Therapeutic effects of calcium & vitamin D supplementation in women with PCOS

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Abstract

Objective

To evaluate the efficacy of calcium & vitamin D supplementation in infertile women suffering from polycystic ovary syndrome (PCOS), and to assess levels of 25-hydroxy vitamin D in these patients.

Methods

In a case control study, 100 infertile PCOS women based on a randomly divided into two groups. Group I \((n = 50)\) were treated with metformin 1500 mg/day, and group II \((n = 50)\) treated with metformin 1500 mg/day plus
Calcium 1000 mg/day and Vitamin D 100000 IU/month for 6 months. Patients were followed by transvaginal sonography at first, 3 and 6 months later for evaluating dominant follicle.

BMI, menstrual regularity, follicle diameter, pregnancy, serum 25-OH-vitamin D level were matured and compared in two groups.

Results

BMI decreased almost significantly (25.49 ± 1.88 vs 26.28 ± 2.15, \( p: 0.054 \)) in group II. A better improvement was gained in regulating menstrual abnormalities (70% vs 58%, \( p: 0.211 \)), follicle maturation (28% vs 22%, \( p: 0.698 \)), and infertility (18% vs 12%, \( p: 0.401 \)) in group II compared with group I, but these results were not statistically significant. Eighty three percent of all the PCOS patients showed vitamin D deficiency while 35% were severely deficient. The serum 25-OH-vitamin D mean levels were 13.38 ± 6.48 ng/ml. Vitamin D deficiency was recompensed in 74% of the PCOS patients who had taken calcium & vitamin D supplementation. There was no correlation between BMI and 25-OH-VD before and after the treatment (\( p \geq 0.01 \)).

Conclusion

This study showed the positive effects of calcium & vitamin D supplementation on weight loss, follicle maturation, menstrual regularity, and improvement of hyperandrogenism, in infertile women with PCOS.

Keywords

- Polycystic ovary syndrome;
- Calcium;
- Vitamin D;
- Metformin
1. Introduction

Polycystic ovarian syndrome which was initially described in 1935 by Stein and Leventhal is one of the most common endocrine disorder affecting women of reproductive age and is characterized by hyperandrogenism, menstrual disturbance and polycystic ovaries on ultrasound. \cite{1} and \cite{2} PCOS is associated with multiple cardiovascular risk factors, including insulin resistance, central obesity, hypertension, impaired glucose tolerance, type 2 diabetes, and the metabolic syndrome.\cite{3} PCOS is also associated with nontraditional markers of cardiovascular risk, such as inflammation, thrombosis, oxidative stress, sleep apnea, endothelial dysfunction, and arterial stiffness. \cite{4} and \cite{5} Insulin resistance and central obesity increase the risk of developing metabolic disorder in polycystic ovary syndrome.\cite{6} Different studies have indicated that weight loss and metformin administration decrease androgen levels and improve hyperandrogenic signs and symptoms.\cite{7} Investigations on hyperandrogenic PCOS patients have shown that, by decreasing insulin levels, androgen concentrations of blood can be suppressed.\cite{8} Some evidence has newly demonstrated effective approaches to nutrition and exercise improve endocrine features, reproductive function and cardiometabolic risk profile even without marked weight loss. Recent studies allow us to make recommendations on macronutrient intake for example, Myo-inositol and B complex vitamin was associated with a decrease of serum testosterone and simultaneously, due to its ability to increase insulin sensitivity, women who received myo-inositol showed a great improvement of the ovulatory function.\cite{9} Omega-3 fatty acid supplementation has a beneficial effect on liver fat content and other cardiovascular risk factors in women with PCOS.\cite{10}

Furthermore, many studies have shown that the metabolism of vitamin D affects glucose and insulin metabolism and plays an important role in Diabetic Mellitus (type II) improvement.\cite{11} Vitamin D is endowed with pleiotropic effects on a wide spectrum of intracellular regulatory mechanisms, including insulin
metabolism, or intrinsic apoptotic pathway, on both classical and nonclassical tissues, such as ovary. Also, investigations on animals have demonstrated a role for calcium in oocyte maturation. It affects the resumption and progression of follicular development. Moreover, disturbances in calcium regulation can be responsible for the follicular arrest. Previous studies have suggested the function of vitamin D in reproduction. Both calcium and vitamin D deficiency are considered as potential risk factors for obesity. Besides, it has been demonstrated that increased body weight has a considerable negative effect on 25-OH-VD and 1,25-OH-VD concentrations in women with PCOS.

