Evaluation of Magnesium, Vitamin D, Calcium, and Metformin Supplements on Cardiometabolic Variables in Infertile Women Diagnosed with Polycystic Ovary Syndrome (PCOS): A Randomized, Controlled, Clinical Trial

CURRENT STATUS: UNDER REVIEW

Roghayeh Anvari Aliabad
Hamadan University of Medical Sciences Medical School

Mohsen Gharakhani
Hamadan University of Medical Sciences Medical School

Marzie Farimani
Hamedan University of Medical Science

dr_farimani@yahoo.com Corresponding Author
ORCiD: https://orcid.org/0000-0003-4161-5143

DOI: 10.21203/rs.3.rs-24236/v1

SUBJECT AREAS
- Preventive Medicine
- Internal Medicine

KEYWORDS
- polycystic ovary syndrome, Metabolic Syndrome, Metformin, Calcium, Magnesium
Abstract

Background

Polycystic ovary syndrome (PCOS) is known as an important etiology of ovulation failure which probably is related to resistance to insulin and other possible related metabolic states. The current study aimed to investigate the effect of supplements such as calcium, vitamin D, and magnesium on cardiometabolic risk factors of infertile PCOS patients.

Methods

This randomized trial was registered at Iranian Registry of Clinical Trial (reference code: IRCT2013110615307N1). First, bio-demographic data were gathered and complete physical examinations were performed, for all cases. After meeting the inclusion and exclusion criteria, 106 individuals were randomized into the metformin and vitamin D/calcium (37 patients), metformin and magnesium (34 patients), and metformin (35 patients) groups. After 12 weeks, cardiometabolic parameters including blood pressure, fasting blood sugar (FBS), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), and dehydroepiandrosterone-sulfate (DHEA-S) were compared among the mentioned groups. In order to compare treatment groups per protocol analysis was performed. All data were analyzed by SPSS and any p-value less than 0.05 was considered statistically significant.

Results

The results showed no significant difference in age, infertility duration, frequency of metabolic syndrome, and the cause and duration of infertility among the groups. Also, no statistically significant difference was found between patients diagnosed with (N=12, 35%) and without metabolic syndrome. Many of the metabolic factors (body mass index (BMI), blood pressure (BP), FBS, LDL, and cholesterol) were decreased significantly in all groups after the treatments (p<0.001). However, this difference was not statistically significant for HDL, DHEA-S, TG, and testosterone for either of the groups. The mean changes of HDL, testosterone, and LDL were statistically different among the groups (P-values= 0.005, 0.004, and 0.008 for group 1, 2, and 3, respectively). Also, in the metformin and magnesium group, the positive changes of some laboratory parameters were more than the other groups;
however, this difference was not statistically significant in most cases. Also, BMI and BP in metformin and magnesium group were more reduced, however, the difference was not statistically significant.

Conclusion
It seems that treatment with metformin and magnesium may have benefits for patients with PCOS but further clinical trials are necessary.

Background
Polycystic ovary syndrome (PCOS), with an estimated general prevalence of 3–10%, has been described as one of the most prevalent endocrine disorders in women (1). This phenomenon is initially accompanied by hyperandrogenism, menstrual cycle disorders, hirsutism, and infertility, which occurs in the form of (enlarged) ovaries containing cysts. PCOS is also known as the main cause of infertility due to anovulation. Other prevalent clinical signs of this syndrome include several metabolic disorders such as hypertension, dyslipidemia, metabolic syndrome, glucose tolerance and insulin resistance, and consequently hyperinsulinemia. Among these signs, hyperinsulinemia has been known as a key role player in the pathogenesis of the syndrome as it has been detected in 50–70% of affected cases. It should be noted that insulin resistance is independent of the weight in these patients, but obesity intensifies metabolic manifestations. In certain studies, obesity has been observed in more than 50% of patients diagnosed with PCOS (2, 3). On the other hand, dysfunction/malfunction in insulin secretion and its response not only increases the risk of diabetes mellitus type II (DM-II) and metabolic syndrome (4, 5), but is also considered as a major factor in occurrence of heart diseases; such way that cardiovascular risk factor levels are higher in these individuals compared to the control group (5).

