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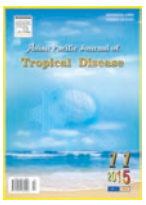

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Vitamin D plus calcium supplementation increased serum 25(OH)D on reproductive age women workers

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ABSTRACT

Objective: To analyze the efficacy of calcium supplementation plus vitamin D on the improved concentrations of serum 25(OH)D and the blood pressure in working women of childbearing age.

Methods: The design used in this research was an experimental study (randomized control trial), with 39 subjects of women at childbearing age who met the inclusion criteria for the study. Subjects were randomly allocated into two treatment groups, the VDC group (400 IU of vitamin D plus 500 mg of calcium) and the VD group (400 IU of vitamin D). Supplements were consumed every day for 12 weeks.

Results: Prior to supplementation, the average level of serum 25(OH)D in the VDC group was (16.7 ± 4.5) ng/dL which was higher than the average level of serum 25(OH)D in the VD group which was (14.9 ± 5.1) ng/dL. After supplementation, the subjects of VDC group showed an average increased 3.6 ng/dL of serum 25(OH)D. The average increase of serum 25(OH)D in VD group was 6.3 ng/dL. The increase of serum 25(OH)D in VDC group was 21.6%, while in the VD group the increase was almost two times higher (42.3%) than that of the VDC group. Statistical test results showed that the average levels of serum 25(OH)D between the two treatment groups were significantly different.

Conclusions: The average systolic blood pressure prior to supplementation of the VDC group was (128.5 ± 22.5) mmHg which was slightly lower than that of the VD group [(131.1 ± 18.0) mmHg].

1. Introduction

Women workers are part of reproductive age women who need attentions because they are vulnerable to problems caused by nutrition issue due to the physiological roles for undergoing pregnancy, childbirth and menstruation. Groups of workers who are rarely being studied, particularly related to vitamin D status, are women workers, especially those who work in the garment industry in which they are rarely exposed to sunlight. This is related to morning to evening working hours and working in closed place, thus they are at risk of vitamin D deficiency due to lack of sunlight[1]. Factors that cause vitamin D deficiency in women include lifestyle. Those women tend to avoid the sun, use sunblock, ingest foods with little vitamin D and work indoors for a long period of time. This vitamin D deficiency can be overcome by increasing the synthesis of vitamin D through sun exposure, food fortification or vitamin D supplementation[2].

Individuals at risk of vitamin D deficiency are characterized by low serum 25(OH)D, limited sun exposure, dark skin which is protected from the sun by the glass, use of covered clothing, use of sunscreen lotions and/or low intake of vitamin D in the diet. One of vitamin D deficiency prevention efforts at an age of 19–50 years can be conducted by taking vitamin D supplements to prevent bone diseases and muscle function loss. However, in order to increase serum 25(OH)D to over 30 ng/mL, it is recommended to take 1 500–2 000 IU/day of vitamin D supplements[3].

The roles of calcium and vitamin D are essential regulating factors in biological systems. Calcium and vitamin D deficiency in the pathogenesis of osteoporosis is generally understood. In addition, other reports suggest that calcium intake may have effects in a variety of unclear diseases, such as arterial hypertension. Study conducted by Major on 63 women aged 38–48 years who were supplemented with 200 IU of vitamin D and 600 mg of calcium for 15 weeks successfully reduced the ratio of low-density lipoprotein cholesterol: high-density lipoprotein cholesterol ($P < 0.01$) and low-density lipoprotein cholesterol ($P < 0.05$), but did not improve the hypertension condition[4].

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Study conducted on women aged 16–50 years in various countries showed that the average intake of calcium is still low, such as in the USA (626 mg/day), Bangladesh (180 mg/day) and Malaysia (386 mg/day). Intake of calcium in Indonesia is about 250 mg/day[5]. These numbers are still far below the recommended dietary allowance in each country. Calcium supplementation in this study also aimed to increase the intake of calcium on women of childbearing age (WCA).