In line with the above-mentioned studies, the present study was conducted to evaluate the effects of calcium-vitamin D supplementation on menstrual regularity, BMI, follicle responses, and pregnancy rate in women with PCOS.

2. Methods

This study was a medical thesis and performed from April 2008 to May 2010 in the Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran. This study was approved by the ethics committee of university and informed written consents were obtained from all the participants.

The experiment population consisted of 100 infertile women with PCOS. The patients in the range of 20–40 years of age were enrolled in this study. All of the participants were originally from Iran. Before entering the study, they all gave a written informed consent including the performance conditions.

The patients were divided into two groups of 50 patients based on a random number table. After collecting the blood samples, all the women in group I were treated with metformin (Osvah Pharmaceutical Company, tablet 500 mg) 1500 mg/day, and all in group II were treated with metformin (Osvah Pharmaceutical Company, tablet 500 mg) 1500 mg/day plus calcium
(Sobhandarou Pharmaceutical Company, tablet 500 mg) 1000 mg/day and vitamin D (Zahravi Pharmaceutical Company, pearl Vitamin D3 50000 IU/week) orally for 6 months (As newer Overview of calcium & vitamin D).

The clinical evaluation consisted of a detailed medical and menstrual history and physical and clinical examinations including transvaginal sonography and hormonal analyses. The laboratory analyses of LH, FSH, serum levels of Calcium, and 25 Hydroxy vitamin D were performed for each woman. In order to determine the follicular growth, transvaginal sonography was performed in the middle of the menstrual cycle at the baseline, 3 months and 6 months after the treatment. In case that a side effect of the drugs such as a severe headache or gastrointestinal complications was observed, the patient was excluded.

The regularity of menses according to time intervals (21–35 days), the BMI according to weight/height ratio, the follicular growth according to transvaginal sonography, and the pregnancy rate according to B-HCG titer were compared between the two groups. Serum 25 Hydroxy Vitamin D was measured by a radioimmunoassay method. The normal range of 25-OH-VD for our assay was 20–49.0 ng/ml, and a level less than 10 ng/ml was considered as severe deficiency.

Using transvaginal sonography at the mid-cycle, a classification was postulated for follicular development which put the participants into three groups based on the size criteria [5–9 mm (not responded), 10–14 mm (borderline) and ≥14 mm (responded)].

Considering a patient with PCOS was based on Rotterdam criteria: a) oligoovulation or anovulation, b) clinical and/or biochemical signs of hyperandrogenism e.g. hirsutism, acne, etc, and c) polycystic ovaries image in ultrasound examination. Indeed, having two of these three features is sufficient for defining PCOS. The exclusion criteria were Cushing’s syndrome, hyperprolactinemia, renal failure (creatinine ≥ 2.0 mg/dl), adrenal androgen
secreting tumors, primary hyperparathyroidism, ovarian androgen secreting tumors, malignancy, gastrectomy, inflammatory bowel disease, the use of anticonvulsants, and mental retardation. There were no evident differences of calcium intake in the participants.

The results were all put into a statistical package for the Social Sciences 16.00 (SPSS, Chicago, IL, USA). The data are presented as mean ± SD or as percentages. The differences between the two groups before and after the treatment were measured by Chi-Square test, Mc Nemar test, and Paired Samples Test. Pearson correlation and linear regression were used to evaluate the associations between BMI and serum 25 hydroxy vitamin D. The correlation is significant at a 0.01 level (2-tailed).