In these patients, coronary artery disease, atherosclerosis, myocardial infarction, and stroke risks are higher than healthy controls (6). The excess secretion of insulin could induce ovarian dysfunction through hyperandrogenism and arresting the development of ovarian follicles (7). Androgen level is negatively associated with sex hormone-binding globulin production and secretion. As a result, testosterone and other free androgens increase dramatically in this situation which in turn increase cardiovascular risk factors (8).
Vitamin D, on the other hand, is crucial to the regulation of pathways involved in glucose metabolism and therefore, insulin secretion. Thus, its deficiency could lead to glucose intolerance, insulin resistance, and finally metabolic syndrome (7–11). It was shown in a meta-analysis that 67–85% of patients diagnosed with PCOS were suffering from vitamin D deficiency (sera level of 25-hydroxyvitamin D (25OHD) < 20 ng/mL) (9). This phenomenon may increase dehydroepiandrosterone-sulfate (DHEA-S) and insulin resistance (10).

According to the studies, low serum magnesium is linked to insulin resistance, hypertension, metabolic-syndrome, DM-II, and cardiovascular disorders (11, 12). It has been shown that low sera magnesium levels increase PCOS risk by up to 19 times (13).

Regarding the prevalence of PCOS in today’s lifestyle and the necessity of disease prevention, the tendency towards treatment with oral supplements has increased. Based on the points discussed earlier, the effectiveness of the above-mentioned supplements on cardiometabolic factors, and disagreements of the reports on the subject, we found it necessary to investigate the effectiveness of these supplements and comparing their therapeutic effects with one another. Results obtained from previous studies show the effectiveness of these supplements on cardiometabolic factors and reduction of chronic diseases risk. However, the increasing rate of cardiovascular diseases, DM, high blood pressure, anti-oxidant shortage, raised oxidative stress levels, pro-inflammatory and vascular endothelial damage in individuals with this syndrome highlights the importance of these supplements in individuals suffering from the above-mentioned conditions (14–16).

Accordingly, this study aims to investigate and compare the effects of three groups of treatments with the supplements of magnesium, vitamin D, and calcium on cardiometabolic variables in PCOS women with infertility.

**Methods**

**Study design**

This randomized, controlled, parallel group, single-blind clinical trial was performed on women between 20 and 40 years old who visited infertility clinic of Fatemiyeh Hospital (Hamadan University of Medical Sciences, Hamedan, Iran) for infertility between June 2014 and Jun 2015.

**Ethical Considerations**
The current study was approved Medical Ethics Committee of Hamadan University of Medical Sciences. All patients participated only voluntarily in this study and were asked (freely) to sign a written informed consent form. The patients were also provided with the required information according to their level of knowledge. They were charged no additional costs aside from the routine charges of the clinic and all of the medications and supplements were given free of charge. It is noteworthy to mention that all participants were allowed to quit the study freely as they will at any time while their information would remain confidential. All procedures and evaluations were performed in accordance with the guidelines laid down in the most recent revision of the declaration of Helsinki. This trial was registered in the Iranian Registry of Clinical Trials under the code of IRCT2013110615307N1.

Study protocol
This study used Rotterdam criteria for the diagnosis of patients with PCOS (17, 18). The inclusion criteria were defined as irregular/abnormal menstrual cycles (amenorrhea or oligomenorrhea), hyperandrogenism laboratory results or clinical symptoms (acne and/or hirsutism), and evidences of polycystic ovary detected on ultrasound evaluation.

Patients who met the inclusion criteria were studied for the defined exclusion criteria as well defined as follows: chronic diseases (rheumatoid arthritis, chronic kidney disease, etc), history of malignancy, pregnancy, smoking, pervious surgery of abdominal and/or pelvic cavity (both open and laparascopic), unusual finding in hysterosalpingography, abnormal prolactin level (sera), consumption of corticosteriods, insulin, statins, anti-obesity medications, and herbal supplements, up to last two months.

The simple sampling method was used for those who met both inclusion and exclusion criteria. Also, block-randomized method was applied for random assignment of participants to the treatment arms of calcium along vit D, magnesium, and control. Regarding the issue that metformin is a backbone particle of routine treatment in mentioned patients, all cases in each group received 1500 mg/day of metformin/day (PO) as well as their assigned supplements (execpt for the controls). The treatment took 12 weeks during which, the first group recieved 1000 mg/day of calcium and 400 IU/dose vitamin
D twice a day (800 IU/day). The second group received 75 mmol/day of magnesium while the third group was treated only with metformin (1500 mg/day).

