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Original Article

Effects of high-dose parenteral vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy: A randomized double-blind clinical trial

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ABSTRACT

Aims: 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
Materials and method: Patients with early diabetic nephropathy were recruited. Selected patients were allocated to two groups by Block randomization method. Treatment group received 50,000 IU of vitamin D3 intramuscularly and the control group was given an equal volume of distilled water (0.25 mL) monthly for six months. Blood and urine were collected at the baseline for biochemical analyses and blood pressure was measured. After six months all the measurements done at the baseline were repeated.
Results: Of 155 patients invited, 85 were randomly assigned to two groups. No significant differences were found between treatment and control groups at the baseline. Vitamin D therapy significantly reduced DBP, total cholesterol and LDL 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 Between groups difference was significant ($P = <0.001$).
Conclusions: There was a significant improvement of serum HDL level with six months therapy of high dose vitamin D in patients with early diabetic nephropathy.

1. Introduction

Diabetic nephropathy is a leading cause of end stage renal disease worldwide [1] and the onset of nephropathy amplifies the already existent risk of cardiovascular disease (CVD) in diabetes [2]. Several metabolic derangements that accompany renal dysfunction in diabetes such as worsening of insulin resistance, albuminuria, high blood pressure, alteration of plasma lipids probably underlie this increased predilection [3]. Therapeutic interventions aimed at reducing blood pressure, controlling albuminuria and optimizing plasma lipids, however, have not significantly reduced cardiovascular morbidity and mortality in patients with diabetic nephropathy [4]. With rising incidence of diabetes and associated adverse cardiovascular outcomes, there is a need for novel therapeutic approaches to reduce the rising burden of CVD in patients with diabetic nephropathy. In this regard, preliminary studies using vitamin D on insulin resistance and cardiovascular risk factors have shown some favorable effects.

Renal dysfunction is associated with vitamin D deficiency due to the reduced synthesis of 1-alpha hydroxylation enzyme. Many observational studies have reported significant associations of vitamin D deficiency with insulin resistance [5–7]. An association between low serum vitamin D level with carotid intima media thickness (CIMT), a surrogate marker of atherosclerosis has been reported in a study conducted among obese children and adolescents [8]. Supplementation of vitamin D for patients with type 2 diabetes and low serum vitamin D level has resulted in favorable changes in systolic and diastolic blood pressure, low density lipoproteins and high density lipoproteins [9]. A study involving 462 patients type 2 diabetes with mild renal impairment has reported an inverse and significant association of serum vitamin D level with prevalent CVD, independent of baseline renal dysfunction and other risk factors [10]. Therefore, vitamin D

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supplementation is an emerging potential approach to reduce burden of CVD in diabetic nephropathy through its favorable effects on insulin resistance and the adverse cardiovascular risk profile. Most of the previous studies on vitamin D and CVD have used conventional doses of vitamin D. We studied the effects of high dose vitamin D supplementation on cardiovascular risk factor profile in patients with early stages of diabetic nephropathy.

This was a randomized, placebo controlled clinical trial on effects of high dose vitamin D therapy on cardiovascular risk factor profile in patients with type 2 diabetes and early nephropathy. In this study, we focused on the effects of high dose vitamin D therapy on systolic and diastolic blood pressure, total cholesterol, low-density lipoproteins (LDL), triglycerides (TG) and high density lipoproteins (HDL).

2. Subjects

Patients with early stages of diabetic nephropathy (urinary albumin >30 mg/g of creatinine and GFR more than 30 mL/min) were recruited from medical clinics in Teaching Hospital, Karapitiya, Galle in Sri Lanka.

3. Materials & methods

Selected patients were informed about the research project and written consent was obtained. Those who had blood pressure >130/80 mmHg over the last two clinic visits, hyperphosphataemia (serum phosphate > 5 mg/dL), hypercalcaemia (serum total Ca > 11 mg/dL), HbA1c > 8% and patients who had liver disease, hyperthyroidism, hyperparathyroidism, or diseases related to calcium or vitamin D metabolism and decompensated congestive heart failure (current) were excluded.

Patients were randomized as blocks of two using a random number table. Concealed envelopes were given to research assistants who assigned participants to test and control groups. Patients were randomly assigned to receive either a placebo or vitamin D. Test group received monthly doses of 50,000 IU of vitamin D3 intramuscularly and the control group received an equal volume of distilled water (0.25 mL) in a similar manner. Participants, those administering the interventions, and those assessing the outcomes were blinded to the group assignment.

Blood and urine were collected for the baseline measurements which included serum creatinine, serum calcium, urine micro-albumin, fasting glucose (FBS) and lipids namely total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG), and high density lipoprotein (HDL) levels. Patients underwent a detailed medical history a physical examination including systolic and diastolic blood pressure (SBP and DBP) measurement.

