Randomised Controlled Trial

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The beneficial influences of vitamin D intake on inflammation and oxidative stress in infertile women with polycystic ovary syndrome

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Objective: Oxidative stress and inflammation play a vital function in the pathophysiology of polycystic ovary syndrome (PCOS) and infertility. The aim of this work was to control the impacts of vitamin D intake on metabolic profiles in infertile subjects with PCOS.
Trial design and methods: This randomized, double-blinded, placebo-controlled clinical trial was carried out among 40 infertile women with PCOS. Subjects were randomly divided into two intervention groups to take either 50 000 IU vitamin D ($n=20$) or placebo ($n=20$) weekly for 8 weeks. Metabolic profiles and few inflammatory cytokines expression evaluated on peripheral blood mononuclear cells (PBMCs) of participants, using real-time polymerase chain reaction (RT-PCR) method.
Results: Vitamin D intake decreased high-sensitivity C-reactive protein (hs-CRP) (-0.9 ± 1.1 vs. 0.3 ± 0.9 mg/l, $P=0.002$) and elevated total antioxidant capacity (TAC) levels (49.2 ± 60.2 vs. -50.6 ± 161.8 mmol/l, $P=0.02$) compared with placebo; but no significant effects on other metabolic parameters were observed. Moreover, a significant downregulation of tumor necrosis factor alpha (TNF-α) expression ($P=0.03$) was observed after taking vitamin D compared with the placebo.
Conclusions: Overall, vitamin D intake for eight weeks had beneficial impacts on hs-CRP, TAC, and TNF-α among infertile women with PCOS.

Keywords: inflammation, oxidative stress, polycystic ovary syndrome, vitamin D intake

Introduction

Background

Polycystic ovary syndrome (PCOS) is an endocrine disorder in woman with a prevalence of 5–20%[1]. Its main characteristics are hyperandrogenemia (HA), insulin resistance (IR), obesity, oligo/anovulation, and/or polycystic ovarian morphology[2]. The possibilities of conception is decreased in PCOS women and the risk of complications during pregnancy is increased[3]. The pathogenesis of PCOS may be due to hyperandrogenemia, oxidative damage and inflammation together, play a main role in its pathophysiology[4,5]. Inflammation is a risk feature of the pathogenesis and expansion of this syndrome[4], which has an important role in female infertility[6]. In addition, several studies confirmed the existence of oxidative stress in PCOS women because aberrant circulating oxidative stress markers has been detected in these patients[7]. Furthermore, reactive oxygen species (ROS) has been established to play an indispensable part in the pathophysiology of subfertility in females[8]. Controlled and adequate ROS levels exert physiologic functions which is an important mediator of conception in females[8].

The occurrence of vitamin D insufficiency in PCOS patients was reported about 67–85%[3]. It was reported that hypocalcemia and vitamin D deficiency have an effect on female reproductive function[9]. Vitamin D is a vital antioxidant for humans because it has the ability of controlling oxidative stress, systemic inflammation, and mitochondrial respiratory function[10]. Evans *et al.*[11] shown that $25OHD_3$ and $1,25(OH)_2D_3$ decrease synthesis of pro-inflammatory cytokines like interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and IL-6 in isolated uterine natural killer cells. Another in-vitro study reported that vitamin D repressed human monocytes production of IL-6 and TNF-α[12]. Vitamin D may have antioxidant

HIGHLIGHTS

- This randomized double-blind, placebo-controlled trial was conducted on 40 infertile polycystic ovary syndrome (PCOS) women candidates for in-vitro fertilization (IVF).
- Eight-week vitamin D supplementation decreased tumor necrosis factor alpha (TNF-α) expression in infertile PCOS women candidates for IVF.
- Vitamin D supplementation reduced inflammation and oxidative damage.

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properties by modulating some antioxidant defense enzymes[13]. Nitric oxide (NO), malondialdehyde (MDA), xanthine oxidase and advanced glycosylated end products (AGEs) increase in women with PCOS[14]. A meta-analysis reported a significant improvement in MDA, high-sensitivity C-reactive protein (hs-CRP) and total antioxidant capacity (TAC) levels in PCOS patients by receiving vitamin D supplement, while no notable effects were reported for glutathione (GSH) and NO levels[15]. Further, vitamin D plus calcium intake reduced oxidative stress and hs-CRP[16]. Moreover, Zhao *et al.*[17] reported that vitamin D intake could regulate TAC, hs-CRP, and MDA values in PCOS women without change in NO and GSH levels. But another study reported no significant change in hs-CRP by receiving vitamin D[18]. Vitamin D supplementation in PCOS patients candidate for in-vitro fertilization (IVF) improved clinical pregnancy[19].

