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The Effect of Vitamin D and Co-enzyme Q10 Replacement Therapy on Hormonal Profile and Ovulation Statusin Women with Clomiphene Citrate Resistant Polycystic Ovary Syndrome

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Abstract:

Background: Polycystic ovary syndrome (PCOS) was one of the most common endocrine disorders that result in anovulatory cycle lead to infertility or sub infertility. Clomiphene citrate used for ovulation induction and about 15% of PCOS patient do not respond to the maximum dose of clomiphene citrate and are considered resistant.

Objectives: To evaluate the effect of combining oral vitamin D3 tablet or CoQ10 capsule with clomiphene citrate on hormonal, oxidative marker, and ovulation outcome in clomiphene citrate resistance PCOS patients.

Materials and Methods: A prospective interventional randomized- controlled, open-label study include 41 PCOS patient aged range (18-34) are clomiphene citrate resistant divided into two groups, group 1 include hypovitaminosis D PCOS patient receive clomiphene citrate 100mg daily (for 5 days in each induction month) plus vitamin D 10000IU daily (2 months), and group 2 include PCOS patient with insufficient vitamin D status receive clomiphene citrate 100mg daily (for 5 days in each induction month) plus CoQ10 200mg daily (2 months). Baseline and after 2 months fasting blood samples used to measure hormonal and oxidative stress biomarkers, transvaginal ultrasound for determination of ovulation, and pregnancy test was done for those patient that have no menstruation for two week after HCG injection.

Results: Vitamin D3 supplementation resulted in significant decrease in free testesterone (P=0.012), LH (P=0.021), LH: FSH ratio(P=0.019), and Anti-mullerian hormone (AMH) (P=0.002), and significant increase in GSH (P<0.001). Meanwhile CoQ10 supplementation result in significant decrease in LH (P=0.021), LH: FSH ratio (P=0.007), and AMH (P=0.022), and significant increase in GSH (P=0.009). Both supplements resulted in improvement in ovulation outcome. The overall ovulation was in 75% of PCOS patient, mean while overall pregnancy was in 15% equal in both groups.

Conclusion: Supplementation with vitamin D and CoQ10 to clomiphene citrate resistance PCOS patients result in improving hormonal profile, oxidative marker, and ovulation outcome.

Keywords: clomiphene-citrate-resistant PCOS, Co-enzyme Q10, ovarian functions, reproductive hormones, Polycystic Ovary Syndrome, Vitamin D supplementation.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is considered as one of the most common endocrine disorders in women of reproductive age with a strong genetic component[1]. Infertility or sub fertility is a frequent complaint in women with PCOS that results from anovulatory cycles[2]. Clomiphene citrate remains still the first line therapy for induction of ovulation in women with PCOS, ovulation can be induced in about 80% of women[3]. Approximately 15% of women with PCOS do not respond to the maximum dose of clomiphene citrate and are considered resistant to this medication[4], therefore many additive medications can be combined to overcome clomiphene citrate resistance[5].

Vitamin D deficiency is one of the most common nutritional deficiencies worldwide.Vitamin D deficiency (VDD) is common among women with PCOS (approximately 67%–85% women with PCOS have VDD)[6]. There is some evidence suggesting that vitamin D deficiency may be involved in the pathogenesis of insulin resistance and metabolic syndrome in PCOS[7,8]. Vitamin D is thought to influence the development of PCOS through gene transcription and hormonal modulation influencing insulin metabolism and fertility regulation[9].

Diverse mechanisms explain Vitamin D3 role in female reproduction; First, the direct stimulatory effect of vitamin D3[1,25(OH)2D3] on the aromatase gene expression in reproductive tissues[10]. Second, it has been shown that HOXA10 expressionwhich is essential for endometrial development is up-regulated by 1,25(OH)2D3 in human endometrial stroma cells. Third, Vitamin D3 and calcium repletion might lead to normalization of menstrual cycles and restoration of ovulation through oocyte activation and maturation[11].

The fundamental role of CoQ10 in mitochondrial bioenergetics and its well acknowledged antioxidant properties constitute the basis for its clinical applications, although some of its effects may be related to a gene induction mechanism[12].Oxidative stress is increased in PCOS patients that linked to IR and infertility[13-16].

Numerous potential mechanisms by which CoQ10 could improve ovarian functions in clomiphene-citrate-resistant PCOS; First, CoQ10 acts directly on the mitochondria, possibly related to electron transfer in the respiratory chain, and plays a crucial role in the production of cellular ATP[17]. Second, CoQ10 could reduce oxidative stress within the ovary and protect DNA from free radical induced oxidative damage[18], also CoQ10 acts as antioxidant itself, since its reduced form, ubiquinol inhibits lipid peroxidation in biological membranes, and in LDL, and protects membrane proteins against oxidative damage[19]. Third, it protects the stability of plasma membranes by maintaining their flexibility and fluidity to proper physical performance as membrane receptors, carriers and enzymes[20]. Fourth, CoQ10 is a micronutrient, and their tissue uptake correlates with the degree of tissue deficiency. Finally, antiapoptotic of CoQ10 against the main mechanism involved in follicular cohort atresia[21].

Taken together all the previous evidence, this study was designed to evaluate the potential effect of vitamin D3 and Co enzyme Q10 supplementationin women with clomiphene citrate resistance PCOS to improve fertility outcome and ameliorating hormonal profile.

METHOD

Patients

A total of 45 PCOS patients were enrolled in the study during their visit to gynecologic and obstetric of the general hospital and private clinic, only 41 patients complete the study are at reproductive aged ranged from 18-40 years and desired to be pregnant. The patients were under the supervision of specialist gynecologist and were treated according to the practice guidelines.

The inclusive criteria of the candidate patients is as follows:

- Patients with PCOS diagnosed according to Rotterdam criteria[22]
- Patients with PCOS whom are resistant to clomiphene citrate (CC) mono therapy following general practice of the physician.
- Patients with PCOS and with vitamin D deficiency(< 20 ng/ml)to be enrolled in one group and Patients with PCOS with insufficient vitamin D (20-30ng/ml) to be enrolled in another group.

Patients previously taken induction therapy for five days with clomiphene citrate alone for at least two or three cycles and were diagnosed as persistent anovulation or ovulate with very thin endometrium (<5 mm) on the day of HCG administration[23].

The candidate PCOS patients presented with oligomenorrhea for more than one month, and menstrual regulation was achieved by using progesterone (Allylestrenol) 5mg tablet twice daily for 10 days, then after enrolled in the study when menstruation cycle is regular. All participants were instructed not to take any medications during the trial except after consulting the physician.

PCOS patients on oral contraceptive or use other ovulation stimulator in the past two months, patients with other comorbid or on other medication, patients with other gynecological and hormonal diseases such as hyperprolactinemia, hypercorticism and thyroid dysfunction, or PCOS patient using supplement other than the study intervention are excludedfrom the study.