A safety visit was scheduled one week after starting the trial to monitor calcium and phosphorus concentrations and to elicit any adverse events. The protocol specified withdrawal from the trial if serum calcium exceeded 11 mg/dL. Monthly injections were given for six months and at the end 3 months and 6 months all the baseline measurements were repeated.

This study was registered in the Sri Lanka Clinical Trial Registry (SLCTR) which is a Primary Registry linked to the Registry Network of the International Clinical Trials Registry Platform of the WHO (WHO-ICTRP). Registration No: SLCTR/2009/008.

To compare baseline characteristics between the treatment and control groups, Student *t*-test (unpaired) was used. Categorical variables were analyzed using the Chi-square test. Differences in

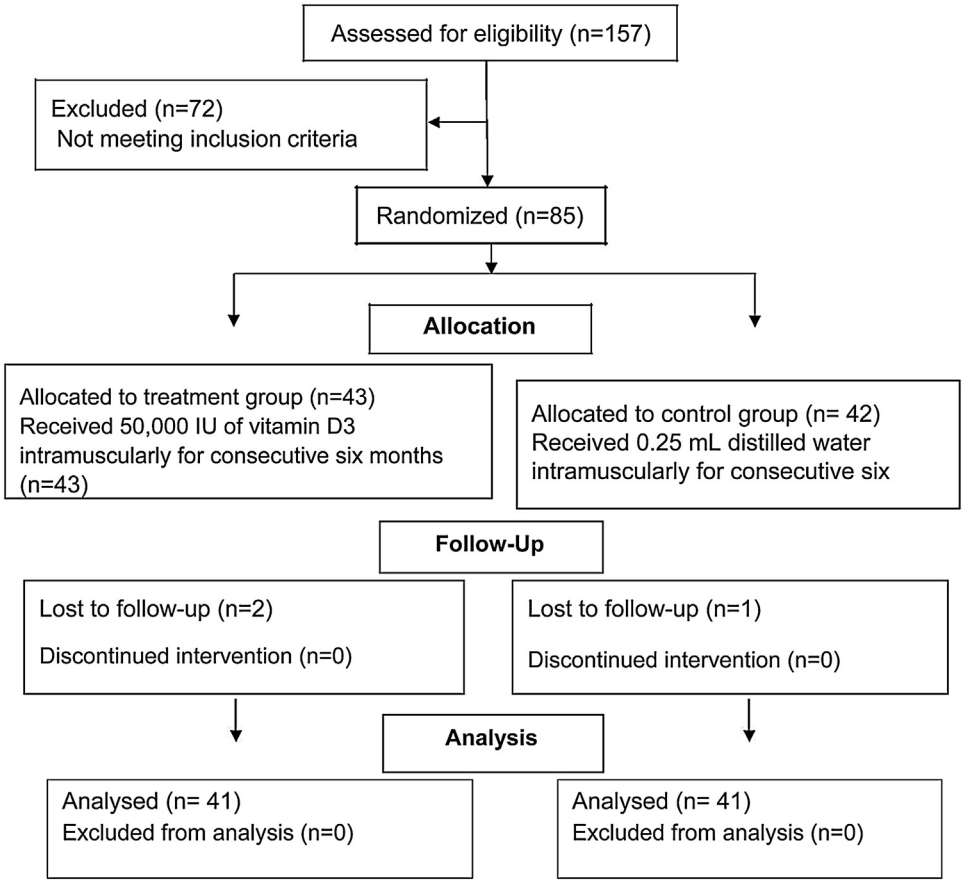

Fig. 1. Flow diagram of the recruitment of the patients for the study.

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
SBP (mmHg)	121 (7)	120 (8)	0.46
DBP (mmHg)	70 (5.9)	71 (5.9)	0.25
25(OH)D (nmol/L)	50.0 (16.5)	55. 9 (12.3)	0.07
Duration of diabetes (years)	7 (4)	8 (5)	0.42
HbA1c%	7 (0.5)	6.9 (0.5)	0.1
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

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

SBP, DBP, TG, LDL, HDL, TC before and after intervention were analyzed by the Repeated measure ANOVA (Within and between group variations). P value was adjusted for multiple comparisons by the Bonferroni method.

4. Results

A total of 157 patients were invited for the study of which 72 were excluded. Eighty five were randomly assigned in to two groups; 43 patients to the treatment group and 42 patients to the control group. Forty one patients from each group completed the study (Fig. 1) and the data were analyzed by the intention-to-treat method. Baseline characteristics of the patients are listed in Table 1. No significant differences were found among the baseline characteristics between the test and control groups. Table 2 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).

5. Discussion

In this randomized placebo controlled trial, we studied the effects of vitamin D therapy on cardiovascular risk profile among patients with early diabetic nephropathy and found a statistically significant increase in HDL cholesterol following high dose vitamin D. No significant change was seen in other CVD risk factors studied.