Objectives

Although the issue of whether vitamin D supplementation has a healing impact on hormones, oxidative stress and inflammation has attracted a lot of attention, but the results have remained debatable[17]. So, this work was aimed to control the impacts of vitamin D intake on biomarkers of oxidative stress and inflammation in infertile women with PCOS.

Methods

Trial design

The research design for this work was a clinical trial using the gold standard of randomization and blinding.

Participants

Subjects included 40 infertile women between the ages of 18 and 40 who had been identified with PCOS consistent with the Rotterdam criteria[20] and who were considering IVF as a treatment option. Between December 2018 and March 2019, people were scouted (recruitment date: December 2018–January 2019 and intervention date: January 2019 to March 2019). People who used anti-diabetic drugs like metformin, as well as hormone and anti-obesity medications, were not allowed to participate. Those with cardiovascular disease, neoplastic disorders, or malabsorptive disorders were also excluded. Before any procedures were performed, all subjects signed an informed consent form.

Interventions

Vitamin D ($n = 20$) or a placebo ($n = 20$) was given to participants on a weekly basis for 8 weeks. All participants were stratified randomization according to BMI (< 25 and ≥ 25 kg/m^2) and age (< 30 and ≥ 30 years). Vitamin D and placebos (sunflower oil) were both manufactured by different firms, but they were indistinguishable from one another in every respect (shape, size, color and packaging). Dosage of medication (Gonal-F therapy) administered based on international protocol, and there was no significant change between two groups.

Outcomes

The principal measure was total testosterone, while secondary measurements were defined as changes in other metabolic markers.

Biochemical assessment

At the start and finish of the experiment, 15 ml of fasting blood were taken. An ELISA kit (IDS, Boldon, UK) was used to measure 25-hydroxyvitamin D concentrations, with coefficient variations (CVs) of less than 7% between individual assays. Inter- and intra-assay CVs for the measurement of serum sex hormone-binding globulin (SHBG) and total testosterone were both less than 5%, thanks to the use of Elisa kits (DiaMetra, Milano, Italy). Inter- and intra-assay CVs for measuring Hs-CRP levels were both less than 7%, thanks to the use of an ELISA kit (LDN). Inter- and intra-assay CVs of less than 5% were achieved when measuring plasma NO with the Griess method[21], GSH using the method of Beutler *et al.*[22], method, TAC with the ferric reducing antioxidant power developed by Benzie and Strain[23], and MDA values with spectrophotometric test[24] were determined with inter- and intra-assay CVs lower than 5%.

RNA extraction and real-time polymerase chain reaction (RT-PCR)

Quantitative RT-PCR was performed on PBMCs to quantity IL-1, TNF-α and IL-8 expression using Light Cycler technology (Roche Diagnostics) and SYBR green detection with an Amplicon Kit (Table 1). Lymphocytes were isolated from blood samples using 50% percoll (Sigma-Aldrich). Extractions of RNA and DNA were made, and trypan blue was used to count and viably test cells[25]. Blood samples were processed using an RNX-plus kit for RNA extraction. In order to extract cDNA, the RNA solution was kept at −20 °C. Each sample's total RNA was measured using UV spectrophotometer. There was no evidence of protein or DNA contamination because the OD 260/280 ratios for all of the samples ranged from 1.7 to 2.1[25]. Using moloney murine leukemia virus reverse transcriptase, the obtained RNA was transformed into a cDNA library (RT). Primers targeting the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were utilized. Primers were created with the use of Beacon designer and Primer Express software (Applied Biosystems).

Sample size

Considerations for type one error (α) of 0.05, type two error (β) of 0.20, and 80% power were used to determine the required number of participants for this clinical research. Based on this, we needed 16 patients in per group. Assuming a dropout of 4 patients in each group, we calculated to have 20 subjects per group.

Table 1

Specific primers used for real-time quantitative PCR.

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG	126	61.3
	R: TCTTCCTCTTGTGCTCTTGCTGG		
IL-1	F: GCTTCTCTCTGGTCCTTGG	174	56
	R: AGGGCAGGGTAGAGAAGAG		
IL-8	F: GCAGAGGGTTGTGGAGAAGT	150	56
	R: ACCCTACAACAGACCCACAC		
TNF-α	F: GTCAACCTCCTCTCTGCCAT	188	52
	R: CCAAAGTAGACCTGCCCAGA		

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin-1; IL-8, interleukin-8; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor α.

