DN patients in Jinshan Hospital Affiliated to Fudan University were selected (from Nov. 2010 to Nov. 2012). Written informed consent was obtained from each patient. The study has been approved by the Ethics Committee of Jinshan Hospital Affiliated to Fudan University. Exclusion criteria were as follows: hypercalcemia (>10.0 mg/dL), high serum phosphate (>5.2 mg/dL), uncontrolled blood pressure (>160/100 mm Hg), hyperparathyroidism, nephrolithiasis, chronic kidney disease, chronic hepatic diseases, heart failure, calcium and phosphorus metabolic disorders such as osteoarticular diseases and bone metastases, pregnancy, breast feeding, malignancy, medicine-taking history of hormones, VD or calcium supplement. In line with the above criteria, a total of 60 patients were enrolled.

Study protocol

In this 12-week study, 60 patients with type 2 DN receiving standard treatment (hypoglycemic reagents including biguanides and sulfonylureas) and then remained relatively stable conditions were enrolled and randomly divided into two groups: control (0.25 μg placebo daily) and calcitriol (Roche Pharmaceuticals, 0.25 μg oral calcitriol daily). In this study, doses of biguanides and sulfonylureas remained the same as pre-trial. That is, exactly, patients in this study were divided into (standard treatment + placebo) and (standard treatment + calcitriol) groups, with each for 30 patients.

Patients were followed for a total of 5 visits: one screening, randomized visit and then subsequently at 1st, 3rd, 6th and 12th weeks. Antihypertensive drugs were used to maintain target blood pressure (systolic: ≤130 mm Hg, diastolic: ≤80 mm Hg). The clinical characteristics of each patient were shown in **Table 1.**

Data collection

The age, gender, height, weight, and duration of DM of each type 2 DN patient were recorded. The body mass index (BMI) was calculated according the formula: BMI (kg/m^2) = weight

Table 2. Effect of calcitriol on glucose and lipid metabolism, pancreatic islet β-cell function and ACR

Index	Control group (n = 30)		Calcitriol group (n = 30)	
	Before treatment	After treatment	Before treatment	After treatment
FPG (mmol/L)	8.03±1.24	7.90±0.96	7.91±1.14	7.23±0.41[a,b]
FINS (mU/L)	9.53±0.64	9.51±0.81	9.47±0.86	8.11±0.53[a,b]
HbA1c (%)	7.52±1.08	7.48±1.13	7.51±1.26	6.62±0.92[a,b]
TC (mmol/L)	5.68±0.67	5.67±0.72	5.69±0.64	5.20±0.49[a,b]
TG (mmol/L)	1.93±0.27	1.92±0.33	1.92±0.34	1.48±0.26[a,b]
HDL-C (mmol/L)	1.04±0.26	1.05±0.21	1.03±0.26	1.48±0.38[a,b]
LDL-C (mmol/L)	3.17±0.44	3.17±0.46	3.16±0.41	2.62±0.51[a,b]
Scr (mg/L)	9.31±1.66	9.29±0.80	9.30±0.87	9.27±0.63
HOMA-IR	3.38±0.65	3.37±0.76	3.38±0.75	2.54±0.55[a,b]
ACR (mg/mmol)	11.33±1.25	11.37±1.41	11.52±1.95	4.23±1.18[a,b]

[a]P<0.05 vs. before treatment in Calcitriol group (ANOVA); [b]P<0.05 vs. after treatment in Control group (independent t-test). FPG, fasting plasma glucose; FINS, fasting insulin; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Scr, serum creatinine; HOMA-IR, homeostasis model of assessment for insulin resistance index; ACR, albumin-to-creatinine ratio.