Firstly, the demographic data were recorded for each patient as well as clinical finding such as systolic and diastolic blood pressure (BP) in the standard situation, weight, and height. Then, in order to evaluate baseline levels of laboratory parameters, before any intervention and after 8–12 hours of night fasting, blood samples were taken from all participants. The evaluated factors were fasting blood sugar (FBS), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), total cholesterol, testosterone, and dehydroepiandrosterone sulfate (DHEA-S). At the end of the 12 weeks, post-intervention blood samples were obtained from the participants for re-evaluation of laboratory variables under the same conditions the pre-intervention blood was gathered.

For the diagnosis of possible metabolic syndrome, Adult Treatment Panel III (ATP-III) were used according to the following criteria. Individuals who met 3 ≤ of follow criteria (below) were considered positive.

1. A waist circumference 102< and 88< cm in men and women, respectively.
2. Elevated TG level (150<) or consumption of any medication for hypertriglyceridemia.
3. Decreased HDL level (40>) or consumption of any medication for low HDL
4. Increased FBS level (100<).
5. Increased systolic and diastolic BP (130< and 80< mmHg, respectively) or consumption of any medication for hypertension.

**Statistical Analysis**

The collected data were analyzed by Statistical Package for Social Sciences version 22.0 (SPSS, Inc., Chicago, IL, USA). Results were reported as percentage and mean ± standard deviation (SD). Any P-value ≤ 0.05 was considered statistically significant.

**Results**

Among 114 individuals who met the inclusion criteria, 106 were enrolled to the study after an evaluation for the already mentioned exclusion criteria. From these 106 cases, 37, 34, and 35 individuals were placed in the metformin and calcium/vitamin D group, metformin and magnesium
group, and metformin group (control), respectively. Due to headache and the recommendation by an internal specialist, one patient in the calcium/vitamin D and metformin group left the study. Moreover, among the patients in the magnesium and metformin group, three and two individuals left the trial due to unwillingness for follow-up and discontent. In the control group, three, two, and one patients discontinued their treatment due to diarrhea, unwillingness to consume the given medication, and pregnancy (through intracytoplasmic sperm injection or ICSI), respectively. Finally, the trial reached its final numbers with 36, 35, and 30 individuals in calcium + vitamin D and metformin, magnesium and metformin, and control groups, respectively.

The average age of the patients in the and calcium + vitamin D along metformin, magnesium and metformin, and the metformin single therapy (control) groups were calculated 26.75, 28.82, and 27.49 years, respectively. There was no statistically significant difference among the groups age-wise (P-value = 0.147). Results of other demographic variables are provided in Table 1.

As it was shown, metabolic syndrome (ATP-III criteria) was diagnosed for 12 patients (35%). Among these 12 patients, two (17%), seven (58%), and three (25%) were in the calcium + vitamin D along with metformin, magnesium and metformin, and metformin monotherapy groups, respectively (P-value = 0.107).

As mentioned, different variables (including both physical examinations and laboratory assessments) were investigated in this trial. The data illustrated in Table 2 expresses any changes in these variables before and after the intervention. In this regard, a statistically significant decrease in LDL was found in magnesium and metformin group when compared to the other groups (P-value = 0.008). Also, in magnesium and metformin group, HDL and testosterone levels showed a statistically

| Groups                        | Age (years)     | Height (cm)    | Weight (kg)   | Waist Circumference (cm) | Hip Circumference (cm) | Duration of infertility (years) |
|-------------------------------|-----------------|----------------|---------------|--------------------------|------------------------|--------------------------------|
| Metformin & Calcium/Vit-D     | 26.76 ± 5.00    | 161.70 ± 3.79  | 69.96 ± 10.58 | 82.59 ± 8.99              | 103.59 ± 8.10          | 4.01 ± 2.80                   |
| Metformin & Magnesium         | 28.82 ± 4.78    | 161.38 ± 6.10  | 72.71 ± 12.93 | 89.38 ± 10.44             | 106.74 ± 12.14         | 3.94 ± 2.91                   |
| Metformin                     | 27.00 ± 4.49    | 158.80 ± 5.18  | 67.54 ± 12.81 | 80.09 ± 11.18             | 101.00 ± 11.91         | 5.01 ± 3.94                   |

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significant increase in comparison to the other two groups (P-value = 0.005 and 0.004, respectively).

