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Research Article

Efficacy of High Dose Vitamin D_3 Supplementation in Improving Serum 25(OH)D Levels of Overweight Women with Polycystic Ovary Syndrome: Randomized Placebo-Controlled Clinical Trial

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Abstract

Background: Vitamin D deficiency is highly prevalent among overweight polycystic ovary syndrome (PCOS) women and higher dose of vitamin D3 supplementation is required for those with serum 25(OH)D levels below 20 ng/ml. The aim of this trial was to study the efficacy of 50,000 IU vitamin D_3 supplementation on improving serum 25(OH)D levels and to examine whether the duration of supplementation is sufficient to normalize 25(OH)D.

Methods: A prospective randomized, double-blinded placebo-controlled clinical trial was conducted on 60 overweight Jordanian women, aged (18 - 49) years with PCOS and vitamin D deficiency. Participants were divided into two groups. Vitamin D group (n = 30) and placebo group (n = 30). Vitamin D group was assigned to receive 50,000 IU/week of vitamin D_3 and placebo group, was assigned to receive placebo tablets orally for 12 consecutive weeks. The serum 25(OH)D concentrations were assessed before (basal) and after day 30, 60, 90 and 104 of intervention.

Results: In the vitamin D group, the serum level of 25(OH)D increased significantly from 12.5 ± 0.61 to 50.2 ± 2.04 ng/mL, ($p < 0.001$), and decreased significantly from 50.2 ± 2.04 to 48.2 ± 2.03 ng/mL, ($p < 0.001$) after 14 days of vitamin D treatment cessation. There were no significant changes in placebo group.

Conclusions: It can be concluded that vitamin D_3 supplementation improving serum 25(OH)D levels and the 12 weeks of supplementation were sufficient to normalize the 25(OH)D concentrations and a maintenance small dose of vitamin D_3 is recommended after reaching the normal concentrations of serum 25(OH)D.

Keywords: Vitamin D_3; 25(OH)D; Polycystic Ovary Syndrome; Overweight

Abbreviations

PCOS: Polycystic Ovary Syndrome; IR: Insulin Resistance; KAUH: King Abdullah University Hospital; IRB: Institutional Research Board; JFDA: Jordan Food and Drug Administration; BMI: Body Mass Index; CBC: Complete Blood Count; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

Introduction

Polycystic ovary syndrome (PCOS) is considered as one of the most frequent endocrine disorders that causes infertility in women of reproductive age [1] and it affects 5 - 10% of them globally [1,2]. Polycystic ovary syndrome is characterized by hyperandrogenism

and chronic anovulation [3]. It is associated with obesity [4], insulin resistance (IR) [5] and features of metabolic syndrome [6].

The relationship between vitamin D levels and different PCOS symptoms, including IR, infertility and hirsutism has been demonstrated in several studies [1,7,8]. Science based evidence suggests that the levels of vitamin D are similar in women with and without PCOS [9]; however, lower levels [10] and higher levels [11] have been reported in women with PCOS. Many studies have reported low levels of 25(OH)D with a range between 11 and 31 ng/ml, [9,12] with the majority having values < 20 ng/ml (67 - 85%) [7-9] in women with PCOS. Vitamin D deficiency is also common in the general population in different countries of the world, with 10 - 60% of adults having values < 20 ng/ml [13].

Several research studies have reported inverse associations between body weight (body mass index, body fat and waist measurements) and serum 25(OH)D levels in women with PCOS and levels of 25(OH)D were reported to be 27 - 56% lower in obese women with PCOS compared with non-obese women with PCOS [7,12]. Muscogiuri and his colleagues in 2012, [12] found that low levels of 25(OH)D were specified by the degree of adiposity and were not directly affected by the development of insulin resistance in women with PCOS. Of the possible explanations of the high prevalence of vitamin D deficiency in women with PCOS is related to obesity [9], because vitamin D is trapped in adipose tissue [14], and obese women may spend less time outdoors exposed to sunlight. It is also possible that dietary preferences and vitamin D metabolism may differ between obese and non-obese individuals [4].

Vitamin D supplementation is required to achieve a desired threshold of serum 25(OH)D concentrations among deficient PCOS women. Although, there is no universally accepted threshold at which initiating vitamin D supplementation would achieve the greatest impact. There is little evidence for the effect of high dose vitamin D supplementation on the increase in serum 25(OH)D at lower baseline serum 25(OH)D levels. Therefore, the aim of this study was to examine the efficacy of 50,000 IU vitamin D_3 once per week over 12 weeks in improving serum 25(OH)D concentrations among overweight PCOS women and whether the duration of supplementation is sufficient to normalize 25(OH)D levels.