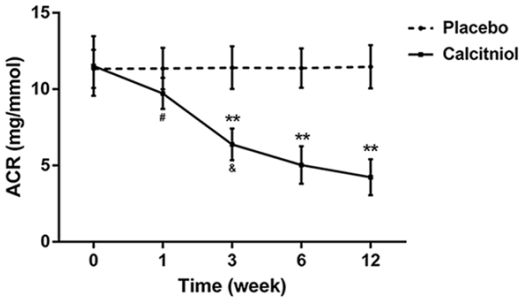

Figure 1. Changes of ACR of placebo and calcitriol groups at 0, 1st, 3rd, 6th and 12th week. ACR in calcitriol group was greatly decreased from the 0 to 3rd week. Compared with placebo treatment, ACR in calcitriol group was significantly down-regulated at 3rd, 6th, and 12th week. $^{**}P$<0.01 vs. Placebo group (independent t-test). $^{\#}P$<0.05: week 1 vs. week 0, $^{\&}P$<0.05: week 3 vs. week 1 (ANOVA).

(kg)/height^2 (m^2). Systolic/diastolic blood pressure was measured twice by a digital sphygmomanometer (BC08, Beurer, Germany). And fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (Scr), serum calcium, and serum phosphorus were measured by 7600-210 biochemical automatic analyzer (Hitachi Data System Corporation, Santa Clara, California, USA). The fasting insulin (FINS) level was determined by immunochemiluminescence (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). The glycosylated hemoglobin (HbA1c) was detected by a colorometric method after an initial chromatographic separation (BioSystems, Barcelona, Spain). The homeostasis model of assessment for insulin resistence index (HOMA-IR) was calculated according the formula: HOMA-IR = FPG × FINS/22.5. Urinary albumin and creatinine were measured by immunoturbidometric and colorometic methods, respectively. And urinary albumin-to-creatinine ratio (ACR) was then measured. Serum osteocalcin level was detected by radioimmunoassay (RIA). BMD was assessed by dual-energy X-ray absorptiometry (Hologic Inc., Bedford MA, USA).

Statistical analysis

Statistical analysis was conducted with SPSS 17.0 (SPSS Inc, USA). Comparison of patient characteristics between control and calcitriol group was conducted by independent t-test. Comparison of patient characteristics between different time points in calcitriol group was performed by one-way analysis of variance (ANOVA). Data were shown as absolute numbers or mean ± standard deviation (SD). P<0.05 was considered statistically significant.

Results

Baseline characteristics of type 2 DN patients

Comparison of baseline characteristics between the two groups was listed in **Table 1**. There were no significant differences of age, sex distribution, duration of DM, BMI, systolic/diastolic blood pressure, serum total cholesterol, serum calcium, and serum phosphorus between groups at baseline (P>0.05).

Effect of calcitriol on glucose and lipid metabolism, pancreatic islet β-cell function, and ACR

As shown in **Table 2**, compared with pre-calcitriol treatment group, levels of glycemic parameters (FPG and HbA1c), pancreatic islet β-cell function-related indexes (FINS and HOMA-IR), serum lipid parameters (TC, TG, LDL-C,)

Table 3. Comparison of serum calcium, phosphorus, osteocalcin and BMD before or after treatment of placebo or calcitriol

Index	Control group (n = 30)		Calcitriol group (n = 30)	
	Before treatment	After treatment	Before treatment	After treatment
Serum calcium (mg/dL)	9.35±0.51	9.34±085	9.27±0.56	9.28±0.44
Serum phosphorus (mg/dL)	3.99±0.68	4.01±0.52	4.05±0.75	4.04±0.83
Serum osteocalcin (μmol/L)	45.91±5.82	46.15±10.74	46.02±7.83	56.33±9.22[a,b]
Lumbar spine BMD (g/cm^2)	0.84±0.04	0.83±0.07	0.84±0.06	0.96±0.03[a,b]
Femoral shaft BMD (g/cm^2)	1.44±0.08	1.45±0.09	1.44±0.07	1.73±0.06[a,b]
Femoral neck BMD (g/cm^2)	0.72±0.05	0.72±0.03	0.73±0.05	0.72±0.04

[a]P<0.05 vs. before treatment in Calcitriol group (ANOVA); [b]P<0.05 vs. after treatment in Control group (independent t-test). BMD, bone mineral density.

