The Effects of Calcium-Vitamin D and Metformin on Polycystic Ovary Syndrome: A Pilot Study

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SUMMARY

Objective: The aim of this study was to evaluate the effects of calcium-vitamin D and metformin on the menstrual cycle and ovulation in patients with polycystic ovary syndrome (PCOS).

Materials and Methods: In this pilot study, 60 infertile PCOS patients were enrolled in a randomized clinical trial and divided into three equal groups. Group 1 received 1,000 mg of calcium and 400 IU of vitamin D per day, orally. Group 2 received the same as Group 1, plus 1,500 mg/day of metformin. Group 3 received 1,500 mg/day of metformin. The patients were treated for 3 months and followed up for a further 3 months. Regularity of menses, number of large follicles (≥ 14 mm) and pregnancy rates were compared among the three groups.

Results: Generalized estimating equation tests showed that the number of dominant follicles (≥ 14 mm) during the 2–3 months of follow-up was higher in the calcium-vitamin D plus metformin group than in either of the other two groups ($p = 0.03$).

Conclusion: The effects of metformin and calcium-vitamin D in regulating the menstrual cycle suggest that they could also be effective for the treatment of anovulation and oligomenorrhea, with possible consequences for pregnancy rates in PCOS patients. [*Taiwan J Obstet Gynecol* 2009;48(2):142–147]

Key Words: amenorrhea, calcium-vitamin D, metformin, oligomenorrhea, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) affects 6–10% of women of reproductive age and is a common cause of infertility [1]. Its clinical manifestations can include menstrual irregularities, signs of androgen excess, and obesity [1]. Trials focusing on pregnancy as an outcome may place greater emphasis on anovulation as the identifying symptom, rather than the presence of polycystic ovaries or clinical hyperandrogenism [2]. Women with chronic hyperandrogenism and irregular menstrual cycles and/or polycystic ovaries appear to be at substantially greater risk of insulin resistance than those with hyperandrogenism and regular cycles [3,4]. It is, therefore, essential that studies into the metabolic features of PCOS should stratify affected women according to ovulatory function (i.e. chronic oligomenorrhea/amenorrhea versus regular cycles). It has been shown that a proportion of PCOS patients do not demonstrate any overt abnormality in circulating androgens [1,5,6]. The optimal management of PCOS is uncertain, and treatment focuses on amelioration of the clinical features. The aim of treatment is to restore ovulatory cycles so that pregnancy can be achieved. Clinical studies have shown that metformin (500 mg three times per day or 850 mg twice daily, with meals) administered to women with PCOS increases the frequency of spontaneous ovulation, menstrual regularity, and ovulatory response to clomiphene [7,8]. However, the long-term use of insulin-sensitizing agents needed to prevent the potential complications of PCOS cannot be recommended because of a lack of evidence regarding their safety and efficacy [9]. Recent animal investigations have established a role for calcium in oocyte activation and maturation, resulting in the resumption and progression

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of follicular development [10]. In 1974, Steinhardt et al [11] proposed that calcium may have a universal role in egg activation. Since then, the importance of calcium in the regulation of both meiotic and mitotic cell division cycles in mammalian oocytes has aroused considerable interest [12]. Because of the importance of calcium in both oocyte activation and maturation, it was hypothesized that abnormalities in calcium homeostasis may play a role in the pathogenesis of PCOS [13]. Previous studies have suggested that vitamin D also plays a role in reproductive functions. Vitamin D receptors are expressed in the ovary and testis, suggesting that vitamin D is active in these organs [14]. In this pilot study, the effects of metformin and calcium-vitamin D on folliculogenesis and menstrual regularity were assessed in PCOS patients.

Materials and Methods

From February 2004, we enrolled 60 infertile women aged 20–40 years old, who were referred to the Vali-e-Asr Reproductive Health Research Center and who met the Rotterdam criteria for PCOS [2]. Permission to perform this study was obtained from the ethical board of the research committee of Tehran Medical University. The subjects volunteered to participate after the purpose of the study was explained to them. All patients signed consent forms.