Based on our previous study, it is shown that the mean serum 25(OH)D on women of 30–44 years was 15.7 ng/mL. On the other hand, Indonesia is a country rich of sunlight throughout the year[6]. One of the efforts that was conducted in this study to overcome vitamin D deficiency was supplementation which aimed at improving the status of serum 25(OH)D on WCA garment workers. Moreover, vitamin D supplementation was expected to lower blood pressure. Different treatments were conducted on the type of intervention: vitamin D supplementation plus calcium (VDC) and vitamin D supplement (VD). The administered dose in the VD group was 400 IU of vitamin D, but in the VDC group vitamin D was added with 500 mg of calcium. This study examined the efficacy of VDC supplementation to improve serum 25(OH)D on WCA worker group and the impact of the improvement on blood pressure. The efficacy was tested by comparing the VDC treatment with VD treatment.

2. Materials and methods

The research design in this study was double-blind randomized controlled trial that has been approved by the Health Research Ethics Commission, Agency for Health Research and Development No. LB.02.01/5.2/KE.093/2013. The research subjects were women workers aged 30–45 years who met the inclusion criteria and were randomly selected. The inclusion criteria required that those subjects were healthy, married, not pregnant and breast-feeding, not a smoker, not an alcohol drinker, not on a diet and willing to sign ethical informed consent form. Whereas the exclusion criteria included women who were suffering from infection-related diseases and unmarried. A minimal amount of research subjects in this study was 13 subjects for each treatment. In anticipation of dropping out, the number of research subjects in each treatment group was prepared to be 21 people. Therefore, the required sample size was 42 people. The research subjects were randomly divided into two treatments in which each treatment consisted of 21 subjects. After the initial blood test, results (baseline) were investigated, then the subject placement for both treatments was randomly conducted to determine the subjects who received VDC and subjects who received VD only.

VDC formulation consisted of 400 IU of vitamin D and 500 mg of calcium, while VD formulation consisted of 400 IU of vitamin D. Each week, both capsule formulae (7 capsules) were transferred into sealed small plastic bags. On each plastic bag, respondents' names and the type of formula that respondents received were randomized at the beginning of the treatment. Each small plastic bag was delivered to the distribution crew, namely, 2 labor union staffs. Type of supplement and differences in the composition contained in capsules provided to each woman worker were not known to researchers and distribution crew. Every Monday morning, a research assistant would recollect the small plastic bags containing capsule strip tears and replace them with small plastic bags containing 7 capsules to be taken in the next week, which then were handed over to the labor union staffs. Supplements were taken daily for 12 weeks.

To improve subject compliance in taking supplements, every morning before going to work at 07:00, subjects took the supplement capsules by using drinking water in front of the labor union staffs and capsule strip tears were recollected into the plastic bags. On Sunday/holiday or when subjects were fasting, the labor union staffs asked the subjects to take the capsules home and bring back capsule tears on the following day. To maintain their compliances in consuming capsules, several efforts were conducted including socialization at the beginning of the study, explanation during the baseline data collection, alerting the subjects to take the capsules by short message service, particularly on Sunday/holiday. During supplementation intervention, subjects were recommended not to take any supplement except medications prescribed by physician. Data collected during the study were subject characteristics, medical history, anthropometric measurements, serum vitamin D [25(OH)D], serum calcium and blood pressure measurement. The identity of the subjects collected were name, date of birth, marital status, education, ethnicity, habit of using cosmetic/sunscreen and sports activities. The identity of the subjects was collected once before the supplementation started.

Anthropometric data included weight and height. Before the anthropometric measurements, subjects were asked to remove wallets and cell phones from their pockets and take off footwears. Microtoise tool was used to measure height with accuracy of 0.1 cm, while "Takana" body scale was used to measure weight. Anthropometric data were collected twice before and after 12-week supplementations. Blood pressure measurement was also conducted twice before and after 12-week supplementations by a physician.

Blood sampling at the beginning and end of the treatment was conducted simultaneously in the morning. Subjects were asked not to eat and drink from 21:00 before blood sampling conducted in the morning. Analysis of serum calcium was conducted in the Health Laboratory, Bogor, while the analysis of serum 25(OH)D was conducted in the Hormones Laboratory, Rehabilitation and Reproduction Unit, Department of Clinical Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University for serum 25(OH)D examination using the 25(OH)D EIA 5396 kit.

