Effect of Vitamin D Supplementation on Phenotypic Characteristics of Polycystic Ovary Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome is considered as the most common endocrine disorder in women of reproductive age (15-44) years. Most polycystic ovary syndrome women exhibit insulin resistance syndrome, which is worsened by hyperandrogenism-related adipose tissue accumulation. Deficiency of vitamin D may aggravate hyperandrogenism and insulin resistance.

Objectives: This study was designed to investigate the role of vitamin D supplement in improvement of phenotypic properties of women with polycystic ovary syndrome including mainly body mass index and insulin resistance.

Methods: This analytical experimental design study included 25 women diagnosed by specialist gynecologist to have had polycystic ovary syndrome with an age range of 18-40 years, without any infertility related treatment for the last two months. These women were only treated with vitamin D3 tablet (50,000 IU/wk.) for 8 weeks. Serum investigations included measurements of fasting serum glucose, insulin, 25 hydroxyvitamin D, luteinizing hormone, follicle-stimulating hormone, prolactin and estradiol before and after vitamin D supplement using enzyme linked immunosorbent assay technique. Homoeostasis model assessment-insulin resistance (HOMA-IR) was calculated and body mass index and waist circumference were also measured and calculated.

Results: The mean value of serum vitamin D as measured by 25 hydroxyvitamin D was significantly corrected and increased after 8 week of treatment ($p < 0.05$). The results of this study found that the mean values of body mass index, waist circumference, and insulin resistance index (HOMA-IR) were decreased and improved after vitamin D supplement ($p < 0.05$). In addition, the mean value of serum LH, FSH and prolactin were significantly lowered after treatment with vitamin D supplement ($p < 0.05$).

Conclusion: Vitamin D with appropriate doses could be used safely in correction of phenotypic characteristics (body mass index, waist circumference and insulin resistance) of polycystic ovary syndrome women and can replace other drugs.

Keywords: Polycystic ovary syndrome, Body mass index, Homeostatic model assessment for insulin resistance, Vitamin D.

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Polycystic ovary syndrome (PCOS) is considered as the most common endocrine disorder in women of reproductive age (15-44) years, with a prevalence of 5-20%[1,2]. Rotterdam criteria represented as the most widely accepted, appropriate and important diagnostic criteria for PCOS, which required that women fulfill at least two of the following three criteria: oligo or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries on ultrasound[2,3].

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Polycystic ovary syndrome is heterogeneous, and women may present with several of reproductive, endocrine, metabolic and psychosocial symptoms[1,3]. Today, 50-80% of patients with PCOS exhibit insulin resistance syndrome, which is worsened by hyperandrogenism-related adipose tissue accumulation and is involved in both the pathogenesis and the progression of the disease[2,4].

Recently, there has been a focus on vitamin D supplementation as an adjunct of PCOS. Many studies have demonstrated that vitamin D deficiency is common among women with PCOS, with the prevalence rate being as high as 67-85%, having serum

25-hydroxyvitamin D concentrations of <20 ng/ml[5,6]. Adequate vitamin D levels (≥30 ng/ml) should be required in women with PCOS. Deficiency of vitamin D may aggravate hyperandrogenism and insulin resistance. Otherwise, lower pregnancy rate, ovulatory and menstrual irregularities, hirsutism, elevated cardiovascular disease risk factors, obesity and infertility are subsequent challenges encountered during the periods of low vitamin D status[5,7].

Vitamin D, as an orally, relatively safe and inexpensive vitamin, could help to treat common ovulation dysfunction in PCOS by promoting follicular development and improve menstruation, and it may be used in infertility in all women of childbearing age[8,9].

This study was designed to investigate the role of vitamin D supplement in improvement of phenotypic properties of women with PCOS including mainly body mass index and insulin resistance and ability to use it in treatment of this syndrome.

Methods

This analytical experimental design study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad, and at Kamal AlSamarraei Hospital for Infertility Management and In Vitro Fertilization, during the period from September 2022 to June 2023. It included 25 infertile women who priory diagnosed with PCOS with age range 18-40 years, without any infertility related treatment for two months at least.

These women were treated with vitamin D3 tablet (50,000 IU/wk) for 8 weeks.

Ethical approval of this study was obtained from the Scientific Committee of Department of Biochemistry, College of Medicine, University of Baghdad along with that obtained from Ministry of Health, Iraq and from each participant woman.

