Original Article

Vitamin D & E Supplemental Therapy could reduce Insulin resistance and control PCOS-specific Inflammatory and Oxidative Stresses

Wagdy M Amer[1] and Hamasat A Alnoury[2]

[1]Department of Obstetrics & Gynecology, [2]Department of Clinical & Chemical Pathology, Faculty of Medicine, Benha University, Egypt

ABSTRACT

Objectives: Evaluation of the effect of 8-wk supplemental therapy (ST) with vitamin D (VD), vitamin E (VE) and calcium on clinical and laboratory findings of women with polycystic ovary syndrome (PCOS).

Patients & Methods: 67 PCOS women fulfilling at least two of the Rotterdam criteria were evaluated clinically and by ultrasonography and gave blood sample for estimation of serum total cholesterol (TC), triglyceride (TG), low-density and high-density lipoprotein-cholesterol (LDL-c & HDL-c), insulin, total testosterone and dehydroepiandrosterone sulfate (DHEA-S) and ELISA estimation of VD, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, superoxide dismutase (SOD) and malonaldehyde (MDA). All patients received 8-wk ST consisted of daily dose of VD and VE and calcium citrate twice daily, and re-evaluated thereafter (T2). Study outcome was the extent of change in clinical, US and laboratory data obtained at T2 in relation to T1 data.

Results: At T1, all studied PCOS women had vitamin D deficiency (VDD) and high serum levels of TC, TG, LDL-c, testosterone, DHEA-S, insulin, MDA, TNF-α and IL-1β, but had low levels of HDL-c and SOD. At T2, all parameters were improved. Spearman's correlation showed negative significant correlations between the extent of change in serum 25OH-VD level and changes of clinical and other laboratory finings and a positive significant correlation with SOD activity level. Regression analysis defined decreases of HOMA-IR score, IL-β and serum TG as the significant predictor for decreased ovarian diameter, while decreases in serum levels of cholesterol, MDA, IL-1β were defined as the predictors for decreased serum testosterone and provision of ST was the significant predictor for increased activity levels of SOD.

Conclusion: PCOS is associated with disturbed immune and redox statuses in conjunction with metabolic and hormonal disturbances. Supplemental therapy with VD, VE and Calcium significantly improved these disturbances with minimal effect on body mass index.

Key Words: Insulin resistance, polycystic ovary syndrome, vitamin E, vitamin D.

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Corresponding Author: Wagdy M. Amer, Department of Obstetrics & Gynecology, Faculty of Medicine, Benha University, Egypt, **Tel.:** +2 010 0563 6992, **E-mail:** wagdyamer24@yahoo.com

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent condition characterized by a range of endocrine, reproductive, and metabolic abnormalities[1]. PCOS commonly affects women in reproductive-age by hyperandrogenism, oligo/anovulation, polycystic ovarian morphology[2], insulin resistance, and cardiometabolic disorders, with overweight/obesity and visceral adiposity[3]. Women with PCOS have reduced muscle insulin-mediated glucose uptake secondary to altered muscle mass, which in turn may aggravate PCOS complications[4].

Currently, the specific mechanism underlying the pathogenesis of PCOS and its associated abnormalities and complications is still unclear[5]. However, the roots of pathogenesis of PCOS and its sequalae are in the ovaries, where it was found that ovariectomized androgenized rats showed lower total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), TG/glucose index and oxidative stress markers in comparison with sham androgenized rats[6].

Oxidative stress (OS) is postulated to be defined as a state in which the pro-oxidative processes overwhelm cellular antioxidant defense due to the disruption of redox signaling and adaptation[7]. Chronic low-grade inflammation syndromes are systemic and chronic pathological conditions characterized by a slight increase in inflammatory markers[8]. The interplay between oxidative stress and inflammation occurs in a reciprocal vicious cycle[9].

Vitamin D deficiency (VDD) is a common comorbidity of PCOS that contributes to development of PCOS and its complications (10). Vitamin D status appears to be closely

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linked with manifestations of PCOS including insulin resistance, obesity, ovulatory and menstrual irregularities, oxidative stress and elevation of parathormone levels[11]. Moreover, both PCOS and VDD are accompanied by increased oxidative stress that may lead to vascular dysfunction with subsequent development of cardiovascular disease in PCOS[10].

Vitamin E (VE) has a potent antioxidant effect against oxidation status via various mechanisms, including its ability to regenerate other antioxidants and to increase antioxidant enzymes[12]. Animal studies showed that VE supplementation decreased lipid peroxidation and enhanced progesterone level and total antioxidant capacity (TAC), and promoted kid survival rate along with increased birth weight[13].