Randomization and treatment allocation

Using a random number table, we assigned duties at random. Participants' 25(OH)D values were measured and supplement containers were collected to calculate the compliance rate. Each patient established a short text message on their phone every day to remind them to intake their supplements, which significantly increased the compliance rate.

Implementation

A trained staff at the clinic, who was not involved in the trial and not aware of assignments, assigned numbered packages of vitamin D and placebos to the subjects.

Blinding

The researchers and the patients had their allocations kept secret by randomization and concealment until the analyses were complete.

Statistical methods

To check if the variables were distributed normally, the Kolmogorov–Smirnov test was carried out. We compared the two intervention groups to assess metabolic profiles, anthropometric parameters, and gene expression of inflammatory markers with an independent samples *t*-test. *P* values below 0.05 were viewed as substantial statistical markers.

Results

Participant flow and recruitment

Totally, 40 persons randomly randomized to two groups receiving: (1) vitamin D ($n=20$) and (2) placebos ($n=20$). Among patients in the vitamin D group, 3 patients (withdrawn due to personal reasons) and in the placebo group, 3 persons (withdrawn due to personal reasons) were excluded (Fig. 1). Finally, 34 participants [vitamin D ($n=17$) and placebo ($n=17$)] completed the trial. In this study, the compliance rate was between 90% and 100% across both groups.

Baseline data and numbers analyzed

Mean age, weight and BMI at baseline and end-of-trial, and weight and BMI change of study participants were not statistically different between the two groups (Table 2).

Outcomes and estimation

TAC levels were significantly higher (49.2 ± 60.2 vs. 50.6 ± 161.8 mmol/l, $P=0.02$) and hs-CRP levels were significantly lower (-0.9 ± 1.1 vs. 0.3 ± 0.9 mg/l, $P=0.002$) after vitamin D supplementation compared with the placebo (Table 3). Other metabolic indicators showed no discernible change after vitamin D supplementation.

Also, a significant downregulation of TNF-α gene expression (0.95 ± 0.15 vs. 1.07 ± 0.14-fold change, $P=0.03$) was observed after taking vitamin D supplements, compared with the placebo, in PBMCs of infertile women with PCOS (Fig. 2). We didn't observe any significant impact on IL-8 and IL-1 expression following vitamin D intake.

Harms

Women with PCOS who were unable to conceive naturally were given vitamin D supplements in this study with no reported adverse effects.

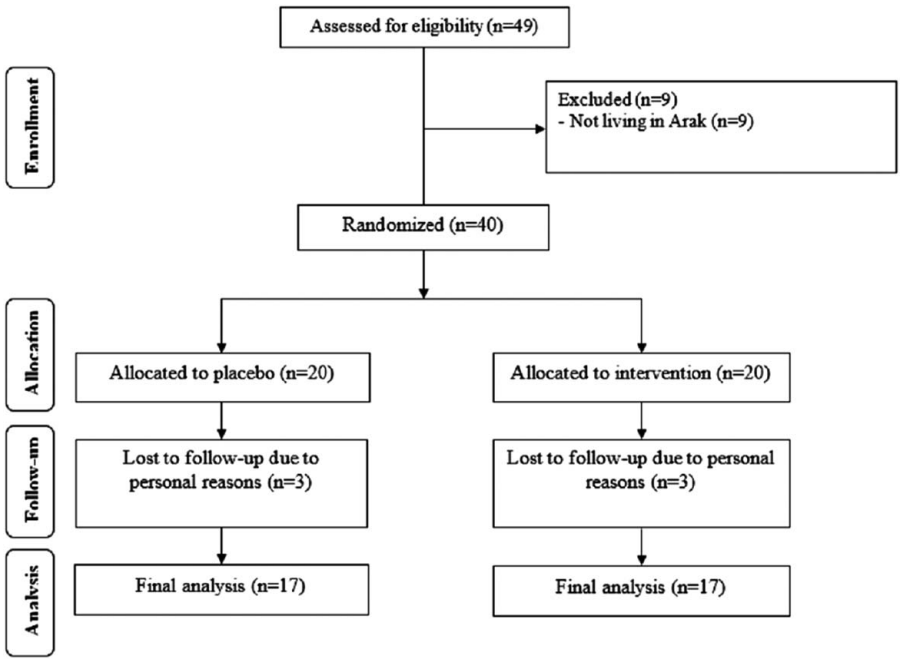

Figure 1. Summary of patient flow diagram.

Table 2

General characteristics of study participants.