The study was approved by the scientific and ethical committee and the agreement of general health directorate was achieved. Patient written consent was taken after full explanation of the aim of the study and ensures the reliability of the collected information.

Study design

This is a prospective interventional randomized-controlled, open-label study to evaluate the effectiveness of combining oral vitamin D3 tablet or oral co-enzyme Q10 capsule with clomiphene citrate tablet, on the hormonal profile and response to ovulation stimulationin clomiphene citrate resistance polycystic ovary syndrome (PCOS) patients. The study was conducted during the period from September 2017 to April 2018.

The eligible patients were allocated into two main groups:

Group 1: include 24 PCOS patients assigned to be treated with clomiphene citrate oral tablets 50mg twice daily after meal plus a daily dose of vitamin D3 10000IU oral tablets after meal for two months period.

Group 2: include 17 PCOS patients assigned to be treated with clomiphene citrate oral tablets 50mg twice daily after meal plus a daily dose of CO-enzyme Q10 soft gel capsule 200mg after meal for two months period.

Both study groups started induction (with conventional ovulation inducer) clomiphene citrate oral tablets for five days only starting at day(2-5) of menstrual cycle, meanwhile the intervention drugs was continued for two months.All Patients were followed up via transvaginal ultrasound at day 10 and day 12 of cycle to determine number of dominant follicle (equal or more than 18). Human chorionic gonadotropin (HCG) injection (Pregnyl 5000 –10,000 IU i.m, Organon, Holland) was given when at least one follicle measuring at least 18 mm was found. Patients were advised to have intercourse throughout 24–36 hrs after HCG injection. Serum HCG was determined 2 weeks after HCG injection in the absence of menstruation for diagnosis of pregnancy. Growing follicles were defined as those size <14 mm, and the mature follicles as those size 18–22 mm. Patients that complete the first month and have pregnancy return for follow up and blood sample was taken, while those did not have pregnancy continue the intervention for the second month starting at day 2-5 of cycle . At the end of two month blood sample was collected from all pregnant and non-pregnant candidates at day 2-5 of cycle.

Among the 41 candidate patients, there was some shortage in the post treatment data particularly in the hormonal test attributed to the specificity and availability of test at the sampling time. Free testosterone test kit was not available in private lab for some weeks. Also some patients return for sampling at days after the scheduled (2-5) days of menstrual cycle and others become pregnant, hence, inaccurate test for FSH and LH may affect the results. Moreover, serum GSH test was done for non-pregnant patients only since pregnancy increase oxidative stress accordingly.

Methods

Ten ml of venous blood were collected using a plastic disposable syringe and placed in to two plain disposable tubes (gel and clot activator) and was separated by centrifuge at a speed of (3000) rpm for (10) minutes. About 1ml serum used directly for measurement of free testosterone using competitive immunoluminometric assay [24], and the remaining serum samples were stored in Eppendorf tube at (-40^{O}C) until the time of measurement of Serum FSH, LH, and (FSH /LH ratio calculation) using the Maglumi FSH (LH) assay which is a sandwich chemiluminescence immunoassay[25,26], measurement of Serum Antimullerian Hormone (AMH) using the Ultra-Sensitive Antimullerian Hormone/ Mullerian Inhibiting Substance (AMH/MIS) ELISA which is a quantitative three step Sandwich immunoassay[27], and measurement of Serum Glutathione (GSH) using competitive type of ELISA[28].

Ethical considerations

The proposal of the research was discussed and approved by the scientific and ethical committee and college of pharmacy -Mustansiriyah University, and the agreement of general health directorate in Babylon Governorate was achieved. Patient written consent was taken after full explanation of the aim of the study and ensure the reliability of the collected information.

Statistical Analysis

Statistical analysis was carried out using SPSS version 20. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Fisher exact test were used to find the association between the categorical variables. Independent samples t-test was used to compare means between two groups. Paired t-test was used to compare means for paired reading. A *p*-value of ≤ 0.05 was considered as significant.

RESULTS

Socio-Demographic and Disease Characteristics of PCOS Patients onVitamin D3 and Co-Enzyme Q10 Supplements

The patient demographic and disease characteristics of 41 PCOS patients, including 24 patients in group 1 (58.5%) and 17 patients in group 2(41.5%) with no statistical significant difference found between both study groups according to type of intervention treatment($P>0.05$), table (1). The age range for all patients were between 18-34 years with the mean age of 24.71 ± 4.07years for group 1 patients, and 22.76 ± 3.8years for group 2 patients. No statistical significant difference found between study groups with respect to age ($P>0.05$).Considering the patients

residence there were (66.7%) of group 1 patients versus (58.8%) of group 2 patients were rural, and (33.3%) of group 1 patients versus (41.2%) of group 2 patients were urban with no significant statistical difference between both groups (P>0.05).The average body mass index (BMI) for group 1 patients and for group 2 patientswas 26.46 ± 4.09 kg/m^2 and 27.59 ± 4.63 kg/m^2, respectively. No statistically significant difference was found between the study groups with respect to the BMI (P>0.05). Positive family history was seen in (37.5%) of patients in group 1 and (35.3 %) of patients in group 2, the history of high diet sugar was positive in (45.8%) of patients in group 1, and (64.7%) of patients in group 2. Regarding earlymenarche was positive in (25%) of patients in group 1, and (11.8%) of patients in group 2. No statistical significant difference between the study group with respect to family history, high diet sugar, and premature adrenerche. The duration of the disease for patients in group 1 and 2 were as follows: 16.7% versus 29.4% for less than 2 years duration, 70.8% versus 58.8% for 2-10 years duration, 12.5% versus 11.8% for more than 10 years duration. No significant difference was found between both groups with respect to the duration of the disease (P>0.05).

Table (1) Demographic data and disease characteristics of PCOSpatients

Study variables	Study groups		*P*-value
	Group 1 (n=24)	Group 2 (n=17)	
Age (years) Range (years)	(24.71 ± 4.07) (19-34)	(22.76 ± 3.8) (18-31)	0.191^{NS}
	n %	**n %**	
Residence			
Urban	8 (33.3)	7 (41.2)	0.607^{NS}
Rural	16 (66.7)	10 (58.8)	
Total	24 (100.0)	17 (100.0)	
BMI (kg/m^2)	26.46 ± 4.09	27.59 ± 4.63	
Normal (18.5-24.9)	9 (37.5)	6 (35.3)	0.41^{NS}
Overweight (25-29.9)	12 (50.0)	8 (47.1)	1.000^{NS}
Obese (≥ 30)	3 (12.5)	3 (17.6)	
Total	24 (100.0)	17 (100.0)	
Family history of PCOS			
Yes	9 (37.5)	5 (29.4)	0.591^{NS}
No	15 (62.5)	12 (70.6)	
Total	24 (100.0)	17 (100.0)	
High sugar diet			
Yes	11 (45.8)	11 (64.7)	0.233^{NS}
No	13 (54.2)	6 (35.3)	
Total	24 (100.0)	17 (100.0)	
Early menarche			
Yes	6 (25.0)	2 (11.8)	0.433^{NS}
No	18 (75.0)	15 (88.2)	
Total	24 (100.0)	17 (100.0)	
Duration of symptoms			
<2 year	4 (16.7)	5(29.4)	
(2-10) years	17 (70.8)	10 (58.8)	1.000^{NS}
More than 10 years	3 (12.5)	2 (11.8)	
Total	24 (100.0)	17 (100.0)	