OBJECTIVES

Evaluation of the effect of 8-wk supplemental therapy (ST) with VD, VE and calcium on clinical and laboratory findings of women with PCOS.

Design

Prospective interventional comparative study

Setting

Departments of Obstetrics & Gynecology and Clinical Pathology, Faculty of Medicine, Benha University

PATIENTS & METHODS

After approval of the study protocol by the Local Ethical Committee, all women presented by manifestations suggestive of PCOS were evaluated. PCOS was diagnosed depending on the presence of at least two of the Rotterdam criteria[14,15], which is documented by recent studies to be the best diagnostic criteria[16,17]; these criteria included the presence of oligomenorrhea, i.e., less than eight spontaneous menstrual cycles yearly for at least 3 years before enrollment or amenorrhea, biochemical hyperandrogenemia with serum total testosterone (TT) level of >0.8 ng/ml, and ultrasonographic detection of polycystic ovaries having >12 follicles of 2–9 mm range and/or an ovarian volume >10 ml per ovary. All women who met the Rotterdam criteria were clinically were evaluated for exclusion criteria.

Exclusion criteria

Exclusion criteria included serum level of 25OH-VD of >50 nmol/L, over or morbid obesity, metabolic syndrome, endocrinopathy inducing obesity or hypocalcemia, anorexia, fear of or non-exposure to sun, attendance during winter to exclude seasonal variations, and renal diseases.

Inclusion criteria and evaluation

Women with PCOS and vitamin D deficiency (VDD), fulfilled at least two of Rotterdam criteria, free of exclusion criteria and attended the clinic during spring or summer were enrolled in the study. Enrolled women underwent evaluation for the following items at time of enrolment and at end of 8-wk supplemental therapy (ST):

1. Ultrasonographic examination to assure the diagnosis of PCOS and to estimate the greatest diameter of the ovary.
2. Laboratory investigations: 5 ml fasting blood were obtained from the antecubital vein under complete aseptic conditions. Samples were divided into two parts:
    a. The 1st part was used for estimation of fasting blood glucose (FBG)
    b. The 2nd part was centrifuged at 2000g for 10 minutes and serum was divided into two parts
        i. One part of the serum was used for estimation of serum levels of TC, TG, LDL-c and HDL-c, insulin, total testosterone and dehydroepiandrosterone sulfate (DHEA-S).
        ii. The other part of serum was used for ELISA estimation of vitamin D, tumor necrosis factor-α (TNF-α), interleukin (IL)-β, superoxide dismutase (SOD) and malonaldehyde (MDA).
3. Obesity was evaluated using body mass index (BMI) as calculating according to the equation: weight (kg) divided by square of height in meter (m^2) as documented previously[18]. BMI was graded according to WHO guidelines as average weight if BMI was <25 kg/m^2, overweight if BMI was ranging between 25 and 30 kg/m^2, obese if BMI was in range of >30-35 kg/m2 and over obese at BMI >35-40 kg/m^2 and morbid obese at BMI >40 kg/m^2[19].
4. Insulin resistance (IR) was diagnosed if the homeostasis model assessment of IR (HOMA-IR) score was >2. HOMA-IR was calculated according Mathews *et al*.[20] using the formula: fasting serum insulin (μU/ml) x [FBG (mg/ml)/18])/22.5.
5. Vitamin D sufficiency state was determined according to Stroud *et al*.[21] as follow: serum level of 25-OHD of ≥75 nmol/L indicates sufficient VD level, 50-75 nmol/L insufficient VD level and <50 nmol/L indicates deficient level. Then, vitamin D deficiency state was categorized as mild, moderate and severe if 25-OHD concentration was 25-50 nmol/L, 12.5-25 nmol/L and <12.5 nmol/L, respectively[21].

Protocol for supplemental therapy

All patients were provided with 8-wk ST consisted of the following:

1. Vitamin D3 ST that was provided as once daily oral dose of 5000 IU softgels (Sunvite Mega Potency Vitamin D3 5000 IU, Puritan's Pride, Inc., Oakdale, NY, USA) to be taken with a mail[22], this daily dose was proved to be safe for correction of VDD[23].

2. Vitamin E ST that was provided in a daily dose of 180 mg (400 IU) as previously used by Fatemi *et al*.[24]. VE was provided as 400 IU softgels containing preservative free VE (Sundown Naturals, Rexall Sundown, Inc., Bohemia, NY, USA).