3. Results

In this study, one hundred infertile women with PCOS were enrolled and divided into two groups of 50 patients. The mean ages of the patients in groups I and II were 28.46 ± 4.16 and 27.96 ± 4.07 respectively. Almost all the cases in group I (92%) and group II (96%) had experienced aligoamenorrhea/amenorrhea in the past 6 months before the study. Out of 100 PCOS patients, a total of 24, i.e. 13(26%) in group I and 11(22%) in group II, were non-obese with a BMI < 25.00 kg/m², as many as 64, i.e. 31(62%) in group I and 33(66%) in group II, were overweight with a BMI 25.00–30.00 kg/m², and the rest of them, i.e. 6(12%) in group I and 6(12%) in group II, were obese with BMI > 30 kg/m².

Regarding the clinical signs of hyperandrogenism, 40% of the PCOS subjects in group I and 50% of the PCOS subjects in group II presented acnes. Also, 46% in group I and 56% in group II were found with hirsutism. The mean levels of serum 25-OH-VD in groups I and II were 13.55 ± 6.39 and 13.21 ± 6.6 respectively.
The clinical and biochemical variables of the two groups are summarized in Table 1. There was no significant difference in age, menstrual disturbances, acne, hirsutism, calcium, LH/FSH ratio, 25 hydroxyvitamin D, and the past history among the two groups ($P > 0.3$).

Table 1. Clinical and biochemical variables of the two PCOS groups.

<table>
<thead>
<tr>
<th>Groups Variable</th>
<th>Met Group</th>
<th>Met-Cal VitD Group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.46 ± 4.16</td>
<td>27.96 ± 4.07</td>
<td>0.544</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.91 ± 2.35</td>
<td>26.89 ± 2.14</td>
<td>0.965</td>
</tr>
<tr>
<td>25-OH-VD (ng/ml)</td>
<td>13.55 ± 6.39</td>
<td>13.21 ± 6.63</td>
<td>0.794</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.35 ± 0.82</td>
<td>9.32 ± 0.81</td>
<td>0.884</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>18.15 ± 3.89</td>
<td>18.33 ± 2.58</td>
<td>0.779</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.45 ± 1.20</td>
<td>6.23 ± 0.96</td>
<td>0.307</td>
</tr>
<tr>
<td>Acne</td>
<td>20 (40%)</td>
<td>25 (50%)</td>
<td>0.315</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>23 (46%)</td>
<td>28 (56%)</td>
<td>0.317</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>38 (76%)</td>
<td>42 (84%)</td>
<td>0.461</td>
</tr>
<tr>
<td>Regular menstrual cycles</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>0.678</td>
</tr>
<tr>
<td>Diabetic Mellitus</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td>1.000</td>
</tr>
<tr>
<td>PCO history in family</td>
<td>7 (14%)</td>
<td>7 (14%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or percentage. Met group: Metformin Group, Met-Cal Vit. D Group: Metformin- Calcium- Vitamin D group, BMI: body mass index, 25-OH-VD: 25 hydroxyvitamin D, FSH: follicle stimulating hormone, LH: luteinizing hormone. $P \leq 0.05$ statistically significant.

In this study, 83 (83%) women were vitamin-D deficient; that is, 42 (84%) and 41 (82%) women in groups I and II respectively. Of these, 35 (35%) were severely deficient; that is, 17 (34%) and 18 (36%) women in groups I and II respectively. In our study, none of the participants had a side effect of the drugs such as a severe headache or gastrointestinal complications, and nobody left the study.

Menstrual regularity was reported six months after the intervention in 58% of the patients in group I and 70% of the patients in Group II. Chi-square tests showed no significant difference of menstrual regularity between the two groups after the treatment ($p = 0.211$).
BMI decreased in both groups, but there was a higher reduction in group II. Statistical analyses show rather noticeable differences between the two groups after the treatment ($p = 0.054$).

