Exploring the pleiotropic effects of vitamin D in diabetes

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Introduction

Vitamin D is a unique vitamin and a hormone which is absolutely essential for bone mineral homeostasis. In addition, in the recent past there has been a growing appreciation for its role as an endocrine regulator having important roles in the renal, cardiovascular, reproductive and immune systems in the body. These actions are named non-classic actions or pleiotropic effects of vitamin D.

Regarding the non-classic actions of the hormonal form of vitamin Din the renal system, inhibition of Renin Angiotensin System (RAS)by reducing renin synthesis is an important action of vitamin D. Renin is the first and the rate limiting step of the RAS cascade.

This action of vitamin D can be used to overcome the unwanted effects resulting from the long term use of Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin Receptor Blockers (ARB) in patients with renal and cardiovascular disease; that is compensatory rise in renin level as a result of disruption of feedback inhibition of renin production.

ACEI and ARB are included in the standard treatment for reducing proteinuria in diabetic nephropathy, which is the commonest cause of end stage renal disease worldwide . Despite the treatment with ACEI and ARB, rate of progression of diabetic nephropathy remains high. Compensatory rise in plasma renin level leading to activation of RAS is one important mechanism which has been postulated as a possible reason for the inadequacy of ACEI and ARBs on reversing diabetic nephropathy.

Current data on the role of vitamin D reducing the progression of renal damage in diabetic renal disease are limited and come from either animal studies or few human studies . Studies which explored the effect of vitamin D on animal models of kidney disease have reported the beneficial effects of such therapy. Randomized control trials examining the effect of vitamin D on the progression of proteinuria are limited. Further, these studies are not sufficiently powered to generate conclusive results. There is a paucity of sufficiently powered randomized controlled trials examining the different reno-protective effects of vitamin D among patients with diabetic nephropathy.

Hence we planned to evaluate the effect of vitamin D therapy in reversing the progression of diabetic nephropathy and also to determine the effect on plasma renin, cardiovascular disease risk (CVDR) profile, bone mineral density (BMD) and bone mineral content (BMC) measurements.

This review includes the following arms exploring the effects of vitamin D on

1. renal functions in patients with diabetic nephropathy.
2. lipid profile and blood pressure in patients with diabetic nephropathy.
3. cardiovascular risk scores in patients with diabetic nephropathy.
4. bone mineral density in patients with diabetic nephropathy.

Methods

This study was conducted on patients with diabetic nephropathy (urinary albumin >30 mg/g of creatinine in two occasions) whose GFR was

more than 30 mL/min. Patients who had albuminuria in a previous cross sectional study six months back were invited and investigations were repeated. This procedure ensured confirmation of albuminuria at least on two occasions over a period 6 months. Selected patients were informed about the study and written consent was obtained. Those who had blood pressure > 130/80 mmHg during the last two clinic visits, hyperphosphataemia (serum phosphate > 5 mg/dL), hyper or hypocalcaemia, uncontrolled blood sugar (current HbA1c > 8) and those with liver disease, hyperthyroidism, hyperparathyroidism, or diseases related to calcium or vitamin D metabolism and congestive heart failure (current) were excluded. Attempts were made to exclude other causes of proteinuria such as ongoing urinary tract infection, urolithiasis, and renal tuberculosis by history, examination and previous investigations. Morning urine samples were collected and urine dipstick test was done to exclude ongoing urine infections. Urine collection was postponed if a patient had fever, urinary symptoms, or menstruation. Only those were negative for nitrates were stored in -80^{0}C for urine albumin analysis. After two weeks second sample of urine was collected. Same procedure was followed as for the first urine sample. If the urinary albumin excretion of the second sample was inconsistent with the first sample, a third urine sample was checked. Presence of microalbuminuria was confirmed only if two consecutive or two out of three samples were positive for urine albumin > 30 mg albumin/g of creatinine. In the same manner, macroalbuminuria was confirmed when urine albumin excretion was > 300 mg/g of creatinine.

