# Vitamin D therapy on diabetic nephropathy

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## ABSTRACT

Diabetic nephropathy (DN) 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) blockade 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 DN.

The aims of the study were to determine the prevalence and associated factors of DN among adult diabetics attending medical clinics in Teaching Hospital, Galle (THG) and to determine the effect of vitamin D therapy on DN, cardiovascular morbidity and bone mineral density (BMD). Phase 1: Cross-sectional study involving patients with diabetes attending medical clinics in the THG. Their serum creatinine and urinary albumin (UA) levels were checked. Phase 2: A double-blind, randomized, placebo controlled study to determine the therapeutic efficacy of vitamin D. Patients with DN (UA >30 mg/g of creatinine) whose estimated glomerular filtration rate (eGFR) was more than 30 mL/min were selected and their plasma renin, Parathyroid hormone (PTH), serum vitamin D, serum calcium, serum creatinine, fasting blood sugar (FBS), lipid profile, ECG and bone mineral density (BMD) were done as baseline measurements. Subjects were randomized into two groups and treatment group was given vitamin D3, 50000 IU (0.25ml) intramuscularly (IM) monthly for 6 months. The control group received same volume of distilled water IM. The investigations were repeated after 6 months of therapy. BMD was measured at 12months in a randomly selected subgroup of patients. The mean (SD) age was 61 (11) years and 75 % of them were females. Among them 66% had albuminuria (microalbuminuria-60.9%; macroalbuminuria-5.1%). The risk factors for albuminuria were poor glycaemic control and duration of the disease. Prevalence of low eGFR was 42.9% (n=174); and it was associated with age and smoking. Retinopathy and neuropathy were associated with albuminuria but not with low eGFR.

Of 155 patients invited, 85 were randomly assigned to two groups after exclusions and 82 completed the study. After six months, mean reduction of urinary albumin to creatinine ratio in the treatment and control group were 51.8 mg/g (P=0.06) and 22.4 mg/g (P<0.001), respectively (between group difference P=0.001). Significant increase in the eGFR was observed in the treatment group while eGFR remained unchanged in the control group (P=0.006 for the between-groups difference). Mean reduction in plasma renin in the treatment group and control group were 5.85 pg/mL (P < 0.001) and 0.95 pg/mL (not sigficant), respectively. After vitamin D treatment, total body BMD, total body BMC (bone mineral content) and BMDs of total spine, femoral neck and total hip regions increased by 2.0%, 2.2%, and 1.8%, 2.1% and 2.6% (P<0.05 for all), respectively in the treatment group. In the same group after 6 months of stopping treatment, marginal but a statistically significant reduction of total BMD and BMC was observed (P=0.009) while all regional BMDs remained unchanged. In the control group, none of the BMD/BMC measurements changed significantly during trial and post-trial period. No significant effect was observed in cardiovascular risk scores. In conclusion, vitamin $D_3$ has beneficial effects on patients with diabetic nephropathy.

*This study was performed at the University of Ruhuna, Sri Lanka and the results were included in a thesis with two published papers for a PhD degree with the University of Ruhuna, Sri Lanka and defended the thesis on 19th of February 2015.*

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## Introduction

Sri Lanka, being a developing country with a population of 20.7 million, is experiencing an epidemic of diabetes mellitus [1]. The burden of diabetic nephropathy should be taken into consideration both in estimating health care cost and in planning health care services in the country.

Although Sri Lanka has a high prevalence of diabetes, prevalence of nephropathy and factors that are associated with nephropathy are not well known. Methodological differences such as definition of

nephropathy and characteristics of study subjects have lead to a wide variation in estimations. Prevalence of nephropathy among patients with diabetes in Sri Lanka varies from 20% to 33% [2,3]. Most of these studies have been conducted in diabetes clinics in Colombo and defining diabetic nephropathy in some analyses is somewhat doubtful. Many studies have used single spot urine sample to detect microalbminuria and also different cut-off values such as 50 mg/L and 20 g/dL [2,3,4].

Microalbuminuria is defined as excretion of 30 - 300 mg of albumin in 24 hr urine collections, 20-200 µg/min in timed urine collection, or 30 - 300 mg/g of creatinine in spot urine collections, on two of three urine collections [50]. Previous studies conducted in Sri Lanka did not follow this standard definition in determining microalbuminuria. Hence phase 1 of this study was carried out to determine the prevalence of nephropathy among patients with diabetes according to the standard methods of defining nephropathy in patients with diabetes in Teaching Hospital Galle (THG).