As compared to group I, follicular response was relatively higher in group II three and six months after the treatment, but the differences were not significant ($p: 0.759$, $p: 0.698$).

Pregnancy occurred during the follow-up period in both groups. There were six pregnancies (12%) in group I and nine (18%) in group II with no significant statistical differences between two groups ($p = 0.401$). All the people who experienced pregnancy were under 35 years of age. A Chi-square test showed significant differences of menstrual regularity, BMI decrease and follicular response within each group before and after the treatment ($p = 0.001$). The results of the treatment are summarized in Table 2.

Table 2. Results of the treatment in the two study groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Met Group</th>
<th>Met-CalVit. D Group</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH-Vitamin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>13.55 ± 6.39</td>
<td>13.21 ± 6.63</td>
<td>0.794</td>
</tr>
<tr>
<td>After treatment</td>
<td>13.79 ± 6.48</td>
<td>24.82 ± 6.54</td>
<td>0.001</td>
</tr>
<tr>
<td>Regulated Menstrual cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>0.678</td>
</tr>
<tr>
<td>After treatment</td>
<td>29 (58%)</td>
<td>35 (70%)</td>
<td>0.211</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>26.91 ± 2.35</td>
<td>26.89 ± 2.14</td>
<td>0.965</td>
</tr>
<tr>
<td>After treatment</td>
<td>26.28 ± 2.15</td>
<td>25.49 ± 1.88</td>
<td>0.054</td>
</tr>
<tr>
<td>Numbers of follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 3-month treatment</td>
<td></td>
<td></td>
<td>0.759</td>
</tr>
<tr>
<td>≤10 mm</td>
<td>38 (76%)</td>
<td>35 (70%)</td>
<td></td>
</tr>
<tr>
<td>10–14 mm</td>
<td>9 (18%)</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>&gt;14 mm</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>After 6-month treatment</td>
<td></td>
<td></td>
<td>0.698</td>
</tr>
<tr>
<td>≤10 mm</td>
<td>27 (54%)</td>
<td>23 (46%)</td>
<td></td>
</tr>
<tr>
<td>10–14 mm</td>
<td>12 (24%)</td>
<td>13 (26%)</td>
<td></td>
</tr>
<tr>
<td>&gt;14 mm</td>
<td>11 (22%)</td>
<td>14 (28%)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>6 (12%)</td>
<td>9 (18%)</td>
<td>0.401</td>
</tr>
</tbody>
</table>

Met group: metformin group Met-calcium vit. D group: metformin- calcium- vitamin D group, $p \leq 0.05$ statistically significant.
The level of serum 25-OH-VD increased from 13.21 ± 6.63 to 24.82 ± 6.54 in group II. In group I, however, where no calcium & vitamin D had been used, the serum level did not change. There was a significant difference between the two groups after the treatment ($p \leq 0.001$). In this study, after 6-month treatment with calcium & vitamin D, only 13 (26%) of the patients remained vitamin D deficient; but the deficiency was severe in none of them.

There was no correlation between BMI and 25-OH-VD before and after the treatment (Table 3). Although there was more improvement in regulating the menstrual abnormalities, follicle maturation, and infertility in group II where the patients received calcium & vitamin D supplementation plus metformin, the trends did not reach the statistical threshold set up for this study.

Table 3. Correlation between BMI and 25-OH-VD in each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment BMI</th>
<th>After treatment BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Group 25-OH-VitD</td>
<td>0.454</td>
<td>0.568</td>
</tr>
<tr>
<td>Met-Cal Vit. D Group 25-OH-VitD</td>
<td>0.367</td>
<td>0.207</td>
</tr>
</tbody>
</table>

Correlation is significant at the 0.05 level (2-tailed).

