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Original article

Effect of multivitamin on serum 25-hydroxy vitamin D level in postmenopausal women: A randomized, double-blind, placebo-controlled trial

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Abstract

Objectives: To determine the effects of multivitamin vitamin D 300 or 600 units on serum 25 hydroxyvitamin D (25(OH)D) level after 4 weeks of supplementation in postmenopausal women with vitamin D insufficiency.
Study design: Randomized double-blind, placebo-controlled trial.
Methods: Postmenopausal women who had vitamin D insufficiency were recruited into the study. The participants were randomized to 3 groups of 4-week treatment period with multivitamin (GPO, Governmental Pharmacy Organization) 2 tablets (contained vitamin D2 amount 600 units), multivitamin 1 tablet (contained vitamin D2 amount 300 units) or placebo. At baseline and after 4 weeks of supplementation, serum 25(OH)D were determined with electrochemilumines-cence immunoassay (Cobas, Roche Diagnostics) and level change of 25(OH)D level were compared among the groups.
Results: Out of 144 participants, 49.3% had vitamin D deficiency (<20 ng/ml) and 50.7% had vitamin D insufficiency (<30 ng/ml). However, after 4 weeks of the GPO oral multivitamin, serum 25(OH)D levels significantly increased from 19.4 ± 6.3 ng/ml at baseline to 22.2 ± 5.2 ng/ml ($p = 0.01$) and from 19.5 ± 5.0 ng/ml to 23.3 ± 5.2 ng/ml ($p < 0.01$) in the groups receiving vitamin D 300 IU and 600 IU/day, respectively. Approximately, 10% of those who took vitamin D had serum 25(OH)D level above the insufficiency level within 4 weeks. There was no significant changes of serum 25(OH)D after 4 weeks in the placebo group.
Conclusions: Daily supplementation of the generic multivitamin containing vitamin D2 300 and 600 IU daily for 4 weeks significantly increased mean serum 25(OH)D from baseline up above the deficiency level.

Keywords: Postmenopause; Multivitamin; Vitamin D insufficiency; Serum total 25 hydroxyvitamin D

1. Introduction

Vitamin D deficiency is defined as a 25-hydroxyvitamin D (25(OH)D) level < 20 ng/ml (50 nmol/L) and vitamin D insufficiency as a 25(OH)D < 30 ng/ml (75 nmol/L) [1]. Vitamin D deficiency is associated with low bone mass and osteoporotic fractures [2]. Nonetheless, vitamin D insufficiency is associated with rising PTH when the level is below 30 ng/ml [1,3]. Vitamin D insufficiency is believed to be common in postmenopausal women [4]. A survey in Thailand, using the cutoff value of 25(OH)D ≤ 35 ng/ml, found that 77.81% of Thai premenopausal women were considered to have vitamin D insufficiency [5].

In 2011, the Endocrine Society Task Force's published guideline for evaluation, treatment, and prevention of vitamin

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D deficiency suggested that all adults age 50–70 and 70 + years require at least 600 and 800 IU/day of inactive vitamin D, respectively to maximize bone health. However, to raise the blood level of 25(OH)D above 30 ng/ml may require at least 1500–2000 IU/day [1].

Although it is still unclear whether we need such high doses of vitamin D, a meta-analysis revealed that vitamin D supplement at the dose of 700–800 IU/day was associated with reduction of hip and non-vertebral fractures in older persons [6].

With the dilemma of which to treat between "vitamin D deficiency" and "vitamin D insufficiency" which may have great impact on health expenditure, we are interested to study the changes of 25(OH)D level after ingestion of a daily dose of either 300 or 600 IU of an inactive vitamin D containing in a generic oral multivitamin (Governmental Pharmacy Organization, GPO) for 4 weeks. The GPO multivitamin is cheap and locally produced within the country. Oral multivitamin has been recommended and prescribed arbitrarily in clinical practice as a supplementation to prevent vitamin D deficiency and insufficiency which were claimed to have high prevalence among Thai postmenopausal women. To address this issue, we conducted a randomized, double-blind, placebo-controlled trial to assess serum levels of 25(OH)D in 3 groups of participants who were blinded to receive a daily dose of placebo or multivitamin containing 300 IU or 600 IU of vitamin D.

2. Material and methods

This study was a randomized, double-blind, placebo-controlled trial conducted from August 2012 to January 2013 at the Menopause Clinic, King Chulalongkorn Memorial Hospital. The study has been approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn Univesity and was conducted in accordance with the Declaration of Helsinki of Good Clinical Practice. The trial is registered at Thai Clinical Trials Registration (TCTR20131206001). All women provided their written, informed consent before recruitment and had the right to withdraw from the study at any time. Participants could also be withdrawn from the study at the discretion of the investigators at any time.