	Placebo group ($n=17$)	Vitamin D group ($n=17$)
Age (year)	30.2 ± 3.3	29.2 ± 3.8
Height (cm)	159.1 ± 2.2	160.9 ± 3.2
Weight at study baseline (kg)	71.5 ± 7.4	71.4 ± 10.7
Weight at end-of-trial (kg)	71.7 ± 7.3	71.6 ± 10.3
BMI at study baseline (kg/m^2)	28.3 ± 2.8	27.6 ± 4.1
BMI at end-of-trial (kg/m^2)	28.3 ± 2.8	27.7 ± 3.9

Data are means ± SDs.

Discussion

Generalizability

This work demonstrated that vitamin D intake for eight weeks had beneficial impacts on hs-CRP, TAC, and TNF-α among infertile subjects with PCOS.

Interpretation

Oxidative stress and inflammation have key roles in the pathophysiology of PCOS[17]. Previously, the beneficial properties of vitamin D intake on metabolic values among subjects with PCOS and other diseases were reported[26–29]. Consistent with our findings, Sharifi *et al.*[30] showed that 50 000 IU vitamin D_3/2 weeks for 4 months decreased hs-CRP in persons with non-alcoholic fatty liver disease. Further, Karonova *et al.*[31] reported that vitamin D intake (40 000 IU/week) for 24 weeks ameliorated some inflammatory cytokines in people with type 2 diabetes. Duggan *et al.*[32] demonstrated that vitamin D supplements (2000 IU/day) for 12 months among postmenopausal women has no beneficial effect on IL-10, IL-1B, IL-8 and TNF-α levels. In another study, no significant changes in some inflammatory markers were seen by taking vitamin D supplements (1000, 2000, or 4000 IU/day for three months) among African Americans population[33]. Inflammatory cytokines may trigger endothelial cell damage and dysfunction in PCOS[34]. Vitamin D has a key role in changeable the creation of inflammatory markers and inhibiting the proliferation of pro-inflammatory cells to modulate the immune/inflammation system[35]. It also plays several other anti-inflammatory effects such as inhibiting prostaglandin action and nuclear factor kappa B (NF-κB) signaling paths[36].

Similar to our findings, Zhang *et al.*[37] exhibited that vitamin D intake (50 000 IU/2 weeks) significantly elevated GSH and TAC levels in women with gestational diabetes mellitus. Also, Cavalcante *et al.*[38] exhibited that supplementation with 200 000 IU vitamin D_3 increased TAC and reduced ultra-sensitive-CRP concentrations in elderly subjects with vitamin D inadequacy after 4 weeks; but no change in MDA levels were detected. Moreover, Heidari *et al.*[39] shown that vitamin D intake (50 000 IU fortnightly for 4 months) increased TAC levels and decreased some inflammatory cytokines in premenstrual syndrome. Similarly, another study showed that 50 000 IU vitamin D_3/weekly for eight weeks significantly increased TAC levels among breast cancer women[40]. In contrast, Mamede *et al.*[41] demonstrated that a single-oral megadose of vitamin D_3 did not improve oxidative damage and inflammation markers levels in obese or overweight women who had a deficiency or insufficiency of vitamin D. Likewise, vitamin D_3 supplementation (50 000 IU/week)significantly reduced MDA concentrations; but the increase in TAC level was nonsignificant in type 2 diabetes[42]. Oxidative stress can cause a number of reproductive diseases like PCOS. The imbalance between pro-oxidants and antioxidants can be an important factor of pregnancy complications including recurrent pregnancy loss, spontaneous abortion, and preeclampsia[43]. On the other hand, vitamin D deficiency disturbs mitochondrial function by decreasing oxygen consumption rate. The exact mechanism of regulating oxidative stress via vitamin D is not yet clarified. In total, vitamin D might directly influence the mitochondrial reactive oxygen species creation by regulating mitochondrial dynamics and function[44].

Limitations

Limitations of this work were: Due to financial limitations, we could not evaluate gene expression related to biomarkers of oxidative stress. The period of the intervention and sample size were low. Future works with a period and larger sample size are required to approve our findings. In addition, in the current study, the range of hs-CRP in most participants was normal. This should be considered in the interpretation of our finding. We believe that further studies should be conducted in different ranges of hs-CRP among infertile women with PCOS. The main outcome of our study was total testosterone. We suggest that further studies should be conducted according to biomarkers of oxidative stress and inflammatory markers. Also, we did not