4. Discussions

PCOS is one of the most common etiologies of menstrual irregularity and androgen excess in women, which is characterized by hyperandrogenic, chronic anovulation, infertility, irregular mense, and hirsutism. Calcium plays a major role in oocyte maturation in mammals. On the other hand, vitamin D deficiency causes insulin resistance and diabetes which induce hyperandrogenism followed by menstrual irregularity. Vitamin D also has a proven role in reproduction. Therefore, this study was conducted to assess the therapeutic effect of calcium & vitamin D supplementation on PCOS patients.

In the present study, the regulation of menstrual dysfunction occurred in 70% of the subjects who had taken calcium & vitamin D and metformin. Later, 18% of them got pregnant. There is the same conclusion reported by Thys-Jacobs et.al. who evaluated the effect of calcium and vitamin D dysregulation on
menstrual disturbances in PCOS patients. Their study assessed the efficacy of calcium & vitamin D therapy in 13 women with PCOS. They demonstrated that menstrual disturbances could be regulated in 7 subjects after 2 months of treatment, and 2 of them got pregnant. In comparison with another study by Rashidi et.al. in Iran conducted on 60 infertile PCOS patients who had improved follicular responses and menstrual disturbances by calcium & vitamin D therapy. Our study showed menstrual regularity in 70% and follicular response in 28% of the patients who had taken calcium & vitamin D and metformin, though the results refer to no statistically significant difference between the two groups of study after the treatment.

In our study, BMI decreased by consumption of calcium & vitamin D as it has been demonstrated in some other studies. [13], [14], [15] and [22].

Many experiments have proved the effect of low calcium intake and hypovitaminosis D on weight gain and body composition. [5], [23] and [24]. Even though many studies have shown an inverse relationship between BMI and vitamin D, several studies do not support this relationship [25] and [26] or show a linear correlation. As for the present study, there was no specific correlation to find between vitamin D levels and BMI.

Our investigation found the prevalence of hypovitaminosis D to be 83% in all the PCOS patients (25-OH-vit D ≤ 20 ng/ml), as Selimoglu’s study that found it at 81.8%. Similarly, Wehr et al. demonstrated that 72.8% of their subjects were vitamin D deficient (25-OH-vit D ≤ 30 ng/ml). In our study, after a 6-month treatment with calcium-vitamin D, the mean serum levels of 25-OH-VD increased from 13.21 ± 6.63 to 24.82 ± 6.54 in group II, and just 26% of the patients remained vitamin D deficient; however, nobody was severely deficient.

This study was performed in the city of Yazd, located in the central desert area of Iran at the latitude of 31.50. The weather is sunny most of the year. There is almost no difference between summer and winter in the number of sunny days in Yazd. As in another study by Mirsaied Ghazi et.al. in Tehran,
there was no significant difference between vitamin D levels in women in summer and winter seasons. Although there is a great deal of sunlight in the city of Yazd, most women are at the risk of vitamin D deficiency. This is because of Iranian clothing styles, specific regional environments, tendency to avoid sunlight, and growing use of different methods for sunlight avoidance. We did not compare these results with a control group, but some studies conducted in Iran have shown a vitamin D deficiency of 60%–80% among healthy women at the reproductive age. [25] and [29]

As the results of this study, owing to vitamin D deficiency in PCOS patients and the positive effects of calcium & vitamin D supplementation on weight loss, follicle maturation, menstrual regularity, and improvement of hyperandrogenism, the use of the supplementation can be advantageous in the treatment of PCOS.

5. Conclusion

Treatment with vitamin D could recompense vitamin D deficiency, increase the vitamin level considerably, and decrease BMI. Furthermore, vitamin D supplementation could play an important role in the treatment of PCOS patients, not only to improve insulin resistance and infertility.

Our study provide further proof for some previous studies that underline the efficacy of calcium and vitamin D in the treatment of PCOS. More widespread, randomized, and controlled empirical attempts seem necessary to determine the potential useful effects of calcium-vitamin D supplementation on different features of PCOS.

Conflict of interest

None.

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