3. Calcium ST was provided as calcium citrate 250 mg twice daily capsule for its easily absorption on a full or empty stomach (Calcium Citrate, Douglas Laboratories, Pittsburgh, Pennsylvania, USA). Dose was adjusted as 500 mg daily as prescribed previous as supplemental therapy for PCOS women[25].

Follow-up

All patients were asked to attend the Gynecology outpatient clinic for clinical, US and laboratory re-evaluation at the end of the 8-wk of ST.

Study outcomes

Study outcome was the percentage of change in clinical, US and laboratory data obtained at end of 8-wk ST (T2) in relation to that obtained at time of enrolment in the study (T1).

Statistical analysis

Data are presented as mean, standard deviation (SD), numbers, percentages, median and interquartile range (IQR). Parametric results were analyzed paired t-test, one-way Anova test and non-parametric results were analyzed using Chi-square test and Mann-Whitney test. Correlation analysis was performed using Spearman's correlation analysis and Regression analysis was performed using the Stepwise method. Statistical analysis was conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. *P value* <0.05 was considered statistically significant.

RESULTS

The study included 89 patients presented by PHH; 22 women were excluded for not fulfilling the inclusion criteria and the 67 women were included in the study (Figure 1). Ten fertile women with cross-matched age and BMI were included as control for laboratory data. Demographic and clinical data of enrolled patients were shown in (Table 1).

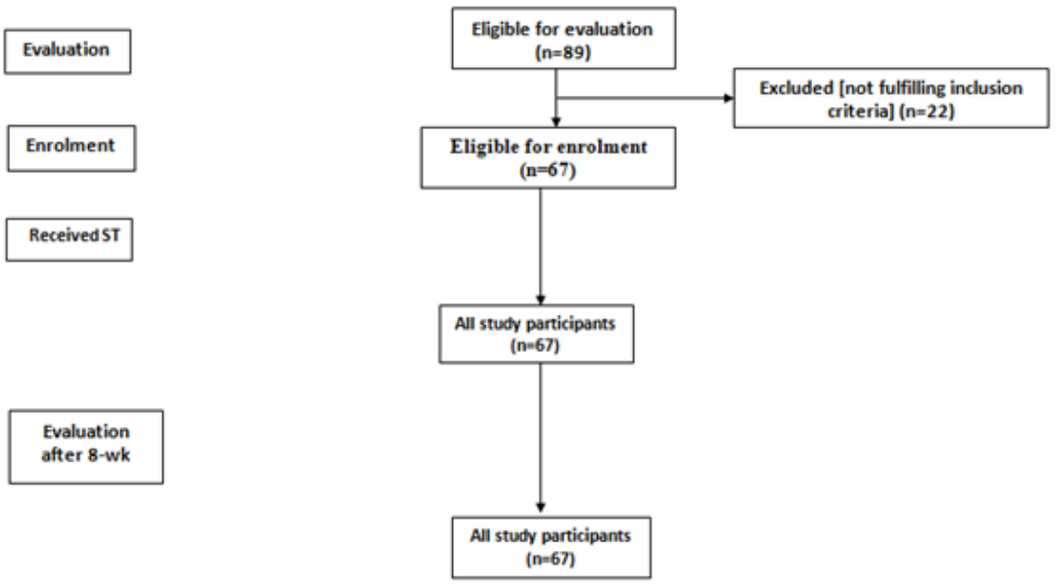

Figure 1: Consort Flow sheet

Fig. 1: Consort flow sheet

Table 1: Demographic and clinical data of patients of both groups

Variables			Findings
Age (years)	Categories	20-25	28 (41.8%)
		26-30	31 (46.3%)
		>30	4 (11.9%)
Body mass index (kg/m^2)	Categories	Mean (SD)	26.5 (3.4)
		Overweight (>25-30)	13 (19.4%)
		Obese (>30-35)	44 (65.7%)
		Over obese (>35)	10 (14.9%)
VD deficiency status	Categories	Mean (SD)	32.4 (2.3)
		Mild (25-30 nmol/L)	12 (17.9%)
		Moderate (12.5-25 nmol/L)	18 (26.9%)
		Severe (<12.5 nmol/L)	37 (55.2%)
Insulin resistance	Categories	Mean (SD)	29.3 (13.9)
		Insulin sensitive (HOMA-IR score <2)	38 (56.7%)
		Insulin resistant (HOMA-IR score >2)	29 (43.3%)
Ovarian diameter (cm)		Mean (SD)	1.81 (0.63)
		Median	10.9
		IQR	9.9-12.4