Diabetic nephropathy is the commonest cause of end stage renal disease worldwide [6,7]. The current management strategies of this condition target the Renin Angiotensin Aldesteron System (RAAS) as derangements in the RAAS are suspected to play a critical role in the aetiology or progression of diabetic nephropathy [8,9]. Inhibitors of RAAS namely Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin Receptor Blockers (ARB) are included in the standard treatment for reducing proteinuria. Despite the optimum use of these agents, renal disease in some tends to progress and this has been attributed to the residual proteinuria not reversed by the above agents [10,11].

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 [12]. Studies which explored the effect of vitamin D on animal models of kidney disease have reported the beneficial effects of such therapy. Li et al. demonstrated that 1, 25 dihydroxy vitamin D3 (1, 25(OH) 2D3), the active form of vitamin D, has an inhibitory effect over RAAS as it reduces renin synthesis, the first and the rate limiting step in the RAAS [13]. In normal mice, vitamin D deficiency stimulated renin expression whereas the treatment with vitamin D reduced the synthesis of rennin [13]. Further, Zhongyi et al. reported that the combined treatment with losartan and vitamin D analogue completely eliminate the albuminuria of diabetic mice [14].

Randomized control trials examining the effect of vitamin D on the progression of proteinuria are limited [15,16]. 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.

Further, Vitamin D supplementation is an emerging potential approach to reduce burden of CVD (coronary vascular disease) in diabetic nephropathy through its favourable effects on insulin resistance and the cardiovascular risk profile. Most of the previous studies on vitamin D and CVD have used conventional doses of vitamin D and not conducted as placebo controlled trials [12,15]. Since the pleotropic effects of vitamin D are reported with higher doses, such doses should be used to determine the effects of vitamin D on cardiovascular risk factor profile in patients with early stages of diabetic nephropathy.

Diabetes which is associated with many chronic, metabolic abnormalities could adversely affect the bone strength. Both type 1 and type 2 diabetes are associated with an increased risk of fragility fractures and osteoporosis [17,18]. Vitamin D has a well-established role in bone health. No studies have examined the effect of vitamin D on BMD/BMC among patients with early diabetic nephropathy. Osteoporosis and diabetes are prevalent diseases in Sri Lanka and the number of patients with this disease combination can be expected to increase in the future [11]. Therefore, it is important to examine the effects of vitamin $D_3$ treatment on BMD in patients with diabetes with mild renal insufficiency.

Hence phase 2 of this study was done to evaluate the effect of vitamin D therapy in reversing the progression of diabetic nephropathy and also the effect on plasma renin, CVD risk profile, BMD and BMC measurements.

## Materials and methods

### *Phase 1*

Cross-sectional study included randomly selected patients with diabetes attending medical clinics at Teaching Hospital Galle. Diagnosis of microalbumin -uria or overt nephropathy was made if urinary albumin excretion was between 30 – 299 mg/g of creatinine and >300 mg/g of creatinine, respectively. We repeated the urine test in positive cases at an interval of two weeks and if the second sample was found negative a third sample was also tested. They were labelled positive only when two samples gave positive results. Ongoing urinary tract infection was excluded by urine strip method in subjects with albuminuria. Demographic data, and presence of

other macrovascular and microvascular changes were also noted in all patients. An estimated GFR (eGFR) <60 mL/min was taken as the cut-off for defining low-eGFR.

***Phase 2***

Patients with early diabetic nephropathy (urinary albumin >30mg/g of creatinine and eGFR more than 30 mL/min) were recruited from phase 1 of the study. Other causes of albuminuria were excluded before urine analysis for microalbuminuria and repeated measurements were done to confirm the findings. Those with uncontrolled blood pressure (>130/80 mmHg over the last two clinic visits), hyperphosphat -aemia (Serum phosphate > 5mg/dL), hypercalc-aemia (Serum total Ca>10 mg/dL), uncontrolled blood sugar (HbA1c>8%) chronic liver disease, hyperthyroidism, hyperparathyroidism, decompensated heart failure or diseases related to calcium or vitamin D metabolism were excluded. Other causes of proteinuria such as current urinary tract infection, urolithiasis, and renal tuberculosis were excluded by history, examination and relevant investigations.

***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 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).

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.

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 [20]. There were no software or hardware changes during the study period.