Data presented as mean ± SD, Number of patients (n), Percentage (%), NS: No significant differences (P>0.05), f: Fisher exact test.
Independent -sample *t*-test is used for statistical analysis of (age, residence, family history, high diet sugar)
Fisher exact testis used for statistical analysis of (BMI, duration of symptom, premature adrenerch)

Effect of Vitamin D3 and Co-Enzyme Q10 Supplements on Hormonal Profile

Free testosterone FT showed a significant higher level in group 1 in pretreatment level compared to group 2 patients (P<0.05), then within each group, a highly significant decrease in FT level was revealed in group 1only after 2 months of treatment (P<0.01). Meanwhile, in group 2, the decrease in FT level was non-significant when compared to pre-treatment levels (P>0.05), table (2).

Pretreatment analysis revealed no significant difference in the mean of FSH, and LH: FSH ratio between group 1 and group 2 patients (P>0.05), except that LH level was significantly lower in group 1 only pretreatment. Two months post treatment, there was no significant change in FSH level within each groups 1and 2 compared to pretreatment levels (P>0.05), nevertheless, a significant decrease in LH level was revealed in group 1 after 2 months of treatment (P<0.05), and a highly significant decrease in LH:FSH ratio in both study groups after 2 months of treatment when compared to pretreatment level(P<0.01).

The pretreatment AMH showed no significant difference in the mean level between group 1 and group 2 patients (P>0.05), then after 2 months of treatment, a highly significant decrease in AMH level was revealed in group 1 (P<0.01), and a significant decrease in AMH level in group 2 compared to pretreatment level(P<0.05), table (2).

The overall changes in the FT, FSH, LH, LH: FSH ratio and AMH was not significant between both study groups after 2 months of treatment(P>0.05).

Table (2) Effect of vitamin D3 and co-enzyme Q10 supplements on hormonal profile

Study variable	Study group				*P* value
	Group 1		Group 2		
Free Testosterone (pg/ml)	**n**	**(Mean ± SD)**	**n**	**(Mean ± SD)**	
Pre treatment	22	2.87± 2.41	17	1.37± 0.81	0.019*
Post treatment	18	1.89± 0.64	15	1.31± 0.96	0.057^{NS}
P-value	0.012**		0.886^{NS}		
Percentage of change (%)	-34.15%		-4.38%		
FSH (mIU/ml)	**n**	**(Mean ± SD)**	**n**	**(Mean ± SD)**	
Pre treatment	23	5.07± 1.69	17	5.69± 1.17	0.210^{NS}
Post treatment	18	5.21± 1.38	14	5.93± 1.84	0.215^{NS}
P-value	0.846^{NS}		0.781^{NS}		
Percentage of change (%)	9.66%		4.22%		
LH (mIU/ml)	**n**	**(Mean ± SD)**	**n**	**(Mean ± SD)**	
Pre treatment	23	5.33 ± 2.53	17	7.44 ± 3.86	0.044*
Post treatment	18	4.09 ± 1.24	14	4.78 ± 2.08	0.283^{NS}
P-value	0.021*		0.021*		
Percentage of change (%)	-23.26%		-35.75%		
LH/FSH ratio	**n**	**(Mean ± SD)**	**n**	**(Mean ± SD)**	
Pre treatment	23	1.06 ± 0.39	17	1.30 ± 0.51	0.101^{NS}
Post treatment	18	0.81 ± 0.28	14	0.87 ± 0.35	0.606^{NS}
P-value	0.019*		0.007**		
Percentage of change (%)	-23.58%		-33.08%		
Anti-mullerian hormone AMH (ng/ml)	**(Mean ± SD)**		**(Mean ± SD)**		
Pre treatment	4.47 ± 2.46		4.08 ± 3.10		0.657^{NS}
Post treatment	3.42 ± 1.43		2.96 ± 1.88		0.382^{NS}
P-value	0.002**		0.022*		
Percentage of change (%)	-23.49%		-27.45%		

Data presented as mean ± SD, Number of patients (n)
NS: No significant differences (P>0.05), *(P<0.05) is considered Significant difference.**(P<0.01)is considered highly significant
Paired *t*-test is statistically used to compare between pre- and post-treatment results in same group.
Independent sample *t*-test is used to compare pre or post treatment between group 1 and group 2 patients.

Effect of Vitamin D3 and Co-Enzyme Q10 Supplements on Serum Glutathione (GSH) Level

Pretreatment, there was no significant difference in the mean GSH level between group 1 and group 2 patients (P>0.05), then after 2 months of treatment, a highly significant increase in GSH was revealed in both group 1 and group 2 when compared to pre-treatment levels (P<0.01), table (3). There was no significant difference between both study groups (P>0.05) after 2 months of treatment where both treatment increased endogenous GSH level.

Table (3) Effect of vitamin D3 and co-enzyme Q10 supplements on GSH level

Study variable	Study group				*P* value
	Group 1		Group 2		
GSH (Mg/ml)	n	(Mean ± SD)	n	(Mean ± SD)	
Pre treatment	21	102.04 ± 90.03	14	95.72 ± 91.72	0.841 NS
Post treatment	21	195.15 ± 124.94	14	177.96 ± 97.69	0.668 NS
P-value	0.001**<		0.009**		
Percentage of change (%)	91.25%		85.92%		

Data presented as mean ± SD, Number of patients (n).
NS: No significant differences (P>0.05), **(P<0.01) is considered highly significantdifferences.
Paired *t*-test is statistically used to compare between pre- and post-treatment results in same group.
Independent sample *t*-test is used to compare pre or post treatment between group 1 and group 2 patients.

The Effect of Vitamin D3 and Co-Enzyme Q10 Supplements on ovulation stimulation and Pregnancy Outcome

The effect of vitamin D3 and CoQ10 supplementation on ovulation and pregnancy outcome is presented in table (4). After 2 months of treatment, there was higher percent of ovulation post treatment in both study groups 1 and 2 (75.0**%** and 76.5**%**) respectively compared to the pre anovulatory status, though no significant difference between both groups (P>0.05).

The effect of vitamin D3 and CoQ10 supplementation on pregnancy outcome revealed that there was 3 patients from each group became pregnant, accordingly, no difference in pregnancy outcome between both study groups (P>0.05).