Participants who were included into the study were Thai postmenopausal women, aged between 50 and 80 years, ambulatory, community-dwellers, body mass index between 18.50–25.00 kg/m^2, having serum 25(OH)D < 30 ng/ml and were willing to participate in the study. Participants were excluded if they were women who used vitamin D-contained medicine within 12 weeks, having a history of malignancy, liver disease, renal disease, hyperparathyroidism disorder, malabsorption, bowel surgery or having abnormal liver or renal function test.

In this study, we define postmenopausal women as those ≥50 years who had permanent cessation of menstruation for at least one year or women who underwent bilateral oophorectomy. Vitamin D insufficiency is defined as a serum 25(OH)D < 30 ng/ml (75 nmol/L) [1]; Vitamin D deficiency: defined as a serum 25(OH)D < 20 ng/ml (50 nmol/L) [1].

Oral multivitamin (GPO®)contained of Vitamin D2 of 300 IU, nicotinamide 7.5 mg, vitamin B2 0.5 mg, vitamin B1 1 mg, vitamin A 2500 IU and vitamin C 15 mg. Independent pharmacist dispensed either pre-packed oral multivitamin 1 tablet (Vitamin D2 300) in one opaque capsule or oral multivitamin (GPO®) 2 tablets in one opaque capsule or placebo used of folic acid (5 mg) (GPO®) 1 tablet in one opaque capsule according to the block of three randomization list.

The multivitamin one tablet, two tablets and placebo were in packages that were identical in appearance. They were packed in one opaque capsule in opaque envelops and consecutively numbered for each women according to the randomization schedule. Each woman was assigned an order number and received the capsules in the corresponding pre-packed envelop.

The serum 25(OH)D, serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase level were assessed at baseline. Serum 25(OH)D was measured by electrochemiluminescence immunoassay (Cobas, Roche Diagnostics Thailand). The inter-assay and intra-assay coefficients of variation (CV) of 25(OH)D were 3.52% and 1.53%, respectively. All analytical platforms and immunoassays were used in the laboratory for routine testing and were strictly run in accordance with the manufacturers' guidelines and according to the laboratories' standard operating procedures (SOP) for good laboratory practice.

During the "screening and baseline visit (first visit)", all participants were interviewed and underwent general physical examination. Baseline characteristics were recorded in the case record form. Blood was drawn between 8:00 and 9:30 a.m. after the subjects had fasted for at least 8 h. These were either analyzed immediately or stored at −20 °C until analyzed. Women with baseline serum 25(OH)D level < 30 ng/ml were enrolled into the study. All participants were explained to take a drug for one capsule at 8.00 p.m. for 28 days. The administration of drug was recorded by patients' self-report on daily record form.

On the follow-up visit (week 2), all participants were interviewed by phone after 2 weeks of drug administration to assess the treatment compliance and adverse effects. On the 3rd visit (week 4), serum 25(OH)D was measured after 28 days of supplement. Blood were collected within 3 days after the last pill intake. Blood were collected and sent to the central laboratory of King Chulalongkorn Memorial Hospital for the preparation of serum. The serum samples were either analyzed immediately or stored at −20 °C until analyzed for 25(OH)D level.

Participants' baseline characteristic data such as age, body mass index (BMI), year since menopause, sun-exposure time, the use of sun screen and baseline serum 25(OH)D level and all relevant laboratory data such as serum 25(OH)D, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), creatinine were obtained. Post-supplement serum 25(OH)D level and change of serum 25(OH)D level were the primary outcome measurement.

Sample size was estimated based on a pilot study. A level of statistical significance of 0.05% and power of study of 90%, we

obtained a sample size of 48 subjects per group which yielded a total sample size of 144 subjects. Descriptive statistics [mean standard deviation or median (range) and percentage] were used to express demographic, baseline and measurement outcome data. Changes of serum 25(OH)D from baseline in each groups were analyzed for statistical significance by paired t-test. Comparisons of measurement outcomes between groups after intervention were analyzed for statistical significance by ANCOVA if the data was normally distributed or Mann–Whitney U test if the data was not normally distributed. A p level of <.05 was considered statistically significant.