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. 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-pictrate 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 and 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 analysed by the Repeated measure ANOVA (SPSS, Chicago, USA). P value was adjusted for multiple comparisons by the Bonferroni method.

**Results**

***Phase 1***

The mean (SD) age of the total study sample (n=480) was 61 (11) years and 75 % of them (n=360) were females. Of the diabetic subjects studied, 286 (60.9%) had microalbuminuria and 24 (5.1%) had macroalbuminuria. Since the number of patients with macroalbuminuria was small we considered both micro and macroalbuminuic patients together in the rest of the analyses. Hence the prevalence of diabetic nephropathy defined according to albuminuria was 66%.

Patients with albuminuria had a longer duration of diabetes when compared with normoalbuminuric patients (P<0.05). Systolic blood pressure, age and poor glycaemic control were significantly higher among patients with albuminuria when compared with normoalbuminuric patients (P<0.05). Smoking, BMI (body mass index), DBP and gender and WHR (waist hip ratio), were not significantly different between patients with albuminuria and normoalbuminuria.

Regression analysis revealed that glycaemic control and duration of diabetes were significant associations of albuminuria in patients with diabetes. Although there were many variables associated with albuminuria, the regression model retained only poor glycaemic control and disease duration as significant associations of albuminuria.

When, nephropathy was defined based on the eGFR regardless of albuminuria, according to the regression analysis, smoking and age were the significant determinants of low eGFR.

One hundred and seventy four (42.9%) patients had low eGFR and 43 (43/174, 24.7%) of them had normoalbuminuria. In the total sample, the proportion of patients with low eGFR and normoalbuminuria was 43 (10.8 %).

When other microvascular complications were considered, retinopathy and neuropathy were associated with albuminuria but not with the low eGFR.

***Phase 2***

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; Forty-one patients from each group completed the study. Data were analysed by the intention-to-treat method.

No significant differences were found with regards to the baseline characteristics between the treatment and control groups. All patients received either an ARB or ACEI at the baseline. During the study period, oral hypoglycaemic drugs were increased in nine patients (six in the treatment group). Losartan was increased in three patients (two in the control group).

Table 1 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 ($P \leq 0.001$) 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=0.006 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 1).

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 2).

Vitamin D therapy significantly reduced DBP, total cholesterol and LDLC but the between group differences were not significant (Table 3). 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 4 shows the changes of the total body BMD/BMC, regional BMDs, total fat and lean masses, during the initial 6 months of treatment in the treatment and control groups.

After six months of vitamin D injections, in the treatment group 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 (Table 5). Increase observed in the BMD measurement in the trochanteric region was not statistically significant among the patients in the treatment group.

In the control group, total body BMC, BMD, or regional BMDs did not change significantly during the initial 6 months. Furthermore, there was no significant difference in either total fat or lean mass in any of the groups before and after treatment.

Table 6 shows the changes observed in the BMD measurements at six months after stopping treatment in subgroup of patients who had undergone the 3rd DXA scan.

After 6 months of stopping treatment, a statistically significant reduction of total BMD and BMC was observed in the treatment group (P=0.009). In the same group, changes in the regional BMDs were not statistically significant.

In the control group none of the BMD/BMC measurements changed significantly during the post-trial follow up 6 months period.

No adverse events, particularly hypercalcaemia were reported during the study period.

## Discussion

The cross-sectional study involving patients with diabetes attending medical clinics, Teaching Hospital Galle, showed the prevalence of diabetic albuminuria to be 66% (95% CI: 61.7 to 70.2). When defined by the degree of albuminuria, the prevalence of microalbuminuria was 60.9% (95% CI: 56.5 to 65.3) while macroalbuminuria prevalence was 5.1% (95% CI: 3.1 to 7.1). The risk factors for albuminuria included poor glycaemic control and the duration of the disease.

Among study subjects, the prevalence of low eGFR was 42.9% (95% CI: 38.1 to 47.7) and age and smoking were the factors related to the low eGFR. Other microvascular complications namely, retinopathy and neuropathy were associated with albuminuria but not with low eGFR.

We found different determinants or associations for albuminuria and eGFR among these adult diabetics. This discrepancy could, partly be due to the fact that these two processes are independent with own risk factors and associations. Another reason could be that the eGFR may not be an accurate reflection of renal functions of these patients since eGFR in these patients were calculated using a formula developed for European patients. Acute or chronic infections too can cause albuminuria without renal insufficiency. The research protocol required to inquire and examine all subjects for the presence of either acute or chronic ongoing urinary infections.