All patients underwent an extensive interview to ascertain their demographic characteristics, completed a questionnaire regarding their dietary habits, and underwent physical and clinical examinations, including sonography and hormonal analyses. Diagnostic endocrine tests included serum levels of prolactin, thyroid-stimulating hormone, follicle-stimulating hormone and luteinizing hormone, and urinary and serum levels of calcium. Baseline transvaginal sonography was performed on the third day of the menstrual cycle. In order to determine follicular growth, transvaginal sonography was performed every 3 days from day 8 of menstruation until the appearance of at least one dominant follicle (14 mm).

According to the Rotterdam criteria, the presence of two of the three following characteristics were required for inclusion in the study: (1) oligomenorrhea/amenorrhea, (2) chemical or clinical findings of hyperandrogenism, and (3) polycystic ovaries on transvaginal sonography. Patients with systemic diseases such as Cushing's syndrome, hyperparathyroidism or hyperprolactinemia, androgen secreting tumors, history of abdominal/pelvic surgery, coexisting male factor infertility, or abnormal hysterosalpingography were excluded from the study.

Patients were divided into three groups, each containing 20 women, using a random number table. Patients in Group 1 received 1,000 mg of calcium and 400 IU of vitamin D (Calcium-D Tab 500 mg, 200 IU; Tehran Darou, Iran) per day, orally. Patients in Group 2 received 1,000 mg of calcium and 400 IU of vitamin D as above, plus metformin 1,500 mg/day (Metformin Tab 500 mg; Minoo Darou, Iran), orally. Patients in Group 3 received metformin 1,500 mg/day, orally. Treatment started on the first day of menses and was continued for 3 months. Patients were followed up 3 months after completion of the treatment.

Regularity of menses according to interval (21–35 days), follicular growth, and pregnancy rate were compared among the three groups. Pregnancy was confirmed by rising serum levels of β-human chorionic gonadotropin and then by identification of a gestational sac 2 weeks later. Follicular growth was categorized into three groups according to the size of the follicle (5–9 mm, 10–14 mm [borderline] and ≥ 14 mm [large]) using transvaginal sonography at mid-cycle. Data analysis was performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). Differences were considered to be statistically significant at $p < 0.05$, and confidence intervals of 95% were used. The data were analyzed using Chi-squared, analysis of variance (ANOVA), Kruskal-Wallis and multivariate regression tests (generalized estimation equation tests [GEE]).

Results

Sixty infertile women with PCOS were enrolled in this study. Their mean age was 25.91 ± 4.23 years, and their mean body mass index (BMI), calculated as the weight in kilograms divided by height in meters squared (kg/m^2), was 26.16 ± 3.95. ANOVA showed no significant differences in these variables among the three groups (Table 1). Dietary habits were compared among the groups using a questionnaire and 24-hour dietary recall. Information was collected on consumption of dairy products, protein and fat. Kruskal-Wallis tests showed that the consumption of dairy products was below the recommended daily allowance in more than 60% of subjects.

Mean serum calcium level (9.62 ± 0.84 mg/dL) and mean urinary calcium concentration (189.81 ± 83.22 mg/dL) were within the normal range (8.6–10.2 mg/dL and 50–300 mg/dL for serum and urinary samples, respectively).

About 80–100% of the subjects in all three groups had a history of oligomenorrhea/amenorrhea during the last 6 months prior to the study (Table 2). Menstrual regularity was reported after the third month of intervention

Table 1. Patients' characteristics*

Variable	Group 1 (Ca-D; $n=20$)	Group 2 (Ca-D + met; $n=20$)	Group 3 (met; $n=20$)	Total	$p^{\dagger}$
Age (yr)	24.95 ± 3.56	25.805 ± 4.61	26.95 ± 4.44	25.91 ± 4.23	0.33
BMI	25.75 ± 3.94	27.81 ± 3.78	25.162 ± 3.860	26.16 ± 3.95	0.108
Duration of infertility (yr)	5.08 ± 3.85	5.52 ± 2.74	4.67 ± 3.31	5.09 ± 3.29	0.727
Plasma calcium (mg/dL)	9.84 ± 0.73	9.43 ± 0.71	9.61 ± 1.03	9.62 ± 0.84	0.306
Urinary calcium (mg/dL)	186.26 ± 86.6	181.7 ± 70.64	201/89 ± 94.44	189.81 ± 83.22	0.738
FSH (mIU/mL)	5.20 ± 1.98	4.95 ± 1.66	5.67 ± 1.70	5.28 ± 1.78	0.438
LH (mIU/mL)	9.90 ± 3.58	10.24 ± 6.38	8.81 ± 4.51	9.65 ± 4.91	0.640
Prolactin (mg/mL)	200.06 ± 198.75	216.66 ± 221.25	334.153 ± 259.02	248.87 ± 231.0	0.145
Serum magnesium (mg/L)	2.17 ± 0.23	2.07 ± 0.24	2.12 ± 0.29	2.12 ± 0.26	0.528