Other variables didn’t show any significant changes among the evaluated groups.

Table 2
The changes in the studied parameters among the three groups compared to the pre-treatment status.

| Groups                | BMI (kg/m²)     | SBP (mmHg)     | DBP (mmHg)     | FBS (mmol/L)  | LDL (mg/dL)    | Total cholesterol (mg/dL) | HDL (mg/dL) | TG (mg/dL) | Testosterone (nmol/L) | DHEA-S (µmol/L) |
|-----------------------|-----------------|----------------|----------------|---------------|----------------|------------------------|-------------|-------------|----------------------|----------------|
| Metformin & Calcium/Vit-D | -0.41 ± 0.50*   | -3.92 ± 9.66*  | -2.36 ± 6.81*  | -3.50 ± 5.85* | -5.31 ± 6.94*  | -23.36 ± 26.79*         | 2.39 ± 3.13 | -4.90 ± 30.16 | -0.08 ± 0.21          | 0.02 ± 0.25      |
| Metformin & Magnesium  | -0.57 ± 0.44*   | -5.00 ± 6.53*  | -4.46 ± 5.67*  | -6.25 ± 10.05*| -11.14 ± 13.53*| -11.96 ± 20.51*         | 1.21 ± 5.04 | -9.96 ± 28.31 | 0.19 ± 0.48           | 0.14 ± 0.77      |
| Metformin             | -0.38 ± 0.56*   | -4.22 ± 5.25*  | -3.59 ± 5.42*  | -5.27 ± 3.53* | -4.37 ± 4.08*  | -15.10 ± 15.92*         | -0.98 ± 4.13| -8.70 ± 11.60 | 0.03 ± 0.19           | 0.02 ± 0.14      |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; DHEA-S, dehydroepiandrosterone-sulfate. *: Statistically significant difference in comparison to the baseline levels (P-value < 0.001).

Discussion

Patients with PCOS are exposed to an increased risk of diabetes, obesity, cardiovascular diseases, and dyslipidemia. While POCS itself is now considered a disorder, its multiple etiology and being always accompanied by metabolic consequences increases the risk of occurrence of metabolic syndrome, obesity, and cardiovascular diseases (3).

The relationship between diet and its components with PCO has been long asserted. According to the studies, it is suggested that consuming high glycemic index food and saturated fatty acids are associated with an increased risk of PCO and subfertility in women (19). According to studies, the role of diet therapy, including reducing simple sugars (using foods of low glycemic index), limiting saturated and trans fatty acid intake, and consumption of supplement (such as vitamin D) in is very important in patients diagnosed with PCO (20).

In this study, the effects of 3 different supplementary-including treatments of calcium + vitamin D and metformin, magnesium and metformin, and metformin monotherapy on patients with PCOS were examined. A wide range of variables including body mass index (BMI), BP, LDL, cholesterol, and FBS showed a significantly decreasing pattern in all the three mentioned groups after the treatments (P-value < 0.001). However, levels of HDL, DHEA-S, TG, and testosterone had no statistically significant change after the 12-week treatment period in any of the studied groups. Furthermore, among all groups, HDL, testosterone, and LDL were statistically different (Table 2). Also, observed positive
changes in the laboratory variables (such as LDL, HDL, and testosterone) appeared to be higher in the magnesium and metformin group when compared to the other groups; however, the differences didn’t reach the statistically cut-off point (P-value ≤ 0.05) for most variables. This condition in the mentioned group was in concordance with BMI and BP.

As it has been shown by Akgül and Bonny (21), Kyrkou et al., (22), Abbasi-Ranjbar et al., (23), and Le et al., (24), the incidence of metabolic syndrome in patients diagnosed with PCOS have been reported to be 45.9% (United State), 12.6% (Greece), 28.8% (Iran), and 10.4% (Vietnam), respectively. Although different prevalence of metabolic syndrome among patients with PCO are available, according to the region within this study was performed, a higher prevalence (35% versus 28.8%) of metabolic syndrome was detected. There was also no significant difference between evaluated parameters in the patients diagnosed with and without metabolic-syndrome in the current study.