Few randomized controlled studies have examined the effect of vitamin D on cardiovascular events such as ischaemic heart disease [11,12]. These studies, however, have not been extended to patients with early diabetic nephropathy and some have used conventional oral forms of vitamin D preparations. Some studies have examined the effects of vitamin D on blood pressure measurements and lipid levels [13–15].

Previous studies examining the effects of vitamin D on blood pressure have shown conflicting results. A recent meta-analysis showed no effect of vitamin D or vitamin D analogues was observed in both SBP and DBP. This was observed in both trial level and individual patient data [13]. Another meta-analysis which included 11 randomized control trials reported a statistically significant but small reduction of diastolic but not systolic blood pressure following vitamin D treatment [14]. Another study which used vitamin D supplementation on patients with diabetes showed a 14 mmHg reduction in systolic blood pressure [15]. In a randomized double blind placebo controlled study, which was done to determine the effect of vitamin D supplementation on 24 h systolic blood pressure did not show any significant change. But in this study all the patients were vitamin D deficient [16].

No reduction of blood pressure was found in Women's Health Initiative Study (WHI) following 400 IU of vitamin D3 per day [17]. The WHI study followed up patients for 7 years and no difference was observed in the occurrence of cardiac events between the two groups. Above trials, however, have used a relatively small dose of vitamin D and the inconclusive results could partly be attributed to this. In addition, poor compliance and bioavailability associated with oral preparations might have influenced the outcome of these studies. Furthermore, co-prescription of calcium supplements with Vitamin D might have hindered the cardiovascular benefits of vitamin D in some of these studies including the WHI study. Calcium has recently been linked to increase cardiovascular risk although the evidence is not strong [18,19]. In our study we planned to overcome hypercalcemic effects of vitamin D therapy by estimating the serum calcium and ensuring normal levels throughout the duration of study but we could not observe any improvement of either systolic or diastolic blood pressures. This is consistent with the results of one of the recent meta-analysis [13].

Table 2
Changes in CVDR factors and risk scores in the treatment and control groups.

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

HDL cholesterol was the only CVD risk factor which improved with high dose vitamin D in our study. Effects of vitamin D on serum lipid components have not been adequately studied. In one study, vitamin D had no significant effect on serum lipids of healthy old people [20]. Similarly, vitamin D showed no effect on serum lipids among patients with diabetes although improvement of triglycerides was observed among patients with chronic renal failure after an intravenous high dose of vitamin D [21]. In a recent meta-analysis which was done to assess the effect of vitamin D on lipid profile demonstrated that vitamin D significantly reduce TC, TG and LDL but not HDL [22].

Low serum HDL cholesterol and high triglyceride are considered as markers of insulin resistance and the TG: HDL ratio is also known as plasma atherogenic index [23]. Even though our findings fail to show a statistically significant reduction in TG, the mean plasma atherogenic index was significantly reduced in the vitamin D treated group, indicating favorable effects of high dose vitamin D on CVD risk factors. As TG: HDL is an indirect indicator of insulin resistance, we can assume that favorable effects of vitamin D on CVD risk factors are mediated through insulin resistance which is increased in diabetic nephropathy.

It is possible that a longer duration of therapy than six months or a higher dose than that we used could make significant changes in other lipid components. Finding that vitamin D in high doses raises HDL level in patients with early diabetic nephropathy is important especially for patients with type 2 diabetes in the South Asian region whose HDL levels are reported to be lower when compared with patients with diabetes from Caucasian and Chinese ethnicities [24]. The observed high CVD among patients with diabetes in the South Asian region are also linked to low levels of HDL in patients with diabetes in this region [25]. Therefore, vitamin D supplementation could be a potential therapeutic option to raise HDL in patients with diabetes in the South Asian region especially, for the category with diabetic nephropathy.

Current study was conducted as a randomized double blind control study conforming to the CONSORT statement to enhance the validity of results. All parties who could influence the outcome of the study were blinded. Also there was a minimal drop-out rate and all patients randomized and received the first dose of vitamin D was included in the final analysis (Intention-to-treat analysis). Since we administered parenteral vitamin D the issues related to compliance and bioavailability did not arise.

There were a few limitations in this study. The duration of the treatment might not have been adequate to observe significant changes in some of the cardiovascular risk factors such as systolic and diastolic blood pressures. Further, patients included in this study had good glycaemic and blood pressure control (mean HbA1c of 7 and 6.95%). That might be a reason for not observing a statistically significant or clinically important reduction in blood pressures or CV risk profile. But since we administered a parenteral vitamin D the problems related to bioavailability and compliance do not apply. Further, we were able to achieve an adequate level of Vitamin D among the patients in the treatment group.

In conclusion, we report statistically significant improvement of serum HDL level and plasma atherogenic index with six months therapy of high dose vitamin D in patients with early diabetic nephropathy.

This 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.

Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this paper.

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