Data are presented as number; percentage; mean; standard deviation (SD), median and interquartile range (IQR), VD: Vitamin D

At end of the 8-wk ST (T2), number of over-obese women was decreased by 50%, however, patients' distribution among BMI grades showed non-significant (p=0.331) difference, while mean level of BMI was significantly (p=0.029) decreased in comparison to preliminary data. On contrary, 13 of 38 IR patients (34.2%) become insulin sensitive, and the frequency of IR patients was significantly (p=0.024) decreased with significantly lower (p=0.017) mean value of HOMA-IR score in comparison to the corresponding T1 measures. Fortunately, at the end of 8-wk ST, there was no patients was still had severe VDD, but 14 women had insufficient and 4 women had sufficient serum 25OH-VD levels. Moreover, patients' distribution among VD sufficiency grades showed significant (p=0.00013) change in direction of sufficiency with significant (p=0.0008) increase of serum 25OH-VD levels. Interestingly, mean ovarian diameter was significantly (0.0014) decreased in comparison to diameter measured before ST (Table 2)

Table 2: clinical data determined at end of 8-wk ST (T2) compared to data determined at time of enrollment (T1)

Variables			T1	T2	*P value*
Body mass index (kg/m^2)	Categories	Overweight (>25-30)	13 (19.4%)	17 (25.3%)	0.331
		Obese (>30-35)	44 (65.7%)	45 (67.2%)	
		Over obese (>35)	10 (14.9%)	5 (7.5%)	
Insulin resistance	Categories	Mean (SD)	32.4 (2.3)	31.5 (2.3)	0.029
		Insulin sensitive (HOMA-IR score <2)	29 (43.3%)	42 (62.7%)	0.024
		Insulin resistant (HOMA-IR score >2)	38 (56.7%)	25 (37.3%)	
VD status	Categories	Mean (SD)	1.81 (0.63)	1.56 (0.55)	0.017
		Severe VDD (<12.5 nmol/L)	12 (17.9%)	0	.00013
		Moderate VDD (12.5-25 nmol/L)	18 (26.9%)	14 (20.9%)	
		Mild VDD (25-50 nmol/L)	37 (55.2%)	35 (52.2%)	
		VD Insufficiency (>50-75 nmol/L)	0	14 (20.9%)	
		VD sufficiency (>75 nmol/L)	0	4 (6%)	
Ovarian diameter (cm)		Mean (SD)	29.3 (13.9)	38.7 (17.5)	0.0008
		Median	10.9	9.9	0.0014
		IQR	9.9-12.4	8.7-11.2	

Data are presented as numbers, percentages, mean, standard deviation (SD), median and interquartile range (IQR); p value indicates significance of difference between both groups; p1 value indicates the significance between the obtained data obtained at end of 8-wk ST in comparison to data obtained before ST; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference

The applied 8-wk ST significantly decreased serum levels of insulin, TC, TG, LDL, and levels of androgenic hormones and inflammatory markers. The effect of ST especially regarding Vitamin E was evident where serum SOD activity level at T2 estimation was non-significantly lower than control activity but significantly higher compared to activity level estimated at T1. However, serum concentration of MDA was decreased at T2 measurement in comparison to T1 measurements, but still significantly (p=0.025) higher than control levels (Table 3).

Table 3: Laboratory data determined at end of 8-wk ST (T2) compared to data determined at time of enrollment (T1)