Methods

Study design, participants and approval

This study is a prospective randomized double-blind placebo-controlled clinical trial conducted on 60 overweight Jordanian women with PCOS and aged 18 - 49 years old who attended the obstetrics and gynecology clinics at King Abdullah University Hospital (KAUH) in the North of Jordan. An informed consent was obtained from each participant before the initiation of the trial and the study was approved by the Institutional Research Board (IRB) committee at KAUH and by Jordan Food and Drug Administration (JFDA). Also, this study was registered under clinical trials.gov identifier No. NCT02328404.

PCOS diagnosis

Diagnosis of PCOS was carried out by the gynecologist based on the Rotterdam criteria (Rotterdam ESHRE-ASRM Sponsored PCOS consensus workshop group, 2004) [15] which necessitated the presence of 2 of the following 3 features: oligo-ovulation and anovulation, biochemical signs of hyperandrogenism; with an exclusion of related disorders such as hyperthyroidism, congenital adrenal hyperplasia, androgen secreting tumor, Cushing syndrome and hyperprolactinemia, and polycystic ovaries on ultrasound examination (defined as the presence of 12 follicles measuring 2 - 9 mm in diameter and/or an ovarian volume > 10 cm^3).

Inclusion and exclusion criteria

Overweight (BMI 25 - 29.9 kg/m^2) women diagnosed with PCOS, having a serum 25(OH)D level < 20 ng/ml, inadequate dietary intake of vitamin D (< 600 IU/day or < 15 µg/day), a normal complete blood count (CBC), aspartate and alanine aminotransferase (AST and ALT), urea and creatinine and who are able and willing to comply with study rules, and to sign an informed consent were included in the study. Meanwhile, participants were excluded from the study if any of these conditions applied; pregnancy, lactation, women aged < 15 or > 49 years old, underweight, normal body weight and obese, diagnosed with diabetes, hypothyroidism, hyperthyroidism, liver disease, renal dysfunction, and cardiovascular diseases, presence of food allergies or intolerance, drug or alcohol abuse, smoking of 10 cigarettes or more or smoking of hookah, adequate dietary intake of vitamin D (600 IU/day or 15 µg/

day), participants who are on medications known to affect metabolic parameters, such as metformin, vitamin D, calcium and corticosteroids, serum 25(OH)D level > 20 ng/ml, abnormal laboratory results of CBC, AST, ALT, urea and creatinine and participation in another clinical or bioequivalence study within 90 days prior to the start of this study.

Vitamin D allocation

Participating women were insured against civil claims of clinical trials, randomly allocated and assigned either to placebo (n = 30) or to vitamin D group (n = 30) using the computer generator random numbers in (SPSS). Randomization for placebo or vitamin D was done using the batch numbers of placebo and vitamin D tablets. Women in the vitamin D group were supplemented with 50,000 IU vitamin D3 every week for 12 consecutive weeks, while women in the placebo group received placebo capsules which were identical to vitamin D3 capsules in color, shape, size, and packaging. Each participant received one bottle contains 4 tablets every 4 weeks according to her randomization. Blood samples for the determination of serum 25(OH)D were obtained at 7 days before the initiation of the study (-7 days), at the beginning of study (0 day), after 30, 60 and 90 days of taking vitamin D_3 or placebo to monitor the changes of serum 25(OH)D levels over the intervention period and after 14 days of the last dose of vitamin D3 or placebo (day 104 of the initiation of the study).

Assessment of 25(OH)D concentration

A morning venous blood sample (approximately 5 ml) was drawn after at least 8 - 12 hours overnight fasting using 10 ml Vacutainer (VACUETTE) plain tubes containing clot activator (Z-serum clot activator) and were stored at room temperature for 30 minutes before centrifugation at 4000-rpm and 23°C for 5 minutes using Eppendorf Centrifuge 5810 R, then it was transferred into endocrine automated analyzer (Beckman Coulter, Access 2 immune assay system, USA) for determination of 25(OH)D level by using an electrochemiluminescence immunoassay system (ECLIA).

Statistical analyses

Collected data was entered twice in data sheets, checked and analyzed using SPSS statistical package (IBM, SPSS version 22, 2013). Descriptive statistics were performed using means and standard error of the means (SEM) to describe continuous variables and frequencies to describe the non-continuous variables. The nonparametric Kolmogorov-Smirnov test was used to examine all continuous variables for normal distribution. The participants were categorized into vitamin D group (n = 30) and placebo group (n = 30) and the differences between the means of the normally distributed variables were examined using student t-test for independent samples.