**Data are presented as mean ± standard deviation; †analysis of variance test, all p values are not significant. Ca-D = calcium-vitamin D; met = metformin; BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone.*

Table 2. Patients' characteristics*

Variable	Group 1 (Ca-D; $n=20$)	Group 2 (Ca-D + met; $n=20$)	Group 3 (met; $n=20$)	$p^{\dagger}$	χ^2
Type of infertility				0.732	0.62
Primary	17 (85)	15 (75)	16 (80)		
Secondary	3 (15)	5 (25)	4 (20)		
Oligomenorrhea				0.126	4.1
Yes	20 (100)	20 (100)	18 (90)		
No	0 (0)	0 (0)	2 (10)		
Amenorrhea				0.308	2.35
Yes	2 (10)	5 (25)	2 (10)		
No	18 (90)	15 (75)	18 (90)		
History of oligomenorrhea (in last 6 months)				0.108	4.44
Yes	18 (90)	20 (100)	16 (80)		
No	2 (10)	0 (0)	4 (20)		
History of amenorrhea (in last 6 months)				0.153	3.75
Yes	2 (10)	5 (25)	2 (10)		
No	18 (90)	15 (75)	18 (90)		

**Data are presented as n (%); †all p values are not significant. Ca-D = calcium-vitamin D; met = metformin.*

in 30%, 50% and 35% of women in Groups 1, 2 and 3, respectively (Table 3). Chi-squared tests showed no significant difference in menstrual regularity among the three groups after treatment ($p=0.400$), although it was more obvious in the calcium-metformin group.

The frequency distribution of menstrual periods showed that normal menstrual cycles were more frequent in women with normal BMIs (BMI, 25–27), compared with women with abnormal BMIs (44.5% in the normal BMI group and 32% in the abnormal BMI groups). Chi-squared and regression analyses showed no significant differences in regularity of menstrual cycles among the three BMI groups (BMI < 25, BMI 25–27, and BMI > 27). There was no significant correlation between follicular response to therapy and BMI.

Multivariate regression analysis and GEE tests were used to compare treatment effects among the three groups (Table 4). As shown in Table 5, follicular response was relatively higher in Group 1 compared with Group 3, but the difference was not significant ($p=0.2896$). The growth of follicles after treatment was significantly higher in Group 2 than in Group 1 ($p=0.0372$). The likelihood of treatment response was about twice as high in Group 2 as in Group 1 (odds ratio, 2.01). The relative frequency of response to treatment in Group 2 was higher than in the other two groups during the fifth and sixth months of the follow-up period (Table 5).

Chemical pregnancy did not occur during the follow-up period in any of the three intervention groups.

Table 3. Regularity of menses after treatment in the treatment groups*†

Menstrual cycle after treatment	Group 1 (Ca-D; $n=20$)	Group 2 (Ca-D + met; $n=20$)	Group 3 (met; $n=20$)
Regular	6 (30)	10 (50)	7 (35)
Irregular	14 (70)	10 (50)	13 (65)

**Data are presented as n (%); †$p=0.400$, $\chi^2=0.83$. Ca-D = calcium-vitamin D; met = metformin.*

Table 4. The regression analysis (generalized estimation equation tests)

Parameter	Estimate	SE	Z	*p*
Intercept 1	−2.4987	0.4119	−6.07	< 0.0001
Intercept 2	−1.9376	0.4075	−4.75	< 0.0001
Group 3 (met)	0.4327	0.6086	1.06	0.2896
Group 2 (Ca-D + met)	0.6995	0.3357	2.08	0.0372
Group 1 (Ca-D)	Reference group			

SE = standard error; Ca-D = calcium-vitamin D; met = metformin.