Study design

Patients were allocated to two groups by block randomization method (block of 2) using a random number table. Concealed envelopes containing treatment allocation were given to research assistants who assigned participants to treatment and control groups. Treatment group received monthly dose of 50,000 IU of vitamin D_3 intramuscularly and the control group was given an equal volume of distilled water (0.25 mL) to the same site in a similar manner. Participants, those administering the interventions, clinicians, and those assessing the outcomes were blinded to the group assignment.

Study procedures

Patients underwent a detailed medical history, a physical examination including systolic and diastolic blood pressure (SBP and DBP) measurement. Blood and urine were collected for the baseline measurements which included serum creatinine, serum calcium, urine microalbumin, fasting blood glucose (FBS), serum calcium, phosphate, creatinine, Parathyroid Hormone (PTH), renin and vitamin D level and lipids namely total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG), and high density lipoprotein (HDL). CVDR was calculated using Framingham risk score (FRS). The CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

A safety visit was scheduled 1 week after starting the treatment to monitor the serum Ca and phosphorus concentrations and to elicit any adverse events. The protocol permitted withdrawal from the trial if serum Ca exceeded 11 mg/dL. During monthly visits patients were inquired about side effects according to a check list in the data collection sheet.

All patients underwent whole body dual-energy X-ray absorptiometry (DXA) scan and BMD and BMC of the total body, total spine (L_1 - L_4) and proximal femur were measured. All scans were performed and analysed by the same technician adhering to the manufacturer's protocol. DXA machine was calibrated using the calibration phantom provided by the manufacturer. The precision error of the machine has been published previously. There were no software or hardware changes during the study period.

At three months blood samples were collected for serum creatinine, serum calcium, serum phosphate, FBS, lipid profile and urine was taken for the assessment of urine micro albumin to creatinine ratio at three months.

After six months of treatment all the measurements done at the baseline, including DXA were repeated. When the trial period of six months was over, a randomly selected subgroup of patients (25 from each group) was followed up for further six months and another DXA testing was performed.

Biochemical assays were performed using commercial kits. Urine albumin was measured by turbid metric method while urinary creatinine concentration was measured using an end-point spectrophotometric method with an alkaline-picrate solution.

Serum creatinine was measured by auto-creatinine calibrated with autocal which is traceable to reference material. Glomerular Filtration Rate (GFR) was estimated by CKD-EPI equation.

Intact PTH (Immunotech, IRMA PTH), renin (Beckman coulter, IRMA Active Renin) by radioimmunoassay and 25-hydroxy vitamin D were measured using immunochemiluminometric (Vitros immunodiagnostic) assays. Serum creatinine was measured by spectrophotometric method with an alkaline-picrate solution.

Ethical aspect

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Clinical trial has been registered in the local clinical trial registry. Informed written consent was obtained from all subjects of the study.

Statistical analysis

The baseline characteristics between the two groups were compared by either unpaired t-test or Chi-square test. Changes in urinary albumin, renal functions, vitamin D, renin, PTH, BMD / BMC during the trial period were analyzed by the Repeated measure ANOVA (SPSS, Chicago, USA). P value was adjusted for multiple comparisons by the Bonferroni method.

Results

A total of 157 patients were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups and 82 subjects completed the study; 41 patients from each group completed the study.

No significant differences were found with regards to the baseline characteristics between the treatment and control groups (Table 1).