Table (4) The Effect of Vitamin D3 and Co-Enzyme Q10 Supplements on ovulation stimulation and pregnancy outcome

Study variables	Study groups		*P*-value
	Group 1 n (%)	Group 2 n (%)	
Ovulation-pre			
Positive (≥18)	2(11.8)	1(7.1)	1.000 NS
Negative (<18)	15(8 8.2)	13(92.9)	
Total	17(100.0)	14(100.0)	
Ovulation-post			
Positive (≥18)	18 (75.0)	13 (76.5)	0.711NS
Negative (<18)	6 (25.0)	4 (23.5)	
Total	24 (100.0)	17 (100.0)	
Pregnancy outcome			
Positive	3 (12.5)	3 (17.6)	0.679NS
Negative	21 (87.5)	14 (82.4)	
Total	24 (100.0)	17 (100.0)	

Data presented as Number of patients (n), Percentage (%),
f: Fisher exact test,NS: No significant differences (P>0.05),P value ≤ 0.05 is significantdifferences.

The overall effect of vitamin D3 and Co-enzyme Q10 supplements on ovulation and pregnancy outcome revealed that the ovulation in clomiphene citrate resistance PCOS patients was in less than (10%) only in the pretreatment overall baseline data (figure 1), then after treatment majority of clomiphene citrate resistance PCOS patients had positive overall ovulation (ovulate mature i.e ova size equal or more than 18mm) about (75%) (Figure 2) after 2 months treatment with vitamin D3 and Co-enzyme Q10 supplements revealed, and (25%) negative ovulation (ova size range from 3-15 mm). Moreover, the overall effect of vitamin D3 and CoQ10 supplemention on pregnancy outcome was (14.6%) of all PCOS patients (figure 3).

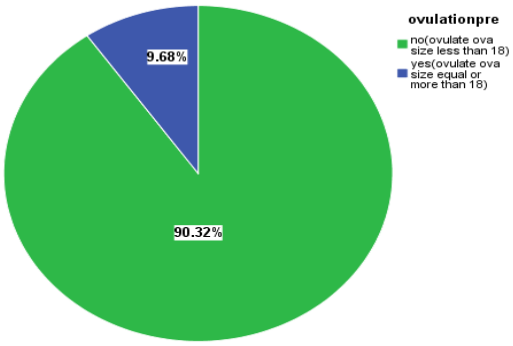

Figure (1) The overall ovulation stimulation before treatment

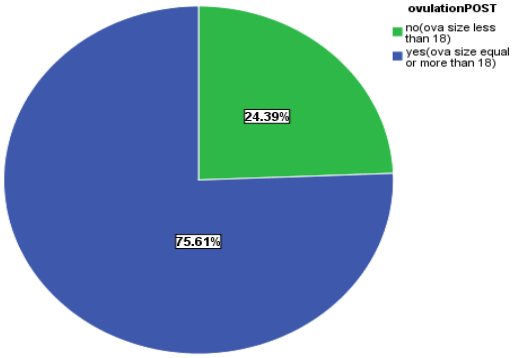

Figure (2) The overall ovulation stimulation after treatment with vitamin D3 and co-enzyme Q10 supplements

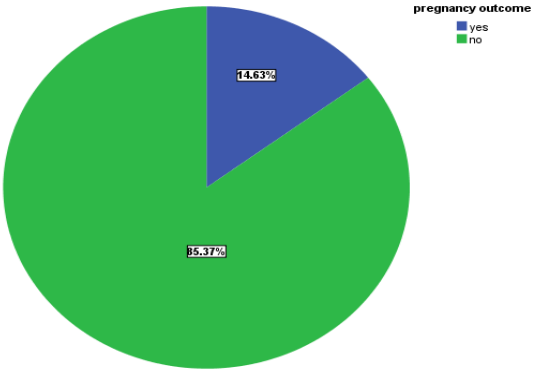

Figure (3) The overall pregnancy outcomes after treatment with vitamin D3 and co-enzyme Q10 supplements

DISCUSSION

Demographic Changes and Disease Characteristics of PCOS Patients

In the present study, all PCOS patients enrolled in this study are resistance to clomiphene citrate and desired to be pregnant and were at the reproductive aged range from 18-34years, and were matched in majority of previous studies[29-32]. Most of them were resident in rural areas than in urban one, willing to have more children**.**

About (65%) of PCOS patients in both group in this study were overweight and obese, meanwhile only (35%) were

normal weight. This finding was consistent with that of Wang *et al*. (2016) and Gomathi *et al*. (2010) where (55.65%) of patients were obese[33,34], that weight gain and obesity occur in approximately (61%) and (76%) of women with PCOS[35], mostly central or abdominal obesity in which hyperinsulinemia, IR, and hyperandrogenemia influence adipocyte function and distribution through inhibition of adipocyte differentiation, which modulates lipolysis and lipogenesis[36]. Moreover, obesitywas worsen reproductive and metabolic abnormalities in women with PCOS[37].

Positive family history of PCOS was found in more than (35%) of patients in both groups of the present study Tehrani *et al.* (2014) found that about (45%) of patients with PCOS have positive family history of PCOS[38]. Additionally Begum *et al.* (2017) reported (60.8%) of patients have positive PCOS family history[39], both studies were consistent with the present one. This is due to the fact presence of a genetic component to PCOS and familialclustering of reproductive and metabolic abnormalities results in increased risk of PCOS among first-degree relatives of PCOS patients[39]. A study found 5 to 6 fold increase in the incidence of PCOS among first-degree female relatives of affected patients when compared with the prevalence of PCOS in general population[40].

Most PCOS patients enrolled in this study presented with (2-10) years duration of symptoms which probably the leading cause to CC resistance or increase symptoms.

Positive high diet sugar was found in more than (45%) of PCOS patients in both study groups of the present study. This finding was in consistent to that of Dabrell*et al*. (2010) who stated that women on high fats and sugars diet and low antioxidants are at higher risk of PCOS compared to participants without these predisposing factors[41]. Similar founding by Begum *et al.* (2017) concerning individuals with fast food diet habits.

Moreover, many studies explained the effect of dietary change to high protein, low carbohydrate diet on many PCOS manifestation such as hyperandrogenemia and menstrual regularity[42,43]. High fat high sugar (HFHS) diet led to metabolic disturbances as well as impaired reproductive health marked by abnormal cycling and the formation of PCOS[44], since fast food usually contains high amounts of saturated fats and steroids , accordingly, consumption of fast food and irregular eating habits leads to fluctuations in glucose levels, insulin resistance and increases hormonal imbalance such as hyperandrogenism[39].

Early menarche was not seems to be associated with strong risk of developing PCOS, since most of patients were not pre-pubertal girls.