Variable		Group	Control	T1	T2	*P value*
Insulin resistance data	FBG (mg/dl)	Level	114.8 (6)	132.5 (14.2)	130.4 (12.1)	0.356
		P1 value		<0.0001	<0.0001	
	FSI (mU/L)	Level	1.97 (0.22)	5.44 (1.58)	4.8 (1.49)	0.018
		P1 value		<0.0001	<0.0001	
	HOMA-IR score	Level	0.56 (0.09)	1.81 (0.63)	1.56 (0.55)	0.017
		P1 value		<0.0001	<0.0001	
Lipid profile data	TC (mg/ml)	Level	140.8 (4)	194.7 (19.5)	186.8 (19.5)	0.045
		P1 value		<0.0001	<0.0001	
	TG mg/ml)	Level	61 (18.2)	134.9 (21)	126.4 (26.8)	0.042
		P1 value		<0.0001	<0.0001	
	LDL (mg/dl)	Level	80.8 (7.5)	96.1 (16.4)	88.8 (17.8)	0.0153
		P1 value		<0.0001	<0.0001	
	HDL (mg/dl)	Level	47.8 (4.6)	40.5 (5.5)	42.6 (7.3)	0.068
		P1 value		<0.0001	<0.0001	
Hormonal profile	Testosterone	Level	0.63 (0.11)	3.15 (0.75)	2.68 (0.72)	0.0004
		P1 value		<0.0001	<0.0001	
	DHEA-S	Level	14.4 (1.27)	24.11(3.77)	21 (3.47)	<0.0001
		P1 value		<0.0001	<0.0001	
Inflammatory cytokines	TNF-α (ng/ml)	Level	1.9 (0.47)	3.16 (0.75)	2.65 (0.67)	<0.0001
		P1 value		<0.0001	<0.0001	
	IL-1β (pg/ml)	Level	14.59 (3.2)	26.92 (8.33)	23.33 (7)	<0.0001
		P1 value		0.00002	0.00025	
Redox status	SOD (nmol/L)	Level	1.726 (0.2)	1.35 (0.16)	1.63 (0.2)	<0.0001
		P1 value		<0.0001	0.152	
	MDA (nmol/L)	Level	0.575 (0.2)	1.318 (0.16)	0.663 (0.1)	<0.0001
		P1 value		<0.0001	0.025	

Data are presented as numbers, percentages, mean, standard deviation (SD), median and interquartile range (IQR); p value indicates significance of difference between both groups; P1 value indicates the significance between the obtained data obtained at end of 8-wk ST in comparison to data obtained before ST; $P<0.05$ indicates significant difference; $P>0.05$ indicates non-significant difference

Spearman's correlation between the extent of change in serum 25OH-VD level and changes of clinical and other laboratory findings showed negative significant correlations, but a positive significant correlation (Rho= 0.353, p=0.003) was detected between the extent of change of serum 25OH-VD and SOD activity level (Table 4).

Table 4: Spearman's correlation between the change of serum 25OH-VD and changes occurred in clinical and other lab parameters at T2 in relation to T1 data

Variables	Rho	*P value*
BMI	-0.329	0.007
HOMA-IR	-0.653	<0.001
Ovarian diameter	-0.367	0.002
TC	-0.224	0.068
HDL-c	0.175	0.156
TG	-0.413	0.001
LDL	0.263	0.045
Testosterone	0.337	0.008
DHEA-S	0.293	0.034
TNF-β	0.426	<0.001
IL-1	0.091	0.463
MDA	0.212	0.084
SOD	0.353	0.001

Rho: Spearman correlation coefficient; $p<0.05$ indicates significant difference; $p>0.05$ indicates non-significant difference; -: indicates negative correlation

Moreover, Regression analysis for predictors for ovarian diameter change defined decreases of HOMA-IR score (β=0.417, $p<0.001$), IL-β (β=0.295, $p=0.005$) and serum TG (β=0.200, p=0.046) as the significant predictor for upcoming decreased ovarian diameter. While decreases in serum levels of cholesterol (β=0.351, $p=0.001$), MDA (β=0.335, $p=0.001$), IL-1β (β=0.291, $p=0.004$) were defined as the predictors for decreased androgenemia as reflected by decreased serum testosterone. On the other hand, provision of ST as reflected by the increased levels of 25OH-VD was the significant predictor for improved oxidative milieu towards antioxidant direction, as reflected by increased activity levels of SOD (β=0.357, $p=0.003$).

DISCUSSION

All the studied PCOS women had vitamin D deficiency (VDD) to varied extents and serum levels of 25OH-VD showed inverse correlation with serum levels of testosterone, DHEA-S, insulin, MDA, TNF-α and IL-1β, and lipid profile but showed positive correlation with serum SOD. These findings illustrate the relation between VDD and disturbed immune and redox status in women with PCOS; either a pathogenesis or a consequence. Similarly, Mu *et al*.[26] out of systemic review documented that vitamin D levels in PCOS women were negatively associated with serum androgen level and with parameters of IR and body fat mass.