Table 1: Baseline characteristics of subjects in the two groups

Variable	Control group (n = 43)	Treatment group(n = 42)	*P* value
Age (years)	59 (8)	56 (10)	0.1
Number of males (%)	42.9	48.8	0.58
SBP (mmHg)	121 (7)	120 (8)	0.46
DBP (mmHg)	70 (5.9)	71 (5.9)	0.25
HbA_1C (%)	7.1 (0.5)	6.9 (0.5)	0.1
Calcium (mg/dL)	8.9 (0.7)	8.8 (0.6)	0.65
Phosphorus (mg/dL)	3.8 (0.6)	3.9 (0.5)	0.31
PTH (pg/mL)	42.5 (19.0)	38.2 (11.3)	0.21
Plasma renin (pg/mL)	15.14 (4.82)	14.64 (5.62)	0.66
25(OH)D (nmol/L)	49.6 (16.5)	56.1 (12.9)	0.07
FBS (mg/dL)	130 (12.5)	128 (13.3)	0.51
Duration of diabetes (years)	7 (4)	8 (5)	0.42
Urine creatinine (mg/dL)	63.6 (10.9)	61.7 (11.9)	0.44
Urine albumin (mg/g of creatinine)	185.8 (50.6)	164.4 (35.8)	0.83
Glomerular Filtration Rate (mL/min)	83.2 (16.1)	86.7 (14.6)	0.28
HDL (mg/dL)	53.5 (10.9)	50.3 (7.5)	0.13
TC (mg/dL)	194.6 (32.1)	194.8 (30.1)	0.87
LDL (mg/dL)	117.0 (28.1)	119.7 (28.7)	0.87
TG (mg/dL)	128.4 (50.8)	122.8 (41.4)	0.66
BMI (kg/m^2)	23.2 (4.0)	24.4 (3.4)	0.14

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), HDL (high density lipoprotein), TC (total cholesterol), LDL (low density lipoprotein), TG (triglyceride), BMI (body mass index)

All patients received either an ARB or ACEI at the baseline. During the study period, oral hypoglycemic drugs were increased in nine patients (6 in the treatment group). Losartan was increased in three patients (2 in the control group). Blood pressure number and doses of anti-hypertensive drugs were quite stable during the clinical trial.

1. Vitamin D therapy on renal functions of patients with diabetic nephropathy

Diabetic nephropathy is the leading cause of end stage renal disease and despite optimum therapy including ACEI/ARBs, a sizable proportion of patients with proteinuria progress to renal failure. It is likely that high renin level induced by RAS (Renin Angiotensin System) blockage may contribute to this and vitamin D is found to have an inhibitory effect over RAS as it reduces renin synthesis.

This study was conducted to examine the effects of vitamin D therapy on renal functions of patients with diabetic nephropathy.

Effect of vitamin D therapy on urinary albumin excretion, renal functions and plasma renin among patients with diabetic nephropathy; a randomized, double-blind clinical trial.

Results

Table 2 shows the changes of the urine microalbumin to creatinine ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBS), serum creatinine, eGFR, PTH, renin and vitamin D levels after three and six months of treatment in the treatment and control groups. After six months, mean reduction of urinary albumin to creatinine ratio was 51.8 mg/g ($p = < 0.001$) in the treatment group, 22.4 mg/g ($p = 0.06$) in the control group and this difference was significant ($p = 0.001$). Significant increase in the GFR was observed in the treatment group while in the control group GFR remained unchanged ($p = 0.03$ for the between-groups difference). There was a significant reduction of serum creatinine in the treatment group but not in the control group. But the change was not significant between groups.

A significant increase of SBP was seen in the control group whereas SBP remained unchanged in the treatment group and the difference was not statistically significant. Significant trends in the DBP was seen in both groups during the study period but the difference between the two groups was not statistically significant ($p = 0.17$). Significant reduction of FBS was seen only in the control group and the difference between groups was not statistically significant ($p = 0.23$).

Significant reduction of PTH was observed in both treatment and the control groups. But the change between two groups was not statistically significant ($p = 0.26$). In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant ($p < 0.001$). Mean reduction in plasma renin in the treatment group was 5.85 pg/mL ($p < 0.001$). In the control group the reduction observed was only 0.95 pg/mL. The difference between the two groups was statistically significant ($p = 0.006$) (Table 2).

A significant inverse correlation was observed in vitamin D with percentage change in plasma renin level ($\rho = -0.66$, $p < 0.01$) and percentage change in urine albumin levels ($r = -0.47$, $p < 0.01$). Furthermore, percentage changes of renin and urinary albumin also showed a significant correlation ($\rho = 0.62$, $p < 0.01$) (Table 3).