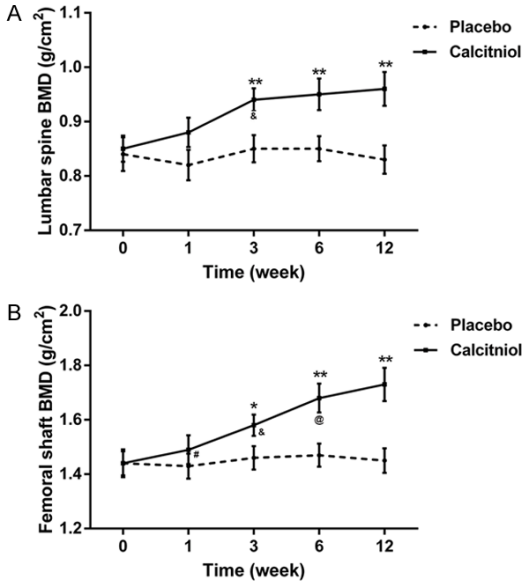

Figure 2. BMD changes of (A) lumbar spine and (B) femoral shaft after treatment of placebo and calcitriol at 0, 1st, 3rd, 6th and 12th week. The lumbar spine BMD in calcitriol group was significantly increased from the 1st to 3rd week, and the femoral shaft BMD in calcitriol group was greatly up-regulated from the 0 to 6th week. The lumbar spine and femoral shaft BMD was significantly increased after calcitriol treatment at 3rd, 6th, and 12th week as compared with placebo group. $^*P$<0.05, $^{**}P$<0.01 vs. Placebo group (independent t-test). $^\#P$<0.05: week 1 vs. week 0, $^\&P$<0.05: week 3 vs. week 1, $^@P$<0.05: week 6 vs. week 3 (ANOVA).

and ACR after calcitriol treatment were significantly decreased, whereas HDL-C was increased markedly. The difference was statistically significant (P<0.05). However, there were no statistical differences between pre-placebo and post-placebo treatment groups (P>0.05). Moreover, as for Scr (renal function-related index), there was no obvious change between pre-calcitriol and post-calcitriol groups. Besides, placebo treatment also failed to change Src (P>0.05). These results indicated that calcitriol may improve glucose and lipid metabolism as well as pancreatic islet β-cell function, and reduce insulin resistance.

ACR changes of placebo and calcitriol groups at 0, 1st, 3rd, 6th and 12th week were shown in **Figure 1**. Before treatment, the mean ACR was 11.33±1.25 mg/mmol in the placebo group and 11.52±1.95 mg/mmol in calcitriol group. At week 1, ACR in calcitriol group was significantly decreased. Then, we found that calcitriol treatment from the 1st to 3ird week still greatly down-regulated ACR. At week 12, the mean ACR was 11.37±1.41 mg/mmol in the placebo group and 4.23±1.18 mg/mmol in calcitriol group. Compared with placebo treatment, ACR was significantly decreased after calcitriol treatment at 3rd, 6th, and 12th week (P<0.01). The results revealed that oral calcitriol significantly decreased urinary albumin excretion as evaluated by reduced ACR level, indicating its renalprotective effect by ameliorating proteinuria.

Calcium-phosphorus metabolism and BMD changes of type 2 DN patients

Then serum levels of calcium, phosphorus, and osteocalcin as well as BMD of various skeletal sites particularly prone to osteoporotic fractures, including lumbar spine, femoral shaft and femoral neck were evaluated. As shown in **Table 3**, there were no significant differences between the serum calcium and phosphorus detected at the beginning and the end of this study. After calcitriol treatment, serum osteocalcin and BMD of lumbar spine and femoral

shaft were increased significantly ($P<0.05$), whereas the difference of femoral neck BMD had no statistical significance between pre-calcitriol and post-calcitriol groups ($P>0.05$). However, there were no statistical differences of these indexes between pre-placebo and post-placebo treatment ($P>0.05$).