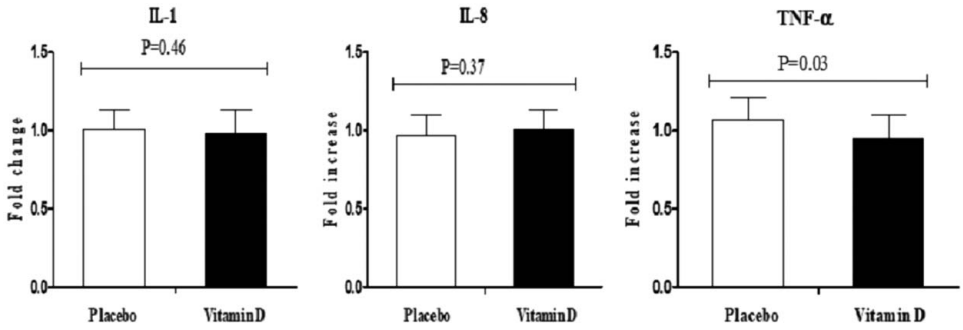

Figure 2. Effect of the 8-week supplementation with vitamin D or placebo on expression ratio of IL-1, IL-8 and TNF-α gene in PBMCs of patients with polycystic ovary syndrome candidate for in-vitro fertilization. IL-1, interleukin-1; IL-8, interleukin-8; TNF-α, tumor necrosis factor alpha.

Table 3

Changes in the levels of metabolic profiles in two groups of patients with polycystic ovary syndrome candidate for in-vitro fertilization.

	Placebo group ($n=17$)			Vitamin D group ($n=17$)			
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	P^a
Vitamin D (ng/ml)	9.3 ± 1.8	9.2 ± 1.9	− 0.1 ± 0.7	10.0 ± 1.9	19.3 ± 4.9	9.3 ± 4.7	< 0.001
Total testosterone (ng/ml)	1.2 ± 0.4	1.3 ± 0.6	0.1 ± 0.6	1.0 ± 0.3	0.9 ± 0.4	− 0.1 ± 0.4	0.35
SHBG (nmol/l)	49.1 ± 18.0	51.3 ± 16.9	2.2 ± 13.2	53.3 ± 13.4	56.3 ± 17.4	2.9 ± 13.8	0.86
hs-CRP (mg/l)	3.4 ± 0.6	3.7 ± 1.0	0.3 ± 0.9	3.6 ± 1.4	2.7 ± 1.2	− 0.9 ± 1.1	0.002
NO (μmol/l)	40.2 ± 3.4	38.4 ± 4.2	− 1.8 ± 3.9	39.3 ± 5.3	37.8 ± 6.3	− 1.5 ± 2.8	0.78
TAC (mmol/l)	694.9 ± 57.2	644.4 ± 160.4	− 50.6 ± 161.8	679.8 ± 179.1	729.1 ± 203.2	49.2 ± 60.2	0.02
GSH (μmol/l)	421.4 ± 42.7	434.8 ± 92.8	13.8 ± 85.2	409.9 ± 41.3	408.3 ± 44.3	− 1.6 ± 35.5	0.51
MDA (μmol/l)	2.7 ± 0.4	2.8 ± 0.4	0.1 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	0.03 ± 0.2	0.57

All values are means ± SDs.

GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; SHBG, sex hormone-binding globulin; TAC, total antioxidant capacity.

[a]*P* values represent independent *t*-test.

evaluate serum vitamin D concentrations at the study baseline. Serum vitamin D concentrations were measured at the end-of-trial to assess compliance to vitamin D supplements.

Conclusions

Overall, we established that vitamin D intake for eight weeks had beneficial effects on hs-CRP, TAC, and TNF-α among infertile women with PCOS.

Ethical approval

The research was approved by the ethics committee of AUMS. Ethics committee reference number: IR.ARAKMU.REC.1397.246. Approval date: 2018-12-16. IRCT code: IRCT20170513033941N53. IRCT record: https://www.IRCT20170513033941N53. Link: https://irct.behdasht.gov.ir/trial/38409

Consent

Before any procedures were performed, all subjects signed an informed consent form.

Source of funding

The current study was founded by a grant from the Vice-chancellor for Research, AUMS, and Iran.

Author contribution

S.A.-A., R.A., E.A. and M.J. involved in drafting and data collection. All authors accepted the final version.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Research registration unique identifying number (UIN)

1. Registry used: IRCT20170513033941N53
2. Unique Identifying number or registration ID: IRCT20170513033941N53
3. Hyperlink to your specific registration: https://irct.behdasht.gov.ir/trial/38409

Guarantor

Guarantor is Mehri Jamilian.

Data availability statement

The datasets analyzed and/or used during the present study are obtainable from the corresponding author on rational request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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