According to the table 4, the microalbuminuria suppression is related to the final serum renin level. The suppression of microalbuminuria is highest in the highest tertile of serum renin and it does not vary across the tertiles of serum vitamin D. The lowest suppression of microalbuminuria is in the lowest tertile of the serum renin. Furthermore, there is a gradient of microalbuminuria suppression across the tertiles of the serum renin concentration.

Serum Ca in patients with vitamin D treated and in the control group were 9.16 (0.61) and 9.045 (0.69) at the end of trial. The difference was not statistically significant. No adverse events, particularly hypercalcaemia were reported during the study period.

Table 2: Changes observed in the treatment and control groups at 3 months and 6 months

Variable		Baseline	At 3 months	At 6 months	*P* within group	*P* between group
SBP (mmHg)	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP (mmHg)	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
FBS (mg/dL)	Control	130.2 (12.5)	130.6 (10.1)	127.8 (10.7)	0.02	0.23
	Treatment	128.3 (13.6)	125.8 (13.4)	125.9 (10.9)	0.08	
PTH (pg/mL)	Control	42.5 (19.0)		37.6 (12.6)	0.003	0.26
	Treatment	38.2 (11.3)		35.7 (7.9)	0.001	
25 (OH)D (nmol/L)	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	
Plasma renin (pg/mL)	Control	15.14 (4.82)		14.19 (4.6)	0.02	0.006
	Treatment	14.64 (5.62)		8.83 (4.81)	< 0.001	
Urine albumin (mg/g)	Control	185.8 (50.6)	160.9 (63.4)	163.4 (56.2)	0.06	0.001
	Treatment	169.4 (35.8)	122.1 (54.4)	117.6 (45.2)	< 0.001	
Serumcreatinine (mg/dL)	Control	0.87 (0.22)	0.87 (0.20)	0.87 (0.20)	0.84	0.10
	Treatment	0.86 (0.13)	0.80 (0.12)	0.77 (0.11)	< 0.001	
GFR (mL/min)	Control	83.2 (16.1)	83.4 (15.6)	83.9 (14.9)	0.74	0.03
	Treatment	86.7 (14.6)	90.7 (14.8)	93.7 (14.1)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), GFR (glomerular filtration rate)

Table 3: Correlations (Spearman rho) between the percentage change in vitamin D, urine albumin, plasma renin and PTH

Percentage change	Urine albumin	Renin	PTH
Vitamin D	-0.47**	-0.66**	-0.02
Urine albumin		0.62**	-0.08
Renin			-0.02

**Correlations are significant at 0.01 level.

Table 4: Percentage change in urinary albumin excretion in relation to of vitamin D and renin

Vitamin D ↓	Renin → Low	Middle	High
Low	32.9 (18.3)	11.9 (7.4)	5.7 (9.1)
Middle	45.0 (8.1)	10.0 (11.6)	2.9 (8.6)
High	33.5 (6.9)	12.6 (11.6)	5.8 (8.6)

Values are given mean (SE)

Conclusions

Randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy showed a significant reduction of urine microalbumin, serum creatinine, renin levels and improvement of GFR among patients in the treatment group compared to the control group after monthly injection of vitamin D for six months.

2. Vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy; a randomized double-blind clinical trial

Aim of this study was to determine the effect of high dose vitamin D given to patients with early diabetic renal disease on systolic and diastolic blood pressure, total cholesterol (TC), low-density lipoproteins (LDL), triglycerides (TG) and high density lipoproteins (HDL) in a randomized controlled trial.

Results

Table 5 shows the changes in SBP, DBP, TC, TG, LDL, HDL and vitamin D at 3 and 6 months of follow up.

In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant ($p < 0.001$).

Vitamin D therapy significantly reduced DBP, total cholesterol and LDLC in the treatment group, but the between group differences were not significant. There was an increase in HDL cholesterol level in the treatment group while there was no change in the control group (the between groups difference was significant).