Our results demonstrated that calcitriol treatment was associated with an increase in BMD at lumbar spine and femoral shaft. While at femoral neck, the increment was not significantly different from placebo-treated patients. BMD changes at lumbar spine and femoral shaft of placebo and calcitriol groups at 0, 1st, 3rd, 6th and 12th week was shown in **Figure 2**. Before treatment, the mean lumbar spine BMD was 0.84±0.04 g/cm2 in the placebo group and 0.84±0.06 g/cm2 in calcitriol group. At week 12, the mean lumbar spine BMD was 0.83±0.07 g/cm2 in the placebo group and 0.96±0.03 g/cm2 in calcitriol group. Calcitriol treatment from the 1st to 3rd week greatly increased lumbar spine BMD. Moreover, the lumbar spine BMD was significantly up-regulated after calcitriol treatment at 3rd, 6th, and 12th week as compared with placebo group. ($P<0.01$, **Figure 2A**). The femoral shaft BMD displayed the similar pattern (**Figure 2B**).

Discussion

The prevalence of diabetes mellitus is estimated at 8.3%, affecting about 387 million people in 2014 and is expected to affect about 592 million people by 2035 [24]. T2DM is the commonest type of diabetes accounting for 90% in diagnosed diabetes cases globally, and is characterized by insulin secretion disorders and insulin resistance (IR) [25]. IR and progressive deterioration of pancreatic islet β-cell function were the two key factors of the pathogenesis of T2DM. VD, an important class of steroid hormones, plays critical roles in regulating calcium, phosphorus metabolism and bone metabolism balance [26-29]. Extensive literature has reported the correlation of VD with diabetes. A prospective study has shown a significant negative correlation between serum 25-hydroxyvitamin D levels and the risk for T2DM in a population from the south Spain [30]. Besides, increased VD levels from 25 to 75 nmol/L leads to a 60% improvement in insulin sensitivity [31-33]. A randomized double-blind clinical trial also demonstrated that, regular VD intake improved glycemic status, lipid profile and endothelial biomarkers in T2DM patients [34]. In this present study, we found that oral calcitriol, an active VD metabolite, may improve glucose and lipid metabolism as well as pancreatic islet β-cell function, and reduce insulin resistance.

Moreover, the higher risk of nephropathy in VD-deficient diabetic patients was shown by a meta-analysis [6]. VD compounds not only lowered parathyroid hormone levels, but reduced overall mortality in patients with chronic kidney disease on dialysis [35, 36]. Additionally, VD has the potential to delay the progression of DN gauged by a reduction in urinary albumin [15]. VD and its analogs are supposed to prevent kidney damage in DN by inhibiting the renin-angiotensin system [16-18]. Our study found that oral calcitriol significantly decreased urinary albumin excretion as evaluated by reduced ACR level, indicating its renalprotective function by ameliorating proteinuria.

DN is accompanied by abnormal bone metabolism and easily results in osteodystrophy and osteoporosis which are serious complications of diabetes that appear in the skeletal system [37]. The decreased BMD is due to the reduced bone matrix synthesis caused by the considerable loss of calcium, phosphorus and magnesium with hyperosmotic diuresis [38]. VD drugs can effectively increase BMD of osteoporosis patients, which is due to the fact that VD may stimulate the differentiation of bone cell precursors and suppress the interaction between the front osteoblast and osteoclast precursor with bone resorption reduced [39, 40]. Previous studies revealed that calcitriol monotherapy improved BMD in elderly osteoporotic Chinese patients [41]. In addition, calcitriol improved streptozotocin-induced diabetes and recovered BMD in diabetic rats [42]. Our study found that, in type 2 DN patients, oral calcitriol appeared to exert a favorable influence on BMD at lumbar spine and femoral shaft, which was associated with a stabilization of serum osteocalcin concentrations.

Conclusion

In summary, oral calcitriol reduced proteinuria and improved BMD in patients with diabetic nephropathy. However, the limitations of the present study must be taken into consider-

ation: the small research sample sizes (n = 60) and the limited treatment periods (12 weeks). Thus, the long-term efficacy and safety of oral calcitriol for type 2 DN patients need to be further investigated.

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Disclosure of conflict of interest

None.

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