3. Results

Out of 202 participants who were screened with the inclusion and exclusion criteria, 144 women were eligible to be included into the intention-to-treat analysis. The participants' mean age was 59.8 ± 6.8 years, year since menopause was 11.6 ± 8.6 years and body mass index was 22.4 ± 1.8 kg/m^2. The estimated mean daily sun-exposure time was 1.1 ± 0.9 h. As a group, baseline characteristics showed no statistical differences of age, BMI, year since menopause and sun-exposure time (Table 1).

At baseline, 49.3% ($n = 71$) of all participants ($n = 144$) had vitamin D deficiency (<20 ng/ml), and 50.7% ($n = 73$) had vitamin D insufficiency (<30 ng/ml). However, after 4 weeks of the GPO oral multivitamin, serum 25(OH)D levels significantly increased from 19.4 ± 6.3 ng/ml at baseline to 22.2 ± 5.2 ng/ml and from 19.5 ± 5.0 ng/ml to 23.3 ± 5.2 ng/ml only in the groups receiving vitamin D 300 IU and 600 IU/day, respectively (Table 2). While there was no significant changes of serum 25(OH)D after 4-week period in the placebo group.

When looked into the percentages of postmenopausal women who had vitamin D deficiency, there was a tendency toward lower percentages of vitamin D deficiency in the groups taking the GPO multivitamin containing vitamin D 300 and 600 IU when compared to the placebo (Table 3). The mean differences between those who received 300 IU or 600 IU of vitamin D and placebo (using ANCOVA) were statistical significant but not between the two vitamin D groups (Table 4).

4. Discussion

Long duration of estrogen deficiency after menopause may result in osteopenia and osteoporosis [14]. Vitamin D deficiency aggravates osteoporosis and causes osteomalacia with diffuse muscle pain and muscle weakness [2]. Vitamin D deficiency has traditionally been defined as a 25(OH)D level below 20 ng/ml [1]. However, higher 25(OH)D levels are probably necessary for optimal musculoskeletal function and normal serum parathyroid hormone level. PTH levels begin to rise when 25(OH)D levels drop below 30 ng/ml [15]. A 25(OH)D level below 30 ng/ml is therefore defined as Vitamin D insufficiency which leads to reduced calcium absorption, elevated serum parathyroid hormone, as a result, increases the rates of bone resorption and proximal muscle weakness that compromise gait and balance which may lead to fall and consequent fracture [15].

The present study reveals that 49.3% of the studied participants had vitamin D deficiency and 50.7% had vitamin D insufficiency. The GPO multivitamin supplementation contained vitamin D2 300–600 IU used in this 4-week study was shown to raise the mean 25(OH)D from baseline up above the deficiency level (20 ng/ml). Only 10.4% of women received vitamin D2 of 300 IU and those received vitamin D2 of 600 IU had 25(OH)D above the insufficiency level within 4 weeks. Nevertheless, this depends upon the baseline 25(OH)D level and the severity of vitamin D deficiency and insufficiency.

The increases of 25(OH)D in our study seems to be less than those from the aforementioned studies [7–12]. This is

Table 1
Participants' characteristics.

	Placebo	300 IU	600 IU
N	48	48	48
Age (y)	59.6 ± 6.4	60.5 ± 7.7	59.5 ± 6.1
Body mass index (kg/m^2)	22.2 ± 1.7	22.7 ± 1.6	22.3 ± 2.1
Year since menopause	11.5 ± 7.4	12.6 ± 9.9	10.5 ± 8.4
Sun-exposure time (h)	1.1 ± 0.9	1.1 ± 1.0	1.0 ± 0.9
Sunscreen % (N)	68.8 (33) use	68.8 (33) use	58.3 (28) use

Data are presented as mean ± SD or percentage.

Table 2
Serum 25(OH)D (ng/ml) before and after 4 weeks of the supplements within group comparison.

Serum 25(OH)D (ng/ml)	Baseline	After 4 weeks	Mean change	p
Placebo	20.5 ± 5.6	21.0 ± 6.0	0.5	0.40
Vitamin D 300 IU	19.4 ± 6.3	22.2 ± 5.2	2.8	0.01
Vitamin D 600 IU	19.5 ± 5.0	23.3 ± 5.2	3.9	<0.01

Paired T-Test: compared pretreatment and post-treatment intra-group.

Table 3
Vitamin D status before and after 4 weeks of the supplements ($N = 144$).