Statistical analysis was conducted to determine differences in diversity of overall variables between the treatment groups (baseline and endline). Different test of independent sample was used to compare differences in parametric variables before treatment. Paired sample test was used to compare parametric variables significance before and after supplementations. Supplementation efficacy test was conducted by *t*-test based on the different value of serum 25(OH)D before and after treatment, systolic and diastolic blood pressure (SBP and DBP) and serum calcium in both treatments and between treatments. Normality test on biomarker data was priorly conducted by the Kolmogorov-Smirnov test, while the variant homogeneity test was conducted by using the Lavene. The test criteria was $P > 0.05$ for accepting the hypothesis that the data were normally distributed.

3. Results

The demographic characteristics of the participants at baseline were shown in Table 1. Based on the inclusion criteria, 42 research subjects were selected. Each treatment group had 21 subjects, but one of them was pregnant on VDC group while on VD group there were 2 subjects who could not complete the intervention due to resigning from the garment factory. The average age of subjects before treatment on VDC group was (37.7 ± 4.3) years, while the age of subjects on VD group was (38.8 ± 4.1) years. Results of independent *t*-test showed that subjects' ages between treatment

groups were not significantly different ($P > 0.05$). Most subjects on VD group (45.0%) were 40–44 years, while on VD group they were mostly at the age of 35–39 years.

Table 1
Baseline characteristics of subjects. Mean ± SD.

Characteristics	VDC ($n = 20$)	VD ($n = 19$)	P value
Age (year)	37.7 ± 4.3	38.8 ± 4.1	0.631
BMI (kg/m^2)	27.7 ± 6.5	27.8 ± 3.4	0.700
SBP (mmHg)	128.5 ± 22.5	131.1 ± 18.8	0.703
DBP (mmHg)	82.5 ± 9.7	86.8 ± 13.8	0.680
Serum 25(OH)D (ng/dL)	16.7 ± 4.5	14.9 ± 5.1	0.263
Calcium serum (mg/dL)	10.2 ± 0.5	10.3 ± 0.7	0.140

BMI: Body mass index.

Assessment of nutritional status was determined by BMI. Before supplementation, the average BMI of subjects in VDC group was 27.7 ± 6.5, while the average BMI of subjects in VD group was 27.8 ± 3.4. Independent t-test results showed that the average BMI was not significantly different between groups ($P > 0.05$). The results also showed that more than half of VDC group subjects (55.0%) had unusual BMI, in which 15.0% was overweight and 40.0% was obese. More than two-thirds of VD group subjects (78.9%) had unusual BMI, in which 10.5% was overweight and 68.4% was obese. A woman's risk of obesity will be increasing, although at the age of 70–80 years the risk will decrease again[7]. It was found that none of the subjects were classified as underweight based on BMI. In this study, the highest percentage was found in the obese person (40.0%) of VDC group and 68.4% in VD group.

Table 2 shows serum 25(OH)D levels before and after the intervention. Before supplementation, the average levels of serum 25(OH)D in VDC group was 16.7 ng/dL with the highest subject's serum at 24.9 ng/dL and the lowest at 8.7 ng/dL. The average levels of serum 25(OH)D in VD group was 14.9 ng/dL with the highest subject's serum at 22.20 ng/dL and the lowest at 3.5 ng/dL. When the average levels of serum 25(OH)D in both groups were being compared, the difference was not significant ($P > 0.05$).

Table 2
The average levels of serum 25(OH)D and calcium serum before and after intervention.

Group	Serum 25(OH)D (ng/dL)		Calcium serum (mg/dL)	
	VDC	VD	VDC	VD
Before	16.7 ± 4.5	14.9 ± 5.1	10.2 ± 0.5	10.3 ± 0.7
After	20.3 ± 6.1	21.2 ± 5.0	10.3 ± 0.3	10.2 ± 0.2
P value	0.03	0.00	0.66	0.37
Delta	3.6 ± 7.0	6.3 ± 3.4	0.1 ± 0.6	-0.1 ± 0.6
P value	0.018		0.851	

After supplementation, there was an average increase of serum 25(OH)D level (3.6 ng/dL) in VDC group, in which the highest serum was 36.50 ng/dL, and the lowest was 12.60 ng/dL. An average increase of serum 25(OH)D in VD group was 6.3 ng/dL, in which the highest serum was 31.90 ng/dL and the lowest was 12.80 ng/dL. It appeared that after 12 weeks of supplementation the serum 25(OH)D in both groups increased. The increase of serum 25(OH)D in VDC group was 21.6%, while in the VD group the increase was almost two times higher (42.3%) than that of the VDC group. Statistical analysis showed that the average levels of serum 25(OH)D were significantly different between the two treatment groups.