Hormonal Changes of PCOS Patient after Vitamin D and COQ10 Treatment

With reference to the results in the current study, PCOS patientswith vitamin D deficiency (<20ng/ml) presented with elevated level of free testosterone (P<0.05) at the baseline compared to the non deficient patients, though still within the normal range in both. In the study of Wehr *et al*. (2009), the prevalence of vitamin D deficiency in 206 women affected by PCOS, vitamin D as an independent predictor of metabolic syndrome in PCOS showed a significant positive correlation of vitamin D levels sex hormone binding protein (SHBG) leading to higher level of free rather than total testosterone which was noticed in hypovitaminosis PCOS patients[45]. In another study Abdulridha *et al.* (2015) reported that SHBG level can be decreased in patients with metabolic disturbance like in diabetes, metabolic syndrome or PCOS patients and consequently lead to decrease hyperandrogenemia particularly increase in testosterone level[46]. After treatment with vitamin D3 supplement, a highly significant decrease in free testosterone level (P<0.01) was produced when compared to CoQ10 treatment, suggesting that restoration of endogenous vitamin D level may play important role in the pathogenesis of hyperandrogenemia in PCOS.

Previous study by Razavi *et al.* (2016) found that vitamin D-K-calcium co-supplementation for 8 weeks resulted in a significant reduction in serum free testosterone (-2.1±1.6 vs+0.1±1.0 pg/ml, P<0.001) and dehydroepiandrosterone sulfate (DHEAS) levels when compared to placebo[47]. Inversely, Jamilian *et al.* (2017) foundthe effect of vitamin D3 replacement therapy on androgen levels in PCOS women revealed significant decrease in total testosterone, and the change in free testosterone was non-significant[5]. Pal *et al*. (2012) found that reductions in total testosterone (P=0.036) and androstenedione (P=0.090) levels were noted following 3 month of vitamin D plus calcium supplementation[48].

On the other hand, Selimoglu *et al*. (2010), Wehr *et al*. (2011), Tehrani *et al.* (2014), and Maktabi *et al*. (2017) did not noticed significant change in both total and free testosterone after vitamin D3 supplementation[49,50,32,38]. Also Karadağ *et al.* (2018) and Bonakdaran *et al*. (2012) found no significant effect for vitamin D3 on total testosterone[51,52]. Recent study Al-Qadhi *et al*. (2017) reported the beneficial effect of CoQ10 in PCOS women administration for three months with metformin revealing significant reduction in total testosterone when compared to group treated with metformin monotherapy[53]. No available interventional studies evaluating the effect of vitamin D3 or CoQ10 on androgen level in CC resistance PCOS women, nevertheless, the present study suggest that vitamin D has beneficial effect on serum free testosterone level.

The present study revealed no significant change in FSH level after both interventions (P>0.05), meanwhile, there is significant decrease in LH level (P<0.05) after two months treatment particularly after CoQ10 supplementation more than post vitamin D3 therapy. No previous study available to interpret these findings, though at the baseline, Park *et al*. (2016) , Almoayad *et al*. (2017) and Al-Qadhi *et al*. (2017) and many others stated that no significant change in the baseline FSH, meanwhile there was higher baseline LH in PCOS patients[54,31,53]. Hsu *et al*. (2009) suggested that an LH: FSH ratio of >1 offered the best combination of sensitivity and specificity for the diagnosis of PCOS[55]. Accordingly, even within normal range of FSH and LH level in this study, the LH:FSH ratio was higher than 1 in both study groups, or greater than 2 in another studies as indicator of PCOS[53,56-58], but there are no exact cutoff values because many different assays are used[59], also the increase in secretion of LH and consequently increase LH: FSH ratio contribute to early growth arrest of antral follicles that reach terminal differentiation before time, producing a greater quantity of steroids and inhibin B that exert negative feedback on FSH production[60],hence, the LH: FSH ratio (1 : 2) to be < 1 produce good ovulation and more mature follicle, according to the Wiser *et al*. (2013) findings that the pregnancy rate in women with LH: FSH ratio of > 1.5 (16.7%) was significantly lower than in those with a ratio of 0.5-1.5 (40.4%), despite the nearly normal levels of both hormones[61].

In the present study, the LH: FSH ratio was significantly reduced (P<0.05) after both vitamin D3 and CoQ10 therapy when compared to pretreatment level. Dravecká *et al*. (2016) studied the effect of alfacalcidiol alone and in combined with metformin in PCOS women and he found no significant change in parameters (FT, DHEAS, LH, LH/FSH ratio) after 6 months therapy in all study groups[62].

As mentioned earlier, serum AMH overproduction (> 3 ng/ ml serum concentration) in PCOS is directly correlated with an increase in testosterone and/or LH concentrations[60,63], in Mahran *et al*. (2013) study of 60 anovulatory women with PCOS who underwent ovulation induction with clomiphene citrate, serum AMH concentration at baseline was found to be predictive

of ovulation and pregnancy. Ovulation and pregnancy rates were significantly higher (97%, $P < 0.001$, and 46%, $P = 0.034$) in patients with low AMH (<3.4 ng/ml) versus women with AMH 3.4 ng/ml or greater (48% and 19%)[64]. Accordingly, in the current study the baseline value of AMH was elevated in both study groups with up ceiling value in the hypovitaminosis PCOS patients, and after two months treatment AMH showed highly significant decrease (P<0.01) to the near normal level after vitamin D3 supplementation, and higher percent of change after Co Q10 supplementation in PCOS patients with normal vitamin D status.

Irani *et al*. (2013) reported that vitamin D3 supplementation 50000IU once weekly for 8 weeks decrease and normalize AMH level in women with PCOS compared with placebo. Appropriate supplementation of patients depleted of vitamin D may result in improved fertility outcomes via normalization of serum AMH[65].

Very recently, Sharma S. (2018) found significant change in AMH levels was observed with mean values changing from 4.88±2.06 ng/ml to 3.79±2.00 ng/ml, the results match the current findings. The proportion of women with normalized AMH levels (<4 ng/ml) increased from 20% to 80% following intervention, accordingly,endogenous vitamin D levels have a crucial regularizing effect on ovarian reserves among PCOS patients with vitamin D deficiency[66]. No previous study was available to interpret the effect of CoQ10 supplementation on AMH level in PCOS, nevertheless the one of the potential mechanisms of CoQ10 in clomiphene-citrate-resistant PCOS patient mentioned earlier, involving the production of cellular ATP, reduce oxidative stress within the ovary, decreasing the production of pro-inflammatory cytokines,protects the stability of plasma membranes, tissue deficiency of CoQ10 a micronutrient, or antiapoptotic mechanism involved in follicular cohort atresia, could have an indirect effect on AMH level.

Changes Reduced Glutathione (GSH) in PCOS Patient after Vitamin D and COQ10 Treatment

Oxidative stress was documented in infertile PCOS women regardless of whether they are lean or have metabolic abnormalities[67]. Glutathione is the major non-enzymatic antioxidant found in oocytes and embryos[68] that their serum level was decreased in PCOS patients[67,69,70].