Table 5: Changes in CVDR factors in the treatment and control groups

Variable		Baseline	At 3 months	After 6 months	*P* value within group	*P* value between group
SBP	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
(mmHg)	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
(mmHg)	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
TC	Control	194.6 (32.1)	193.6 (30.8)	196.9 (31.4)	0.24	0.50
(mg/dL)	Treatment	194.8 (30.1)	191.5 (28.1)	185.7 (27.2)	< 0.001	
TG	Control	128.4 (50.8)	127.9 (49.5)	128.7 (45.3)	0.62	0.44
(mg/dL)	Treatment	122.8 (41.4)	121.8 (40.1)	118.2 (32.4)	0.062	
LDL	Control	117.0 (28.1)	114.6 (28.9)	117.1 (30.2)	0.34	0.7
(mg/dL)	Treatment	119.7 (28.7)	115.7 (27.6)	106.10 (26.5)	< 0.001	
HDL	Control	35.5 (10.9)	53.7 (10.7)	53.9 (9.7)	0.40	< 0.001
(mg/dL)	Treatment	50.3 (7.5)	51.5 (7.1)	55.7 (6.8)	< 0.001	
25 (OH)D	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
(nmol/L)	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), TC (total cholesterol), TG (triglyceride), LDL (low density lipoprotein), HDL (high density lipoprotein)

Conclusions

Randomized double blind placebo control clinical trial conducted among patients with diabetic nephropathy has shown significant improvement in HDL levels but no significant effect on blood pressure after monthly injection of vitamin D for six months.

3. Vitamin D therapy on cardiovascular risk scores of patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy cardiovascular risk scores in patients with diabetic nephropathy; a randomized double-blind clinical trial

Diabetes is considered as a coronary vascular disease equivalent. Cardiovascular disease risk (CVDR) can be assessed by cardiovascular risk scores. The aim of this study was carried out to assess the effects of vitamin D therapy on CVDR scores among patients with diabetic nephropathy.

CVDR was calculated using Framingham risk score (FRS). Further, CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

Results

No significant differences were found between treatment and control groups at the baseline. FRS, CVDR, FCVDR, SR and FSR values before and after the intervention were 20.32 and 21.41, 13.62 and 11.94, 8.97 and 7.951, 7.66 and 7.50, 0.95 and 0.94 respectively in the treatment group. Respective values of the control group were 19.96 and 22.49, 12.76 and 13.59, 7.902 and 8.80, 6.11 and 6.66, 0.75 and 0.87.

No significant effect was found with vitamin D_3 treatment on CVDR scores, measured by FRS and UKPDS (Table 6).

Table 6: Changes in CVD risk scores in the treatment and control groups

Variable		Baseline	At 3 months	At 6 months	*P* value within	*P* value between
FRS	Control	19.96 (11.09)	19.79 (12.02)	22.49 (11.69)	< 0.001	0.92
	Treatment	20.32 (14.14)	19.69 (13.73)	21.41 (21.61)	0.62	
CVDR	Control	12.76 (08.33)	12.72 (08.37)	13.59 (08.69)	.004	0.88
	Treatment	13.62 (11.27)	12.57 (09.85)	11.94 (09.55)	< 0.001	
F CVDR	Control	7.902 (6.32)	8.05 (6.10)	8.80 (6.60)	0.006	0.96
	Treatment	8.97 (9.40)	8.09 (7.82)	7.951 (7.10)	0.005	
Stroke risk	Control	6.11 (4.35)	6.15 (4.40)	6.66 (4.70)	< 0.001	0.51
	Treatment	7.66 (9.60)	6.80 (7.70)	7.50 (9.00)	0.11	
F Stroke risk	Control	0.75 (0.55)	0.76 (0.56)	0.87 (0.62)	< 0.001	0.55

Framingham risk score (FRS), Cardiovascular disease risk (CVDR), fatal coronary vascular disease risk (FCVDR), fatal stroke risk (F Stroke risk)

Conclusions

Monthly injections of high dose vitamin D_3 but did not have a significant effect on cardiovascular risk scores among patients with diabetic nephropathy.