The average SBP before supplementation in VDC group was (128.5 ± 22.5) mmHg. It was slightly lower than that in VD group [(131.1 ± 18.8) mmHg]. After supplementation, both treatment groups showed a small decrease in SBP. Paired t-test which was conducted on the levels of SBP before and after supplementations showed no significant differences ($P > 0.05$) in both VDC and VD groups. Statistical analysis showed that the average SBP between two groups did not differ significantly ($P = 0.87$).

The average DBP before supplementation in VDC group was (82.5 ± 9.7) mmHg, which was slightly lower than that in VD group [(86.8 ± 13.8) mmHg]. After supplementation, the average DBP in VDC group increased by 1.5 mmHg to (84.0 ± 14.3) mmHg, whereas in VD group decreased by 2.1 mmHg to (84.7 ± 10.7) mmHg. Paired t-test on the levels of DBP before and after supplementations showed no significant differences ($P > 0.05$) between VDC and VD groups. When the average DBPs in both groups were compared, they were not significantly different ($P > 0.05$) (Table 3).

Table 3
The averange of blood pressure before and after intervention. mmHg.

Group	SBP		DBP	
	VDC	VD	VDC	VD
Before	128.5 ± 22.5	131.1 ± 18.8	82.5 ± 9.7	86.8 ± 13.8
After	127.0 ± 23.0	130.5 ± 20.7	84.0 ± 14.3	84.7 ± 10.7
P value	0.65	0.91	0.59	0.54
Delta	-1.5	-0.5	1.5	-2.1
P value	0.87		0.45	

4. Discussion

4.1. Efficacy of supplementation on serum 25(OH)D

The present study aimed to clarify the effects of vitamin D plus calcium supplementation on the increase of serum 25(OH)D in reproductive age women workers. After vitamin D supplementation, the VDC group showed an average increase of serum 25(OH)D level at 3.6 ng/dL and the VD group at 6.3 ng/dL. The VDC group demonstrated an increase in serum 25(OH)D by 21.6%, while in the VD group there were almost two times higher increase in serum 25(OH)D at 42.3% as compared to the VDC group.

In our study, capsules were given once a week (7 capsules), thus for 12 weeks of supplementation subjects received 84 capsules. VDC participants were 20 people while VD participants were 19 people. Three participants were dropped out due to pregnancy and resigning from the company. Capsules that were consumed by subjects ranged between 64–77 capsules. It indicated that subjects had consumed the capsules for at least 9 weeks. The average numbers of capsules consumed in the two treatment groups were not significantly different ($P = 0.848$). The average number of capsules consumed by VDC group was 75 capsules, while in VD group that was 76 capsules.

During the 12-week supplementation, the VDC group showed an average increase of serum 25(OH)D level (3.6 ng/dL) with the highest subject's serum at 36.50 ng/dL and the lowest at 12.60 ng/dL. An average increase of serum 25(OH)D in VD group was 6.3 ng/dL with the highest subject's serum at 31.90 ng/dL and the lowest at 12.80 ng/dL. It showed that after 12 weeks of supplementation, serum 25(OH)D in both groups was increased. The VDC group demonstrated an increase in serum 25(OH)D by 21.6%, while in the VD group there were almost two times higher increase in serum 25(OH)D at 42.3%.

However, the average intake of vitamin D from food source was very low, which was only around 4%–6% of the recommended dietary allowance. The infrequent eating of mushrooms, orange juice, milk, cheese and cereal led to low consumption of vitamin D. Foods that were often consumed by a minority of subjects as vitamin D sources were eggs, energen, sardines, milk and yogurt. Sources of vitamin D in their diet were only around 10%. The food frequency questionnaire results showed that the habit of taking

supplements was not often carried out by the subjects before this study was conducted.