In the present study there is highly significant improvement in endogenous GSH level (P<0.01) after treatment with both study interventions, due to antioxidant effect of vitamin D and Co Q10 supplements. This was in agreement with Foroozanfard *et al.* (2015) when he found that after 8 weeks the overweight and vitamin D-deficient women with polycystic ovary syndrome taking calcium plus vitamin D supplements had significant increases in plasma glutathione (GSH) levels 216·0 vs 3·9μmol/l (-47·5 and -160·8 μmol/l) respectively (P = 0·001) compared with calcium alone, vitamin D alone and placebo groups[71], also Nasri *et al*. (2017) found that vitamin plus evening primrose oil (EPO) for 12 weeks among vitamin D-deficient women with PCOS, a significant increase in plasma GSH[72].

To the best search, no previous study was found to highlight the effect of CoQ10 on GSH level in PCOS patients, however, this observed result in the present study probably due to the potent antioxidant effect of CoQ10.

Additionally, the excluded PCOS patients that became pregnant throughout this study showed marked decrease in their serum GSH posttreatment in both study groups, this is due to increase oxidative stress during pregnancy the fact supported by several studies observed a slight increase in oxidative stress, even in the presence of antioxidant systems since the beginning of pregnancy, such as catalase, glutathione peroxidase (GPX), vitamin C, glutathione, among others[73-75].

The Change in Ovulation and Pregnancy Outcome after Vitamin D3 and CoQ10 Supplementation

Several previous studies reported controversial finding concerning the effect of vitamin D3 as adjuvant treatment to improve outcome in a variety of PCOS symptoms including menstrual regularity and ovulation. Rashidi *et al.* (2009) found that higher number of dominant follicles in patients receive calcium-vitamin D plus metformin groups comparing to only calcium-vitamin D or only metformin throughout the follow up period (3 months intervention+3 months follow up), no pregnancy occur during the follow up period in any of the three intervention groups[76].

Meanwhile, Dravecká *et al*. (2016) reported pregnancy rate of (25%) after vitamin D (alphacalcidiol) plus metformin treatment for 6 months[62]. Similarly, Tehrani *et al*. (2014) found that dominant follicles was higher in metformin plus calcium and Vitamin D for 4 months compared with pretreatment[38]. Also Bonakdaran *et al*. (2012) found that calcitriol group had higher percent of ovulation (73%) compared to pretreatment (26%) and no effect in metformin vs. placebo treatment[52]. He also stated in his report that 2 infertile PCOS patients treated by calcitriol thereafter 2-3 month cut in treatment with calcitriol they experience natural gestation, the effect not seen after metformin therapy.

These finding came in agreement with the present study where vitamin D3 supplementation for 2 months in clomiphene citrate PCOS patients produce 18/24 (75%) positive ovulation and 3/24 pregnancies (12.5%), and there was 4/21 PCOS infertile patients became pregnant after discontinuation of vitamin D3 treatment, and this pregnancy occurs within the first month after correction their endogenous vitamin D status in this study.

On the other hand, several previous demonstrated the role of CoQ10 supplementation in PCOS patients, El Refaeey *et al*. (2014) found that ovulation occurred in 54/82 cycles (65.9%) in the CoQ10 treated group (which receive clomiphene citrate plus Co Q10 capsules orally 60mg t.i.d. for 2 induction months compared with control (which receive only induction with clomiphene citrate 150 mg per day for 5 days) where ovulation was (15.5%) only. Consequently, clinical pregnancy occurred in 19/51 in the CoQ10 treated women (37.3%), mean while the clinical pregnancy was 3/50 women (6.0%) in control group[29]. Also Lakshmi *et al*. (2018) found that the number of follicles >14 mm and >18 mm were significantly higher in the Co Q10 treated PCOS patients (who receive clomiphene citrate 100 mg daily for 5 day plus CoQ10 100mg daily for 45 days) compared to control group (clomiphene citrate only group) where ovulation occurred in 9 (45%) in the CoQ10 treated group and 10 (50%) in the control group, and the clinical pregnancy occurred in 2/20 women (10%) vs. 1/20 women (5.0%) in the control group[30].

In this study, CoQ10 supplement for 2 months in clomiphene citrate PCOS patients produce 13/17 (76.5%) positive ovulation and 3/17 (17.6%) pregnancy outcome, a result match the previous findings.

The overall therapeutic outcome of both interventions in the present study revealed improvement in ovulation stimulationin PCOS patients resistant to clomiphene citrate compared to the clomiphene citrate alone according to the pre treatment baseline data, hereby ovulation occur in (75%) of PCOS patient whom almost patient ovulate mature ova (ova size ≥ 18mm) in diameter, nevertheless, the overall pregnancy outcome was (15%) with equally distribution in both study groups.

Accordingly, it can be stated that both CoQ10 and vitamin D3 supplementation seems to be a promising adjuvant to oral ovulatory agents such as clomiphene citrate, and this combination proved to be effective, inexpensive, and safe for stimulating follicular development in PCOS patients resistant to clomiphene citrate and can be tried successfully before a more

complicated treatment such as gonadotrophins and laparoscopic ovarian drilling.

CONCLUSION

From this study we can conclude that supplementation with oral vitamin D and CoQ10 improve fertility outcome and ameliorating hormonal profile in clomiphene citrate resistance PCOS patients. Extended study is warranted to explore the effect of vitamin D3 and/or CoQ10 supplement in PCOS patients on metabolic status and other hormonal profile such as SHBP, TSH, estradiol, prolactin, particularly after restoration vitamin D status in those patients.

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Conflict of interest

The authors report no conflicts of interest in this work.

REFERENCES

[1] The Rotterdam EAsPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction. 2004;19(1):41-7.

[2] STEPHEN F, I MM, KATE H. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. International Journal of Andrology. 2006;29(1):278-85.

[3] Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. International Journal of Women's Health. 2011;3:25-35.

[4] Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics. 2015;70(11):765-9.

[5] Jamilian M, Foroozanfard F, Rahmani E, Talebi M, Bahmani F, Asemi Z. Effect of two different doses of vitamin d supplementation on metabolic profiles of insulin-resistant patients with polycystic ovary syndrome. Nutrients. 2017;9(12):1280.

[6] Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clinical endocrinology. 2012;77(3):343-50.

[7] Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber T, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. European Journal of Endocrinology. 2009;161(4):575-82.

[8] Ngo DTM, Chan W, Rajendran S, Heresztyn T, Amarasekera A, Sverdlov A, et al. Determinants of insulin responsiveness in young women: impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. Nitric Oxide. 2011;25(3):326-30.

[9] Mahmoudi T. Genetic variation in the vitamin D receptor and polycystic ovary syndrome risk. Fertility and sterility. 2009;92(4):1381-3.

[10] RatnabaliChakravorty D. The Relationship between Vitamin D, Insulin Resistance and Infertility in PCOS Women. Gynecology & Obstetrics. 2015;05(05).

[11] Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. Nutrition research reviews. 2009;22(1):82-92.

[12] Littarru GP, Tiano L. Clinical aspects of coenzyme Q10: an update. Nutrition. 2010;26(3):250-4.