4. Vitamin D therapy on bone mineral density of patients with diabetic nephropathy

Effect of vitamin D therapy on bone mineral density among people with diabetic nephropathy; a randomized, double-blind placebo controlled clinical trial.

Aim was to determine the effect of vitamin D given to patients with diabetic nephropathy on bone mineral density (BMD) and bone mineral content (BMC).

Results

A total of 157 people were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups; 43 subjects in the treatment group and 42 subjects to the control group. No significant differences were found with regards to the baseline characteristics between the treatment and control groups.

Two participants from the treatment group and one participant from the control group did not complete the study. They were not contactable probably due to the change of the residence. Forty one subjects from each group completed the intervention. At the end of 6 months DXA results were available in 39 participants in the treatment group and 38 in the control group. At the end of one year, 25 from each group underwent the 3rd BMD measurement.

Table 7 shows the changes of the total body BMD/BMC, regional BMDs, total fat and lean masses, during the initial six months of treatment in the treatment and control groups.

After six months of vitamin D injections, total body BMD, total body BMC and BMDs of spine, femoral neck and total hip regions increased by 2.0%, 2.2%, 1.8%, 2.1% and 2.6% ($P < 0.05$ for all), respectively from the baseline figures. However, increase observed in the trochanteric BMD among them was not statistically significant. In the control group, compared to the baseline values, total body BMC, BMD, or regional BMDs did not change significantly during the initial six months.

Table 7: Changes in bone mineral density and fat mass in the treatment and control groups

Variable		Baseline	After 6 months	Percentage difference	*P* within groups	*P* between groups
BMD	Control	1.038 (0.121)	1.031 (0.191)	-0.67	0.75	0.61
	Treatment	1.038 (0.120)	1.059 (0.107)	2.02	0.01	
BMC	Control	1775.63 (412.76)	1721.64 (369.70)	-3.04	0.074	0.73
	Treatment	1757.95 (383.68)	1795.85 (373.27)	2.16	0.007	
Spine BMD	Control	0.848 (0.132)	0.836 (0.119)	-1.41	0.27	0.72
	Treatment	0.845 (0.153)	0.860 (0.142)	1.78	0.04	
Femoral neck BMD	Control	0.722 (0.109)	0.712 (0.094)	-1.38	0.23	0.43
	Treatment	0.731 (0.153)	0.746 (0.142)	2.05	0.03	
Trochanter BMD	Control	0.607 (0.089)	0.604 (0.08)	-0.49	0.5	0.46
	Treatment	0.615 (0.111)	0.627 (0.103)	1.95	0.07	
Hip BMD	Control	0.857 (0.113)	0.852 (0.105)	-0.58	0.56	0.25
	Treatment	0.876 (0.148)	0.899 (0.149)	2.62	0.008	
Total fat mass	Control	15.85 (6.67)	16.48 (6.16)	3.99	0.20	0.2
	Treatment	17.41 (5367.460)	18.21 (5.56)	4.6	0.06	
lean mass	Control	37.04 (6.94)	36.98 (6.38)	-0.18	0.86	0.16
	Treatment	38.92 (8.32)	39.64 (7.73)	1.85	0.09	

BMD (bone mineral density), BMC (bone mineral content)

Six months after the cessation of vitamin D treatment, significant reductions of both total BMD and BMC were observed (P = 0.009) while regional BMDs remained unchanged (Table 8). In the control group none of the BMD / BMC measurements changed significantly during the post-trial follow up six months period.