The patients' conditions were diagnosed by a gynecologist after proper physical, biochemical and gynecological examinations and confirmed by ultrasound. According to Rotterdam consensus; PCOS is defined by the presence of two of three of the following criteria: oligo and/or anovulation, hyperandrogenism and polycystic ovaries (≥ 12 follicles measuring (2-9) mm in diameter and/or an ovarian volume > 10 ml in at least one ovary)[10].

This study excluded women with any type of cancer, acute and chronic illness, diabetes, chronic liver disease, pregnant women, smokers, endocrine disorders and chronic renal failure.

Blood samples were withdrawn from each included women after 10-12 hours of overnight fasting state, in the follicular phase between the 2nd and 7th day of the menstrual cycle before starting of their designed treatment and after that, between 08:00 and 10:00 hr. a.m. from an antecubital vein after 5 minutes rest in the supine position. The blood samples were separated by centrifugation at 3000 rpm for 5 minutes, to obtain serum after remain to clot at room temperature for 10-15 minutes. All the resulting serum samples (25 patients before and 13 patients after treatment) were aliquot, frozen and maintained at -20 °C. Measurement of fasting serum glucose and insulin (The electrochemiluminescence immunoassay "ECLIA" is intended for use on Auto analyzer by Cobas e 411, c 111 Roche Switzerland company immunoassay analyzers), 25 hydroxyvitamin D, luteinizing hormone (LH) and Estradiol (E2) (competitive enzyme immunoassay technique by Cloud-Clone USA and ELK Biotechnology Chinese companies) and follicle-stimulating hormone (FSH) and prolactin (sandwich enzyme immunoassay technique by ELK Biotechnology Chinese company) for the in vitro determination of human serum and plasma.

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated by equation: HOMA-IR = [insulin (mU/L) x glucose (mg/dl)]/ 405[11].

Weight and height of the included women was measured and the body mass index (BMI) was calculated by equation: BMI (Kg/m^2) = weight (Kg)/height^2 (m^2)[12].

Transvaginal ultrasound scan of both ovaries was performed at Kamal Al-Samarraei hospital for infertility management and IVF, in order to determine the total number of early antral follicles using a 6.5 MHz transducer.

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010. The descriptive statistics including range, mean and standard deviation were measured to describe the data. The groups were compared by applying paired sample t-test (Paired t-test of single variable with two periods in one group). The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r). The results were considered statistically significant when *p* value was equal to or less than 0.05.

Results

The results of this study represented that 13 of the 25 women who were treated with vitamin D supplement were completed the 8 weeks follow up of their treatment, the rest 12 patients did not completed their follow up treatment so they not included in the result. The mean value of serum vitamin D levels represented by 25 hydroxyvitamin D was increased after treatment to 18.20 ± 1.93 ng/ml compared to that before treatment 12.26 ± 1.61 ng/ml, (Table 1).

Polycystic women after vitamin D treatment in comparison with before showed significantly lower mean (±SD) values of BMI (24.75 ± 3.28 kg/m² *vs.* 28.62 ± 6.15; p=0.007), waist circumference (89.46 ± 8.59 cm *vs.* 97.04 ± 12.07; p=0.012) and HOMA-IR (2.88 ± 1.72 *vs.* 4.17 ± 1.69; $p < 0.001$).

The comparison of hormonal levels before and after vitamin D treatment is demonstrated in table 2. Women after vitamin D treatment in comparison with before showed significantly lower mean values of LH (5.29 ± 0.58 mIU/ml *vs.* 6.33 ± 0.80; $p < 0.001$), FSH (5.42 ± 0.66 mIU/ml *vs.* 5.99 ± 0.65; p=0.002) and prolactin (16.44 ± 2.75 ng/ml *vs.* 22.05 ± 2.99; $p <$ 0.001). While, there was no significant changes in the levels of E2 (p=0.388).

Table 1: Mean (±SD) values of body mass index, waist circumference, and HOMA-IR before and after vitamin D treatment.