The effect of vitamin D_3 (50,000 IU/week), [placebo and both vitamin D and placebo spontaneously on serum 25(OH)D concentrations of PCOS women over time] was analyzed using the repeated-measures procedure of the General Linear Model (GLM). All reported P values are 2-tailed and $P < 0.05$ was considered to be statistically significant.

Results

Descriptive statistics of the study participants

Seventy (N = 70) women with PCOS were assessed based on anthropometric indices and serum 25(OH)D concentrations, out of them 64 women had serum 25(OH)D level < 20 ng/ml and 4 women were dropout after screening. Therefore, only 60 women were participated in the intervention trial and 2 of them were dropout on day 90 and day 104 of the study. Therefore, only 29 participants in each group were included in statistical analysis. The prevalence of overall vitamin D deficiency among all screened patients was 91.4%. The mean age of the study participants was (23.67 + 0.66) years, body weight (67.87 + 1.28) kg, height (158.0 + 0.99) cm and BMI was (27.04 + 0.23) kg/m^2. Table 1 shows the minimum, maximum and means + SEM of the biochemical characteristics of the clinical trial participants. Furthermore, descriptive statistics of the study group showed that 64.3% were single, around 48.6% were students and 70% have a university degree. Also, 94.3% covering their heads, 83% do not expose directly to sunlight, 73% had sedentary physical activity level, 91.4% drink soft drinks, 75.7% had irregular meals intake and 64.3% were skipping breakfast meal.

Changes in means of 25(OH)D overtime

Changes in means of serum 25(OH)D for vitamin D and placebo groups showed a highly significant ($p < 0.001$) increase in serum 25(OH)D overtime in vitamin D group compared with placebo group except the basal. However, there were a minor fluctuation in the means of serum 25(OH)D overtime in placebo group (Table 2).

Variable	Minimum	Maximum	Mean ± SEM
Age (year)	18.0	44.0	23.67 ± 0.66
Hirsutism score	5.0	32.0	16.65 ± 0.71
Weigh (kg)	52.0	95.0	67.87 ± 1.28
Height (cm)	144.0	180.0	158.0 ± 0.99
BMI (kg/m^2)	25.0	30.0	27.04 ± 0.23
25(OH)D ng/ml	6.41	19.69	10.80 ± 0.40
Calcium (mmol/L)	2.15	2.48	2.30 ± 0.01
Phosphorus (mmol/L)	0.84	1.50	1.13 ± 0.02
Parathyroid Hormone (pg/ml)	21.40	166.40	64.84 ± 3.74
Alanine Transaminase (U./L)	4.70	41.90	11.35 ± 0.84
Aspartate Aminotransferase (mlU./mL)	5.10	31.20	18.17 ± 0.58
Urea (mmol/L)	1.80	5.60	3.28 ± 0.11
Creatinine (Umol/L)	34.14	68.40	49.88 ± 0.95

Table 1: Descriptive statistics of the study participants age, anthropometry and biochemical characteristics (N = 60).

	Vitamin D (50,000 IU/day) (n = 29)	Placebo (n = 29)	
Intervention Days	**Mean ± SEM**	**Mean ± SEM**	***p*-value**
Day 0 (basal)	12.5 ± 0.61	12.4 ± 38.85	0.876
Day 30	33.5 ± 1.30	10.8 ± 0.63	0.001
Day 60	39.9 ± 1.75	8.0 ± 0.48	0.001
Day 90	50.2 ± 2.04	10.3 ± 2.04	0.001
Day 104	48.2 ± 2.03	11.8 ± 0.76	0.001

Table 2: Changes in means of serum 25(OH)D for placebo and vitamin D groups overtime.

1- Values are presented as Means ± SEM.

2. *p*-value < 0.05 is statistically significant.

The effect of vitamin D and placebo overtime on serum 25(OH) D among both groups were assessed by comparing the least significant means differences using the repeated measure analysis (Table 3). Results of all possible means differences of the vitamin D group showed a significant (p < 0.001) steady increase in serum 25(OH)D up to day 90 of the interventional study period. While there was a significant (p < 0.05) decrease in serum 25(OH)D on day 104 compared with day 90 of the study period (Figure 1).

Figure 1: Estimated marginal means of 25(OH)D for placebo and vitamin D groups overtime.

In general, for the placebo group there was a fluctuation in the marginal means of serum 25(OH)D over the intervention study period; whereas, the least mean was on day 60 (Table 3).

Discussion

Comparing the results of this study with other studies conducted in different parts of the world should be done with caution due to many factors such as differences in study design, criteria used for PCOS diagnosis, cut-off points of biochemical and clinical parameters and indices used to determine signs and symptoms of PCOS. In addition, there are other factors should be taken into consideration such as, differences in socio-demographic characteristics, culture, dietary habits, ethnicity, geographical locations, religious rites and lifestyle characteristics.