Table 8: Changes in bone mineral density and fat mass in the treatment and control groups

Variable		After 6 months		After 12 months		Percentage difference	*P* within groups	*P* between groups
BMD	Control	0.999	(0.134)	1.006	(0.112)	0.70	0.47	0.21
	Treatment	1.054	(0.120)	1.041	(0.131)	-1.23	0.009	
BMC	Control	1735.92	(430.22)	1716.05	(402.87)	-1.14	0.26	0.54
	Treatment	1808.19	(450.57)	1795.94	(458.27)	-0.68	0.04	
Spine BMD	Control	0.823	(0.128)	0.828	(0.126)	0.61	0.19	0.69
	Treatment	0.847	(0.168)	0.837	(0.163)	-1.18	0.07	
Femoral neck BMD	Control	0.711	(0.109)	0.711	(0.105)	0	0.92	0.28
	Treatment	0.756	(0.166)	0.753	(0.169)	-0.4	0.48	
Trochanter BMD	Control	0.601	(0.823)	0.598	(0.851)	-0.5	0.35	0.55
	Treatment	0.617	(0.112)	0.615	(0.110)	-0.32	0.33	
Hip BMD	Control	0.851	(0.114)	0.848	(0.116)	-0.35	0.69	0.3
	Treatment	0.889	(0.160)	0.895	(0.160)	0.67	0.48	
Total fat mass	Control	16.10	(6.75)	15.66	(6.57)	-2.71	0.06	0.22
	Treatment	18.28	(5.31)	17.98	(5.20)	-1.61	0.79	
Lean mass	Control	37.39	(7.36)	37.29	(7.16)	-0.26	0.52	0.2
	Treatment	40.25	(8.54)	40.35	(8.65)	0.23	0.54	

BMD (bone mineral density), BMC (bone mineral content)

Conclusions

The improvement of total body BMC, total body BMD, BMDs of spine, femoral neck and hip were statistically significant among vitamin D treated patients compared to patients in the control group. Six months after stopping treatment the improvement in the regional BMD remained unchanged while only a marginal loss was observed in total body BMD and BMC.

Discussion

Most striking outcome of this randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy was the significant reduction of urine microalbumin after monthly injection of vitamin D for six months. In addition, there was a significant reduction of serum creatinine and improvement of GFR among patients who received vitamin D. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy.

Further, vitamin D increased BMD / BMC compared to placebo given to patients in the control group. This improvement was observed in the total body BMD/BMC and BMDs of total hip, total spine and femoral neck. The regional BMDs remained unchanged six months after withdrawing vitamin D treatment while only a marginal loss was observed in total BMD and BMC.

Vitamin D caused no significant effect on cardiovascular risk scores, blood pressure or major serum lipid components except HDL.

In our sample we recruited patients in the early stages of renal disease (eGFR > 30 mL/min) and majority of them were not vitamin D deficient. Therefore we were able to increase their vitamin D levels to above physiological limits in order to examine for the non-classic benefits of vitamin D. These benefits were independent of the conventional treatment offered for these patients according to current treatment guidelines. According to the studies discussed above there was a significant reduction of urine microalbumin, serum creatinine and improvement of GFR after monthly injection of vitamin D for six months. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy. Furthermore, we observed a significant reduction of renin levels in the treatment group compared to the control group.

The dose of vitamin D used in this study raised serum vitamin D level substantially. Although this was sufficient to demonstrate reno-protective effect and benefit on BMDs the period of trail was insufficient to demonstrate a positive effect on CV measurements except HDL.

Based on the results of these studies vitamin D can be considered as an add-on therapy to patients with increasing microalbuminuria despite optimum glycaemic and blood pressure control and receiving maximum tolerable doses of ACEI or ARB.

Due to the paucity of data, however, further clinical trials should be done to reproduce the results observed in this study. If the same benefits are proven, use of vitamin D for complete suppression of albuminuria can be recommended.

Conclusions

Monthly injections of high dose vitamin D_3 has improved the renal functions, BMD and BMC in patients with diabetic nephropathy.

This treatment did not have a significant effect on cardiovascular risk scores or blood pressure except HDL levels.

Further studies involving longer durations of treatment at different doses of vitamin D may be needed to reconfirm these findings.

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