A sizable proportion of patients with low eGFR had normal albumin excretion (normoalbuminuric renal insufficiency). Among patients with renal insufficiency 26.7% were normoalbuminuric. The majority of patients with normoalbuminuric renal insufficiency did not have either retinopathy or neuropathy suggesting that the aetiology of renal insufficiency could be related to causes other than diabetes in these patients.

Results from the Phase 1 of the study indicate the magnitude of the growing epidemic of diabetic nephropathy in the local set up. It can be recommended that all diabetics be screened to assess their renal function by both albuminuria and by eGFR.

The randomized, double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy showed 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.

Vitamin D increased BMD/BMC compared to placebo. 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 6 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.

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 cardiovascular measurements.

Based on the results of this study, 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.

Cardiovascular benefits of high dose of vitamin D cannot be ruled out, completely, based on the data observed in this study. Since patients recruited had good blood pressure and glycaemic control, further reductions of these measurements were not possible. Further studies on patients with uncontrolled blood pressure and poor glycaemic control may reveal these benefits.

BMD/BMC increase is an important finding so that the treatment of vitamin D in early diabetic nephropathy will delay the occurrence of renal bone disease. But further clinical trials are needed to test this hypothesis in patients with diabetes.

## Conclusions

The prevalence of albuminuria was 66% (95% CI: 61.7 to 70.2) in patients with diabetes who were attending medical clinics, Teaching Hospital Galle. The risk factors for albuminuria included poor glycaemic control and the duration of the disease.

The prevalence of low eGFR was 42.9% (95% CI: 38.1 to 47.7) and age and smoking were the factors related to low eGFR. Other microvascular complication namely, retinopathy and neuropathy were associated with albuminuria but not with low eGFR.

Monthly injections of high dose vitamin D3 has improved the renal functions in patients with diabetic nephropathy, BMD and BMC. This treatment did not have a significant effect on cardiovascular risk scores or blood pressure. Further studies involving longer durations of treatment at different doses of vitamin D may be needed to reconfirm these findings.

**Table 1:** Changes observe 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) | | 1419 (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 | |
| **Serum creatinine (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 | 68.7(20.3) | 68.2 (19.3) | 68.8 (20.5) | 0.81 | 0.006 |
| | Treatment | 77.2 (20.9) | 85.8 (26.3) | 83.1 (24.4) | < 0.001 | |

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

**Table 2:** Correlations (Spearman rho) between the percentage change in vitamin D, urine

| 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 3:** Changes in CVDR factors and risk scores in the test 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)** | Test | 120 (8) | 120 (8) | 121 (7) | 0.59 | |
| **DBP** | Control | 70 (6) | 72 (6) | 72 (6) | < 0.001 | 0.17 |
| **(mmHg)** | Test | 71 (6) | 69 (6) | 68 (6) | < 0.001 | |
| **TC (mg/dL)** | Control | 194.6 (32.1) | 193.6 (30.8) | 196.9 (31.4) | 0.24 | 0.50 |
| | Test | 194.8 (30.1) | 191.5 (28.1) | 185.7 (27.2) | < 0.001 | |
| **TG (mg/dL)** | Control | 128.4 (50.8) | 127.9 (49.5) | 128.7 (45.3) | 0.62 | 0.44 |
| | Test | 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)** | Test | 119.7 (28.7) | 115.7 (27.6) | 106.10(26.5) | < 0.001 | |
| **HDL** | Control | 53.5 (10.9) | 53.7 (10.7) | 53.9 (9.7) | 0.40 | < 0.001 |
| **(mg/dL)** | Test | 50.3 (7.5) | 51.5 (7.1) | 55.7 (6.8) | < 0.001 | |

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

**Table 4:** Changes 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) | 179585 (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 | |

**Table 5**: Percentage changes in BMDs, fat mass and lean mass in different regions in the control and the treatment groups

| | BMD | BMC | Spine BMD | Femoral neck | Trochanter BMD | Hip BMD | Total fat mass | Lean mass |
|---|---|---|---|---|---|---|---|---|
| **Control** | -0.67 | -3.04 | -1.41 | -1.38 | -0.49 | -0.58 | 3.99 | -0.18 |
| **Treatment** | 2.02 | 2.16 | 1.78 | 2.05 | 1.95 | 2.62 | 4.6 | 1.85 |

**Table 6**: Changes 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)

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