Vitamin D status in each group	Placebo $N = 48$	300 IU $N = 48$	600 IU $N = 48$
Vitamin D deficiency,			
- Baseline (%), (n)	47.9 (23)	47.9 (23)	52.0 (25)
- After 4 weeks (%), (n)	41.6 (20)	25.0 (12)	22.9 (11)
Vitamin D insufficiency			
- Baseline (%), (n)	52.0 (25)	52.0 (25)	47.9 (23)
- After 4 weeks (%), (n)	54.1 (26)	64.5 (31)	66.6 (32)
Vitamin D sufficient			
- Baseline (%), (n)	0	0	0
- After 4 weeks (%), (n)	4.2 (2)	10.4 (5)	10.4 (5)

Table 4
Change of serum 25(OH)D levels between groups in relation to dosage of vitamin D supplementation after 4 weeks.

Comparison between vitamin D groups	Mean difference	Std. Error	p
Vitamin D 600 IU – Placebo	3.270	1.046	0.002
Vitamin D 300 IU – Placebo	2.229	1.046	0.035
Vitamin D 600 IU – Vitamin D 300 IU	1.041	1.046	0.321

probably because our study used lower doses of inactive vitamin D. The shorter follow-up duration of which vitamin D, as one of the fat soluble vitamins, is accumulative with time may explain a lower mean level at 4 weeks. Recently, a meta-analysis reveals that vitamin D3 is more efficacious at raising serum 25(OH)D concentrations than is vitamin D2 [16].

In this study we did not evaluate serum calcium level to monitor hypercalcemia or investigating for renal calculi due to the type, dose and duration of vitamin D used in this study was inactive, low doses and short duration. With the very wide therapeutic range of inactive vitamin D, we did not expect to see symptoms of hypercalcemia or occurrence of renal calculi in this short-term study. This may be in accordance with previous studies which showed no case of hypercalcemia or renal stones [17] of which one of the studies gave a weekly 50,000 IU of vitamin D2 to participants for 8 weeks.

As seasons in Bangkok are not much different from one to the other, the daytime and sunlight exposure may be less distinct in different seasons of the year when compared with other different latitudes. Moreover, our subjects were recruited in the same studied period during September to December 2012. Thus, it may have less significant effects from varying sunlight exposure. However, we could not eliminate other confounders such as different intakes of vitamin D from varieties of food source, different percentages of sunscreen used or the way our participants exposed to sunlight.

Although the study was carefully designed as randomized, double-blind, placebo-controlled trial in order to minimize bias that might incur from treatment awareness, practically it might not be able to absolutely eliminate the clinical difference between the placebo and treatment. Since the oral multivitamins used in this study contained various types and doses of vitamin B which might have typical smell that participants could detect the distinction from placebo. This may compromise the blinding of the study. Whether the small but significant increase of 25(OH)D levels among the treatment groups over the placebo has any clinically implication is needed to be elucidated. With respect to the timing of blood collection after 4 weeks, the period of 1−3 days from the last pill will have effect over 25(OH)D levels, and is still needed to be clarified.

5. Conclusion

Daily supplementation of the generic multivitamin containing vitamin D2 of 300 and 600 IU daily for 4 weeks significantly increased mean serum 25(OH)D from baseline up above the deficiency level. However, only 10.4% of postmenopausal women taking the oral multivitamin D2 containing vitamin D2 (300 IU or 600 IU daily) were able to achieve vitamin D levels considered to be sufficient (>30 ng/ml).

Conflicts of interest

The authors declare that there was no conflict of interest. The Roched Diagnostic Thailand who supplied the 25(OH)D assay solution did not involve or participate in the study analysis and manuscript development.

What is already known on this topic

Previous policy in the Menopause Clinic, King Chulalongkorn Memorial Hospital was to provide daily supplementation of the generic multivitamin containing vitamin D2 of 300 or 600 IU to postmenopausal women for prevent vitamin D insufficiency and osteoporosis.

In 2011, the Endocrine Society Task Force's published guideline for evaluation, treatment, and prevention of vitamin D deficiency suggested that all adults age 50−70 and 70 + years require at least 600 and 800 IU/day of inactive vitamin D, respectively to maximize bone health. However, to raise the blood level of 25(OH)D above 30 ng/ml (vitamin D insufficiency) may require at least 1500−2000 IU/day [1].

What this study adds

Daily supplementation of the generic multivitamin containing vitamin D2 of 300 or 600 IU seem less effective to prevent vitamin D insufficiency and osteoporosis in postmenopausal women.

The findings support the increasing of daily supplementation vitamin D dosage should necessary for prevent vitamin D insufficiency and osteoporosis recommended by the guideline.

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