Table 5. Follicular growth after treatment in the three treatment groups*

Follicular response	Group 1 (Ca-D; $n=20$)	Group 2 (Ca-D + met; $n=20$)	Group 3 (met; $n=20$)
Follicular response to treatment in third month			
No	19 (95%)	15 (75%)	19 (95%)
Borderline (10–14 mm)	1 (5%)	2 (10%)	0 (0)
Yes (≥ 14 mm)	0 (0)	3 (15%)	1 (5%)
Follicular response to treatment in fourth month			
No	19 (95%)	13 (65%)	15 (75%)
Borderline (10–14 mm)	1 (5%)	5 (25%)	2 (10%)
Yes (≥ 14 mm)	0 (0)	2 (10%)	3 (15%)
Follicular response to treatment in fifth month			
No	16 (80%)	12 (60%)	14 (73.7%)
Borderline (10–14 mm)	0 (0)	2 (10%)	4 (21.1%)
Yes (≥ 14 mm)	4 (20%)	6 (30%)	1 (5.3%)
Follicular response to treatment in sixth month			
No	15 (78.9%)	12 (63.2%)	15 (78.9%)
Borderline (10–14 mm)	0 (0)	2 (10.5%)	1 (5.3%)
Yes (≥ 14 mm)	4 (21.1%)	5 (26.3%)	3 (15.8%)

**Data are presented as n (%). Ca-D = calcium-vitamin D; met = metformin.*

Discussion

PCOS is recognized as one of the most common female endocrine disorders, and is characterized by hyperandrogenic chronic anovulation with infertility, irregular menses, and hirsutism [15].

Previous studies demonstrated a role for calcium in oocyte maturation in invertebrates and amphibians, and this role has been confirmed in mammals [16–18]. Another study showed that the calcium channel blocker, verapamil tetracaine, caused oocyte arrest in meiosis [19].

Recent research suggests that low levels of vitamin D may be a primary factor in the initiation and development of PCOS, and that dietary repletion of this important vitamin could help to restore normal menstrual cycles in women with this condition [20,21]. The combination of dietary calcium insufficiency and

vitamin D deficiency may be largely responsible for the menstrual abnormalities associated with PCOS [13].

Our study included a 3-month treatment period and a 3-month follow-up period in three groups of 20 women with PCOS, most of whom had oligomenorrhea. We assessed the effects of calcium plus vitamin D on menstrual irregularities and infertility. Improvements in menstrual irregularities were more noticeable in the calcium-metformin group, though the differences among the groups were not significant, possibly because of the small sample sizes. It is possible that a large, randomized clinical study with an increased sample size might be able to demonstrate a clearer relationship between calcium-metformin treatment and menstrual regularity. A study performed by Thys-Jacobs et al [13] on the effects of calcium-vitamin D (1,500 mg) in 13 women with PCOS showed that menstrual irregularities were corrected in seven subjects after 2 months of treatment.

We found a statistically significant difference between the calcium-metformin and calcium groups in terms of follicular growth, using GEE regression analysis. This means that there was a better response to treatment in the calcium-metformin group than in the other two groups, especially in the second and third months of follow-up. Follicular development following calcium therapy has been confirmed in animal studies [10]. In the study performed by Velazquez et al [22], fertility was restored in PCOS patients after 6 months' metformin therapy that reduced insulin and androgen levels, thus regulating menstrual cycles.

In the current study, the consumption of dairy products was below the recommended daily allowance in more than 60% of patients in all the groups (less than 1,500 mg/day of calcium-rich foods, such as milk, yoghurt, lassi, cheese, butter or dried whey). However, the mean serum calcium concentrations in all the subjects were still within the normal range. Another study found low levels of intracellular calcium and abnormal oocyte function, even though extracellular calcium levels were within the normal range [12].

The inadequate dietary calcium intake by these patients and the role of calcium-vitamin D in bone health and fertility suggest possible routes for treatment of these patients. It appears that the combined use of these two drugs (calcium-vitamin D plus metformin) is more effective for correcting menstrual disorders and maturation of follicles than either drug alone. More widespread epidemiologic, laboratory and clinical studies on the role of calcium-vitamin D in follicular maturation in humans are needed to clarify their roles in oocyte maturation. Better understanding of these mechanisms would allow us to take more cost-effective steps in the treatment of PCOS patients.

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