In a case-control study by Firouzabadi et al., 100 infertile women with PCOS were examined for 6 months. The control group received 1500 mg/day of metformin and the test group received 1000 mg/day of calcium and 100000 IU/month of vitamin D/day accompanied with 1500 mg/day of metformin. It was finally observed that these two supplements along with metformin have positive effects on weight loss, follicular maturation, and recovery from hyperandrogenism in these women (25).

As Szczuko et al., shows, the level of minerals in patients diagnosed with PCOS is lower than normal healthy controls. As their study illustrates, these patients were at the risk of calcium, potassium, and magnesium deficiency (26). Also, according to Sharifi et al., decreased hypo-magnesiumia was associated with an increased risk of PCOS up to 19 folds (13).

In the current trial, it was demonstrated that the evaluated variables endured more positive changes in the magnesium and metformin group in comparison to the other groups. However, the mentioned changes were not statistically significant for most variables except for HDL, LDL, and testosterone (Table 2).

In a prospective study lasted 20 years, Kim et al., prescribed magnesium for 4497 Americans with no diabetes history among whom 330 diabetic cases were reported. It was then revealed that the use of
magnesium was significantly and inversely associated with diabetes incidence, insulin resistance, and systemic inflammation (27).

In a review study by Champan (2008), sufficient dosage of magnesium was proved effective on blood pressure control, weight loss, and improvement of risk of chronic diseases (28).

Upon examining nine RTC studies in the form of a meta-analysis, Song et al., (2006) concluded that prescription of a daily dose of 360 milligrams of oral magnesium for 4 to 16 weeks increases HDL levels and significantly decreases fasting glucose levels while not changing HbA1C, SBP and DBP, TG, total cholesterol, and LDL levels (29).

Conclusion
In this study, we examined the effects of simultaneous consumption of calcium, vitamin D, and magnesium as supplements in patients with PCOS. Our results affirmed the effectiveness of using these supplements in patients. The post-treatment increased rate of HDL was significantly higher in the metformin & calcium/ vitamin D group patients compared to the metformin group patients.

Regarding the other parameters studied, except for some cases in the metformin & calcium/ vitamin D group with better conditions than the metformin group, there was no significant difference between the patients in the two groups. Moreover, in the patients receiving Magnesium, the rates of the positive changes in the studied parameters were higher compared to the other groups. Although this difference was not statistically significant in most cases, in the case of LDL, HDL, and testosterone, the rates of the positive changes in the metformin and magnesium group patients were significantly better than in the other two groups. The decrease rates of BMI and BP were higher in the patients receiving magnesium, but this difference was not statistically significant.

A total of 12 patients among all cases had a history of metabolic-syndrome before the study. There was no significant difference between rates of changes in the parameters under study in patients with and without metabolic syndrome.

List Of Abbreviations

BMI; body mass index, BP; blood pressure, SBP; systolic blood pressure, DBP; diastolic blood pressure, PCOS; polycystic ovarian syndrome, TG; triglyceride, LDL; low-density lipoprotein, HDL; high density
lipoprotein, DHEA-S; dehydroepiandrosterone-sulfate.

Declarations

Ethics approval and consent to participate
This study was approved Medical Ethics Committee of Hamadan University of Medical Sciences. All patients participated only voluntarily in this study and were asked to sign a written informed consent form. The patients were also provided with the required information according to their level of knowledge. This trial was registered in the Iranian Registry of Clinical Trials under the code of IRCT2013110615307N1.

Consent for publication
This section is not applicable to the current study.

Availability of data and material
The datasets used and/or analyzed during the current study could become available through the corresponding author on reasonable request.

Funding
The authors declare that the current study was conducted in the absence of any commercial or financial relationships.

Authors’ contributions
MF and MG contributed to the conceptual design of the study. MF and RA designed the project. RA contributed to data analysis. As well as data collection and writing the draft, all authors have read and approved the final manuscript.

Acknowledgments
The authors deeply acknowledge all the patients who contributed to this study. Also, we thank colleagues at the Clinical Research Development of Hamadan Fatemieh Hospital who provided insight and expertise that greatly assisted the research.

Competing interests
Authors declare no actual or potential competing interests related to this study.

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Figures

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