Moreover, all women showed biochemical evidence of oxidative stress and disturbed immune milieu in direction of inflammation as presented by low serum levels of SOD and high levels of MDA, TNF-α and IL-1β. Moreover, there were positive significant association between high lipid profile and high serum MDA, TNF-α and IL-1β with significant inverse relation to serum SOD levels. These results go in hand with Wang *et al*.[27] who detected decreased serum concentrations of SOD, total antioxidant activity, vitamin E (VE) and retinol with increased MDA concentration in PCOS women and found the decrease was more significant in PCOS with metabolic syndrome with an inverse relation between serum levels of SOD and total antioxidant activity and TG and LDL-c. Also, Fatima *et al*.[28] found women with PCOS had poor antioxidant status as reflected by significantly low levels of glutathione, vitamin C and E and considerably increased oxidative stress with a positive correlation between oxidative stress and insulin parameters. Moreover, the obtained results coincided with the research survey that showed a close interaction between oxidative stress, low-grade inflammation, and PCOS[3,29].

The applied 8-wk ST significantly improved the disturbed inflammatory and oxidative milieus with improvement of parameters of IR and hypertriglyceridemia and disappearance of bony ache. In line with these results multiple recent studies documented that oral VD supplemental therapy significantly decreased serum fasting blood glucose and serum insulin, TC, TG and VLDL-C, thus exerting favorable effects on glucose metabolism and lipid metabolism, especially in VD deficient PCOS women[30,31,32]. Furthermore, other recent studies documented the favorable effect of VD supplemental therapy on patients' lipid profile, oxidative stress and inflammatory status[33,34,35] and additionally reported improvement of endometrial receptivity[33], hirsutism[34] and ovulation rate in subfertile women with PCOS undergoing induction of ovulation[35].

Concerning the effect of adding calcium (Ca) to the applied therapy, role of Ca was documented by earlier studied that reported significant reductions of serum insulin concentrations with decreased HOMA-IR and increased quantitative insulin sensitivity check index and improved serum lipid concentrations after 8-wk supplemental therapy for PCOS women with Ca and vitamins D and K[36] or magnesium-zinc-calcium-vitamin D[37]. Thereafter, other studies found Ca/VD supplementation for 8 weeks for PCOS women receiving metformin resulted in a significant increase in 25-OH-VD and calcium levels, significantly decreased IR parameters, and serum TG, VLDL-c, TC and LDL[38,39,40]. Moreover, Ca/VD-metformin for PCOS women improved menstrual cycle irregularity[38,40], follicular maturation and significantly decreased hirsutism and testosterone levels Shojaeian *et al*.[40] in comparison to metformin-placebo

It is to be noted that the reported improvement could not be attributed to improvement of VDD alone, as there is a role for VE also as previously documented by Izadi *et al*.[41] who detected significant decrease in serum TC, TG and LDL-c with increased levels of HDL-c and significant effects on atherogenic index of plasma, lipid accumulation product, visceral adiposity index and blood pressure in women with PCOS who received vitamin E and Coenzyme 10. Thereafter, Sadeghi *et al*.[42] reported significantly higher levels of total antioxidant capacity, catalase activity

and glutathione levels with a significant reduction of MDA levels in PCOS women who received omega-3 and vitamin E co-supplementation. Moreover, in addition to these improvements with VE therapy regarding metabolic, oxidative and inflammatory statuses of PCOS women, Hager *et al*.[43] found multi-nutrient supplementation including VE for a minimum of 3 months significantly reduced PCOS-specific parameters; namely, the LH:FSH ratio, testosterone and anti-Mullerian hormone levels. Also, Chen *et al*.[44] found short-term vitamin E supplementation for infertile women with PCOS who underwent ovulation induction with clomiphene citrate (CC) and human menopausal gonadotropin (HMG) improved oxidative stress, and when taken during the follicular phase reduced exogenous HMG dosage. Moreover, Morsi *et al*.[45] detected significantly increased endometrial thickness with vitamin E supplementation for PCOS CC-resistant women and Shirazi *et al*.[46] found vitamin E supplementation significantly reduced body weight, fat mass, angiopoietin-1, angiopoietin-1/ angiopoietin-2 ratio and vascular endothelial growth factor.

CONCLUSION

PCOS is associated with disturbed immune and redox statuses in conjunction with metabolic and hormonal disturbances. Supplemental therapy with VD, VE and calcium significantly improved these disturbances with minimal effect on body mass index.

LIMITATIONS

Short-duration of supplemental therapy, absence of adjuvant weight reduction regimen and missing the effect on menstrual or fertility problems are limitation of this preliminary study.

RECOMMENDATIONS

Wider scale studies to evaluate the outcomes of various dosing regimen and durations of supplementation on the evaluated parameters and on menstrual and fertility problems.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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