Parameter	Before vitamin D treatment (n=25)	After vitamin D treatment (n=13)	p value
BMI (kg/m²)	28.62 ± 6.15	24.75 ± 3.28	0.007 Ŧ S
Waist circumference (cm)	97.04 ± 12.07	89.46 ± 8.59	0.012 Ŧ S
HOMA-IR	4.17 ± 1.69	2.88 ± 1.72	< 0.001 Ŧ S
25 hydroxyvitamin D (ng/ml)	12.26 ± 1.61	18.20 ± 1.93	< 0.001 Ŧ S

BMI: Body mass index, Ŧ: Paired sample t test, S: Significant (p ≤ 0.05).

Table 2: Mean (±SD) values of hormonal levels before and after vitamin D treatment.

Parameter	Before vitamin D treatment (n=25)	After vitamin D treatment (n=13)	p value
LH (mIU/ml)	6.33 ± 0.80	5.29 ± 0.58	< 0.001 Ŧ S
FSH (mIU/ml)	5.99 ± 0.65	5.42 ± 0.66	0.002 Ŧ S
Basal E2 (pg/ ml)	48.96 ± 4.82	47.11 ± 3.19	0.472 Ŧ NS
Prolactin (ng/ml)	22.05 ± 2.99	16.44 ± 2.75	< 0.001 Ŧ S

LH: Luteinizing hormone; FSH: Follicle stimulating hormone; E2: Estradiol;
Ŧ: Paired sample t test, NS: Not significant (p>0.05); S: Significant (p ≤ 0.05).

Discussion

Polycystic ovary syndrome women after vitamin D supplementation improved significantly lower levels of BMI, WC and HOMA-IR. Also, there were significantly increased levels of 25 hydroxyvitamin D. Williams *et al.,* reported that, the use of vitamin D supplementation for PCOS patients improved insulin resistance and other metabolic profiles (reduction of BMI and HOMA-IR)[2], and significantly increased serum 25 hydroxyvitamin D, which was consistent with this study. While, Xue YP *et al.,* found that vitamin D supplementation did not change the HOMA-IR in women with PCOS[13].

Vitamin D has been proven to have therapeutic uses in PCOS based on the prognosis of PCOS. Vitamin D significantly enhances insulin synthesis and increases insulin receptor expression while suppressing pro-inflammatory cytokines, thus improving glucose metabolism[14]. Vitamin D's influence on PCOS metabolic and reproductive dysfunctions might be mediated through an overall effect on insulin resistance. Vitamin D treatment significantly reduced fasting plasma glucose; insulin resistance, or serum fasting insulin, showed significant improvement[15].

The present study revealed that PCOS women after vitamin D treatment also had improved LH, FSH and prolactin compared with before treatment. A study by Alomda *et al.,* confirmed that vitamin D supplementation can help significantly in decreasing the hormonal profile in women with PCOS[16].

The presence of vitamin D receptors (VDRs) in the granulosa cells and the cumulus oophorus cells of humans and animals supports the claim that vitamin D plays a significant role in the proper regulation of the female reproductive cycle[17]. Vitamin D receptors and traces of vitamin D metabolizing enzyme were found in syncytioblast procured from human culturing. VDR was detected in the decidua as well as the placenta. Several studies have linked the elevated level of androgen with precursors of vitamin D plasma levels (25 hydroxyvitamin)[17]. So, vitamin D had an important role in the development of metabolic, endocrine and reproductive abnormalities or dysfunctions in PCOS women[15,18].

In a study conducted by Dastorani *et al.,* infertile women with PCOS who were candidates for IVF were given 50,000 IU of vitamin D every week for 8 weeks, which resulted in significant reductions in blood levels of AMH and improved insulin metabolism[19].

According to Firouzabadi *et al.,* treatment with vitamin D might compensate for vitamin D insufficiency (significantly raise vitamin D levels), and lower BMI. In addition, vitamin D supplementation may be beneficial in treating PCOS patients by improving insulin resistance and infertility[20].

In conclusion, vitamin D with appropriate doses could be used safely in correction of

phenotypic characteristics (BMI, WC and insulin resistance) of PCOS women and can replaced other drugs. Also, hormonal profile of PCOS were significantly corrected.

Recommendations for future research and clinical practice are; early screen, diagnosis, and treatment of PCOS women to decrease the incidence of irregular menstruation, assess serum level of vitamin D in all patients with PCOS and treating the insufficiency. This study revealed that vitamin D supplementation had a significant effect on metabolic and hormonal profiles, suggest to advice patients with PCOS to take vitamin D supplementation for the beneficial effects.

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Conflict of Interest: The authors declared that there are no conflicts of interest.

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