[13] Özer A, Bakacak M, Kıran H, Ercan Ö, Köstü B, Kanat-Pektaş M, et al. Increased oxidative stress is associated with insulin resistance and infertility in polycystic ovary syndrome. Ginekologiapolska. 2016;87(11):733-8.

[14] Blair SA, Kyaw-Tun T, Young IS, Phelan NA, Gibney J, McEneny J. Oxidative stress and inflammation in lean and obese subjects with polycystic ovary syndrome. The Journal of reproductive medicine. 2013;58(3-4):107-14.

[15] Moti M, Amini L, Ardakani SSM, Kamalzadeh S, Masoomikarimi M. Oxidative stress and anti-oxidant defense system in Iranian women with polycystic ovary syndrome. Iranian journal of reproductive medicine. 2015;13(6):373.

[16] Turan V, Sezer ED, Zeybek B, Sendag F. Infertility and the presence of insulin resistance are associated with increased oxidative stress in young, non-obese Turkish women with polycystic ovary syndrome. Journal of pediatric and adolescent gynecology. 2015;28(2):119-23.

[17] Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. BiochimicaetBiophysicaActa (BBA)-Biomembranes. 2004;1660(1):171-99.

[18] Luce K, Weil AC, Osiewacz HD. Mitochondrial protein quality control systems in aging and disease. Protein metabolism and homeostasis in aging: Springer; 2010. p. 108-25.

[19] Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. Mitochondrion. 2007;7:S41-S50.

[20] Quinzii CM, Hirano M, DiMauro S. CoQ 10 deficiency diseases in adults. Mitochondrion. 2007;7:S122-S6.

[21] Ochiai A, Itagaki S, Kurokawa T, Kobayashi M, Hirano T, Iseki K. Improvement in intestinal coenzyme Q10 absorption by food intake. YakugakuZasshi. 2007;127(8):1251-4.

[22] Kawwass J, Loucks T, Berga S. An algorithm for treatment of infertile women with polycystic ovary syndrome. Middle East Fertility Society Journal. 2010;15(4):231-239.

[23] Homburg R. Clomiphene citrate—end of an era? a mini-review. Human Reproduction. 2005;20(8):2043-2051.

[24] Human serum free testosterone Maglumi kit (catalog No: 130202011M)[product insert on the internet]. China: Snibe.

[25] Human serum FSH Maglumi kit (catalog No: 130202001M)[product insert on the internet]. China: Snibe.

[26] Human serum LH Maglumi kit (catalog No: 130202002M)[product insert on the internet]. China: Snibe.

[27] Human serum AMH ELISA kit (catalog No: 032417) [product insert on the internet] .USA: AnshLabs.

[28] Human serum GSH ELISA kit (catalog No: E-EL-0026) [product insert on the internet].China: Elabscience.

[29] El Refaeey A, Selem A, Badawy A. Combined coenzyme Q10 and clomiphene citrate for ovulation induction in clomiphene-citrate-resistant polycystic ovary syndrome. Reproductive biomedicine online. 2014;29(1):119-24.

[30] Lakshmi B, Bhavani K, Sudhakar M, Parveen H. A study on quality of life and effect of coenzyme-Q10 in polycystic ovarian syndrome patients. International Journal of Advances in Pharmacy Medicine and Bioallied Sciences. 2018;6(1)14-21.

[31] Almoayad HA, Abdulrasul EA, Jumaa NA. COMPARISON OF ANTIMULLERIAN HORMONE LEVEL BETWEEN WOMEN WITH POLYCYSTIC OVARY SYNDROME AND NORMAL OVULATORY INFERTILE WOMEN OF REPRODUCTIVE AGE. Iraqi Journal of Medical Sciences. 2017;15(3):234-41.

[32] Maktabi M, Chamani M, Asemi Z. The effects of vitamin D supplementation on metabolic status of patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hormone and Metabolic Research. 2017;49(07):493-8.

[33] Wang F, Dai W, Yang X-h, Guo Y-h, Sun Y-p. Analyses of optimal body mass index for infertile patients with either polycystic or non-polycystic ovary syndrome during assisted reproductive treatment in China. Scientific reports. 2016;6:34538.

[34] Gomathi K, Shaafie I, Mummigatti K, Shahid S, Sreedharan J. Biochemical parameters in women with polycystic ovary syndrome in Ajman, UAE. Nepal Journal of Obstetrics and Gynaecology. 2012;6(2):7-10.

[35] El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G. Poly Cystic Ovarian Syndrome: An Updated Overview. Frontiers in Physiology. 2016;7(124).

[36] Vilmann LS, Thisted E, Baker JL, Holm J-C. Development of obesity and polycystic ovary syndrome in adolescents. Hormone research in paediatrics. 2012;78(5-6):269-78.

[37] Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Alvero R, et al. The Pregnancy in Polycystic Ovary Syndrome II study: baseline characteristics and effects of obesity from a multicenter randomized clinical trial. Fertility and sterility. 2014;101(1):258-69. e8.

[38] Tehrani HG, Mostajeran F, Shahsavari S. The effect of calcium and vitamin D supplementation on menstrual cycle, body mass index and hyperandrogenism state of women with poly cystic ovarian syndrome. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2014;19(9):875.

[39] Begum GS, Shariff A, Ayman G, Mohammad B, Housam R, Khaled N. Assessment of Risk Factors for development of Polycystic Ovarian Syndrome. Diabetes. 2017;1:2.

[40] Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R. Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. Fertility and sterility. 2001;75(1):53-8.