Previous studies reported the number of vitamin D deficiency in the group of women aged 60–75 years was 35.1%[8]. While the study conducted by Green in a group of women aged 18–40 years concluded that vitamin D deficiency was 63.0% which was lower than the rate of vitamin D deficiency obtained from this study (82.0%)[9]. These findings indicate that the latitude coordinate of a country does not guarantee the status of vitamin D. As a tropical country, Indonesia which is exposed to the sun throughout the year does not ensure vitamin D fulfillment to its people.

Oral consumption of vitamin D is not directly associated with elevated levels of 1.25-vitamin D because it is highly dependent on the physiological state of enzyme that acts in activating 25-hydroxyvitamin D3 1-α-hydroxylase. In addition, macrophages in atherosclerotic lesions which are associated with vascular calcification can express 1-α-hydroxylase activities and produce 1.25-vitamin D. Several macrophages may share the osteoclastic capacity to eliminate phagocytic activity of calcium from arterial walls and this resorption will provide a source of serum calcium but has the potential to reduce the activation of vitamin D[10].

4.2. Efficacy of supplementation on blood pressure

Subjects with hypertension based on SBD were categorized as high at 35.0% in VDC group and 31.6% in VD group. The average and standard deviation of SBD in VDC group was (128.5 ± 22.5) mmHg, while in VD group was (131.1 ± 18.8) mmHg. Statistical analysis showed no significant difference in SBD before treatment between groups ($P > 0.05$). Subjects who suffered from hypertension based on DBP at 47.3% in VD group were higher than that in VDC group (35.0%). The average and standard deviation of DBP in VDC group was (82.5 ± 9.7) mmHg, while in VD group was (86.8 ± 13.8) mmHg. Statistical analysis showed no difference in DBP before treatment between groups ($P > 0.05$) (Table 1).

This study showed that calcium intake both in VDC group or VD group was still below the recommended dietary allowance (800 mg/day). The average calcium intake was 21.0%–25.0%. The cause of low calcium intake is due to the very low calcium intake from animal sources. The routines of consuming milk in both intervention groups were very low. Milk often consumed was sweetened condensed milk (25% in VDC group and 21.1% in VD group), followed by ultra heat treated milk (10.0% in VDC group). None of the subjects in both intervention groups consumed full cream milk powder, skim milk or yogurt in the past month. While the plant-derived calcium source was obtained from “tempe” (more than 60% of subjects in both groups) and tahu/tofu (70% of subjects in VDC group and 42.1% of subjects in VD group). Meanwhile, legumes consumption was less than 20% of total subjects (15% in VDC group and only 5.3% in VD group).

Burgaz in the meta-analysis conducted 18 studies consisting of 4 prospective studies and 14 cross-sectional studies published until 2010 which stated that serum 25(OH)D was inversely proportional to the hypertension incidence[11]. In his study, Li detected the effect of vitamin D injection in mice as antihypertensive agent to control the production of renin and blood pressure. In this study, mice deficient in vitamin D showed an increase in renin and angiotensin II productions, which caused hypertension, cardiac hypertrophy and increased water intake. However, vitamin D-injected mice were able to reduce the synthesis of renin by the transcription of a gene that suppressed renin[12].

The average DBP before supplementation in VDC group was (82.5 ± 9.7) mmHg, which was slightly lower than that in VD group [(86.8 ± 13.8) mmHg]. After supplementation, the average DBP in VDC group increased by 1.5 mmHg to (84.0 ± 14.3) mmHg, whereas on VD group decreased by 2.1 mmHg to (84.7 ± 10.7) mmHg. A study related to vitamin D supplementation with a dose of 400 IU of vitamin D plus 1 000 mg of calcium given daily to women aged 50–79 years showed that there was no significant change in SBP and DBP indicating 0.22 mmHg decrease in SBP and only 0.11 mmHg decrease in DBP[13].

After taking supplementation, the VDC group demonstrated an increase in serum 25(OH)D by 21.6%, while in the VD group there were almost two times higher increase in serum 25(OH)D at 42.3% compared to the VDC group. Supplementation can increase serum 25(OH)D, but can’t decrease SBP and DBP in both intervention groups that received VDC or VD.

Conflict of interest statement

We declare that we have no conflict of interest.

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