Despite the fact that Jordan is a sunny country except during the winter season, the prevalence of vitamin D deficiency (25 - OH-D < 20 ng/ml) among the study population was 91.4%. This rate agrees with the rate found in a previous study conducted among Jordanian women in their reproductive age, which showed that the prevalence of vitamin D deficiency (< 12 ng/ml) was 60.3% and 95.7% for insufficiency (< 20 ng/ml) [16]. Moreover, most women in this study were either covering their heads (94.3%) and/or veiled covering their faces for religious or cultural reasons

Comparisons		Vitamin D (50,000 IU/week) (n = 29)		Placebo (n = 29)	
Day	Day	Mean Difference ± SEM	*p*-value *	Mean Difference ± SEM	*p*-value *
0	30	-21.00 ± 1.26	0.001	1.51 ± 0.61	0.020
0	60	-27.43 ± 1.74	0.001	4.36 ± 0.55	0.000
0	90	-37.76 ± 2.03	0.001	1.95 ± 0.65	0.005
0	104	-35.72 ± 2.07	0.001	0.55 ± 0.75	0.469
30	0	21.03 ± 1.26	0.001	-1.51 ± 0.61	0.020
30	60	-6.40 ± 1.05	0.001	2.85 ± 0.43	0.000
30	90	-16.73 ± 1.34	0.001	0.45 ± 0.48	0.359
30	104	-14.69 ± 1.42	0.001	-0.96 ± 0.50	0.066
60	0	27.43 ± 1.74	0.001	-4.35 ± 0.55	0.000
60	30	6.41 ± 1.05	0.001	-2.85 ± 0.43	0.000
60	90	-10.32 ± 1.02	0.001	-2.40 ± 0.29	0.000
60	104	-8.28 ± 1.06	0.001	-3.81 ± 0.42	0.000
90	0	37.76 ± 2.03	0.001	-1.95 ± 0.65	0.005
90	30	16.72 ± 1.34	0.001	-0.45 ± 0.45	0.359
90	60	10.32 ± 1.02	0.001	2.40 ± 0.27	0.000
90	104	2.04 ± 0.87	0.001	-1.40 ± 0.39	0.001
104	0	35.72 ± 2.07	0.001	-0.55 ± 0.75	0.469
104	30	14.69 ± 1.42	0.001	0.96 ± 0.50	0.066
104	60	8.28 ± 1.06	0.001	3.81 ± 0.42	0.000
104	90	-2.04 ± 0.87	0.026	1.40 ± 0.39	0.001

Table 3: Comparisons of the Least Significant Differences of 25(OH)D for Placebo and Vitamin D Groups over Time.

1-* Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

2- Values presented as Mean Differences ± SEM.

3- *p*-value ≤ 0.05 is statistically significant.

and 83% not exposed directly to sunlight. This might support the hypothesis raised previously which claimed that "covering heads and/or wearing veil" may affect serum 25(OH)D levels in Jordanian women [16,17].

There was a highly significant increase ($p < 0.001$) in means of 25(OH)D over the study period (104 days) when compared to the basal levels among the vitamin D group whereas, the placebo group showed a decrease in their serum 25(OH)D level compared to the basal level. Also, no significant differences were found between the basal levels of the placebo and vitamin D groups. The most significant increase in the means of serum 25(OH)D was observed on day 90 of the intervention period, which was expected since the day 90 is the last day of taking vitamin D3. There was a significant reduction in serum 25(OH)D levels on day 104; after 14 days from the last week of the intervention period (12 weeks), which was not expected since vitamin D is a fat-soluble vitamin that is stored in the liver. Similar significant increases in serum 25(OH)D levels were reported in previous clinical intervention trials [5,18-20]. There were not any vitamin D3 interventional clinical trial that followed the participants after 2 weeks or more of the intervention period to study the changes in serum 25(OH)D levels. In addition, controlling for the vitamin D_3 treatment residue showed significant increase in serum 25(OH)D levels within days of interventions except on day 104 compared to day 90. Similar results were reported recently by Rahimi-Ardabili., *et al*. (2013) and Khan., *et al*. (2014) [19,20].

Conclusion

It can be concluded that vitamin D supplementation in treatment dose of 50,000 IU weekly for at least 12 weeks improving serum 25(OH)D levels and the duration of supplementation was suf-

ficient to achieve the greatest impact. In addition, a maintenance smaller dose of 1000 IU vitamin D_3 daily is recommended to help in maintaining the normal concentration of serum 25(OH). The positive improvements in 25(OH)D levels may help in improving PCOS prognosis which will reflect better fertility and reproductive life for the overweight women with PCOS.

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