[41] Dabrell R. Differences in the intake of foods high in fats, sugars and low in antioxidants in women with polycystic ovary syndrome and a control group. 2016.
[42] Sørensen LB, Søe M, Halkier KH, Stigsby B, Astrup A. Effects of increased dietary protein-to-carbohydrate ratios in women with polycystic ovary syndrome–. The American journal of clinical nutrition. 2011;95(1):39-48.
[43] Stamets K, Taylor DS, Kunselman A, Demers LM, Pelkman CL, Legro RS. A randomized trial of the effects of two types of short-term hypocaloric diets on weight loss in women with polycystic ovary syndrome. Fertility and sterility. 2004;81(3):630-7.
[44] Roberts JS, Perets RA, Sarfert KS, Bowman JJ, Ozark PA, Whitworth GB, et al. High-fat high-sugar diet induces polycystic ovary syndrome in a rodent model. Biology of reproduction. 2017;96(3):551-62.
[45] Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber T, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. European Journal of Endocrinology. 2009;161(4):575-82.
[46] Khalaf BH, Abdulridha MK, Tuma WE, Najim HD. Effect of Endogenous Insulin Levels on Serum Testosterone, Glycemic, and Obesity Parameters in Premenopausal Women with Type 2 Diabetes Mellitus. UK Journal of Pharmaceutical and Biosciences. 2015;3(2):60-7.
[47] Razavi M, Jamilian M, Karamali M, Bahmani F, Aghadavod E, Asemi Z. The effects of vitamin DK-calcium co-supplementation on endocrine, inflammation, and oxidative stress biomarkers in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hormone and Metabolic Research. 2016;48(07):446-51.
[48] Pal L, Berry A, Coraluzzi L, Kustan E, Danton C, Shaw J, et al. Therapeutic implications of vitamin D and calcium in overweight women with polycystic ovary syndrome. Gynecological Endocrinology. 2012;28(12):965-8.
[49] Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, et al. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. Journal of endocrinological investigation. 2010;33(4):234-8.
[50] Wehr E, Pieber T, Obermayer-Pietsch B. Effect of vitamin D3 treatment on glucose metabolism and menstrual frequency in polycystic ovary syndrome women: a pilot study. Journal of endocrinological investigation. 2011;34(10):757-63.
[51] Karadağ C, Yoldemir T, Yavuz DG. Effects of vitamin D supplementation on insulin sensitivity and androgen levels in vitamin-D-deficient polycystic ovary syndrome patients. Journal of Obstetrics and Gynaecology Research. 2018;44(2):270-7.
[52] Bonakdaran S, Khorasani ZM, Davachi B, Khorasani JM. The effects of calcitriol on improvement of insulin resistance, ovulation and comparison with metformin therapy in PCOS patients: a randomized placebo-controlled clinical trial. Iranian journal of reproductive medicine. 2012;10(5):465.
[53] Al-Qadhi HI, Kadhim EJ, Ali RH. Coenzyme Q10 effects on body weight, serum testosterone level and oxidative stress in women with polycystic ovarian syndrome (PCOS). International Journal of Research in Pharmaceutical Sciences. 2017;8(3):377-82.
[54] Park C-H, Chun S. Association between serum gonadotropin level and insulin resistance-related parameters in Korean women with polycystic ovary syndrome. Obstetrics & gynecology science. 2016;59(6):498-505.
[55] Hsu M-I, Liou T-H, Liang S-J, Su H-W, Wu C-H, Hsu C-S. Inappropriate gonadotropin secretion in polycystic ovary syndrome. Fertility and sterility. 2009;91(4):1168-74.
[56] Saucedo de la Llata E, Moraga-Sánchez M, Romeu-Sarrió A, Carmona-Ruiz I. LH-FSH ratio and Polycystic Ovary Syndrome: A forgotten test? Ginecologia y obstetricia de Mexico. 2016;84(02):84-94.
[57] Al-Taee HA. The Role of Antimullerian Hormone in the Diagnosis of Poly Cystic Ovarian Syndrom. Journal of the Faculty of Medicine. 2013;55(4):374-9.
[58] Sheri FH, Jaccob AA, Hadi AM, Naser AA. Serum Leptin, Ghrelin and Insulin Resistance in Iraqi Women with Clomiphene Resistance Polycystic Ovary Syndrome. kufa Journal for Nursing sciences. 2015;5(1):243-54.
[59] Williams T, Mortada R, Porter S. Diagnosis and Treatment of Polycystic Ovary Syndrome. American family physician. 2016;94(2).
[60] Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Human Reproduction Update. 2008;14(4):367-78.
[61] Wiser A, Shehata F, Holzer H, Hyman JH, Shalom-Paz E, Son W-Y, et al. Effect of high LH/FSH ratio on women with polycystic ovary syndrome undergoing in vitro maturation treatment. The Journal of reproductive medicine. 2013;58(5-6):219-23.
[62] Dravecká I, Figurova J, Javorský M, Petrikova J, Valkova M, Lazurova I. The effect of alfacalcidiol and metformin on phenotype manifestations in women with polycystic ovary syndrome-a preliminary study. Physiological research. 2016;65(5):815.
[63] La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Human Reproduction Update. 2010;16(2):113-30.
[64] Mahran A, Abdelmeged A, El-Adawy AR, Eissa MK, Shaw RW, Amer SA. The predictive value of circulating anti-Müllerian hormone in women with polycystic ovarian syndrome receiving clomiphene citrate: a prospective observational study. The Journal of Clinical Endocrinology & Metabolism. 2013;98(10):4170-5.
[65] Irani M, Seifer D, Minkoff H, Merhi Z. Vitamin D supplementation appears to normalize serum AMH levels in vitamin D deficient premenopausal women. Fertility and Sterility. 2013;100(3):S338.
[66] S Sharma. Evaluation of effect of vitamin D supplementation on serum AMH in vitamin D deficient PCOS women, 2018; available from http://isge2018.isgesociety.com/wp-content/app/abs/pdf/abs6552.pdf.
[67] Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease☆ 1. Clinical biochemistry. 2001;34(5):407-13.
[68] Behrman HR, Kodaman PH, Preston SL, Gao S. Oxidative stress and the ovary. Journal of the Society for Gynecologic Investigation. 2001;8(1_suppl):S40-S2.
[69] Di˙ ncer Y, Akcay T, Erdem T, IlkerSaygi˙ li˙ E, Gundogdu S. DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scandinavian journal of clinical and laboratory investigation. 2005;65(8):721-8.
[70] Humadi EH, Al-Azzawie HF. Oxidative Stress and the Antioxidant Mechanisms in a Sample of Iraqi Patients with Polycystic Ovary Syndrome (POS). IRAQI JOURNALOF COMMUNITY MEDICINE. 2010;23(3):196-200.
[71] Foroozanfard F, Jamilian M, Bahmani F, Talaee R, Talaee N, Hashemi T, et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clinical endocrinology. 2015;83(6):888-94.
[72] Nasri K, Akrami S, Rahimi M, Taghizadeh M, Behfar M, Mazandaranian MR, et al. The effects of vitamin D and evening primrose oil co-supplementation on lipid profiles and biomarkers of oxidative stress in vitamin D-deficient women with polycystic ovary syndrome: A randomized, double-blind, placebo-controlled trial. Endocrine research. 2018;43(1):1-10.
[73] Tiwari D, Akhtar S, Garg R, Manger PT, Khan MM. A comparative study of oxidative status in pregnant and non-pregnant women. Int J Basic Appl Med Res. 2016;5(3):225-30.
[74] Patil S, Kodliwadmath M, Kodliwadmath SM. Study of oxidative stress and enzymatic antioxidants in normal pregnancy. Indian Journal of Clinical Biochemistry. 2007;22(1):135-7.
[75] Awusha OF, Elochukwu AC, Ogar IK, Augusta N, Maisie E. Assessment of Total Antioxidant Capacity and Lipid Profile among Pregnant Women Attending Ante Natal Clinic in University of Calabar Teaching Hospital, Nigeria. J Med Pharm Sci. 2016;2(4):72-5.
[76] Rashidi B, Haghollahi F, Shariat M, Zayerii F. The effects of calcium-vitamin D and metformin on polycystic ovary syndrome: a pilot study. Taiwanese Journal of Obstetrics and Gynecology. 2009;48(2):142-7.