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Research Article

Ova Size and Endometrial Thickness Post-treatment with Vitamin D and Coenzyme Q10 Supplements in PCOs Women Resistant to Clomiphene

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Abstract

Clomiphene Citrate resistance is a term that refers to the persistence of ovulation after standard Clomiphene Citrate therapy, and about 15% of Polycystic ovary syndrome were resistant to the maximum dose of clomiphene citrate. The study aims to evaluate the effect of combining oral vitamin D3 and CoQ10 supplements to overcome Clomiphene resistance on hormonal profile and ovulation outcome(ova size and endometrial thickness) in clomiphene citrate resistance PCOS patients. The study was a prospective intervention comparative, open-label study include 35 PCOS patient aged (18-34) years are resistant to clomiphene citrate ellocated in two groups, group 1 include vitamin D deficient PCOS patient received clomiphene citrate 100mg daily clomiphene for five days in each month with 10000IU daily vitamin D for 2 months, group 2 include PCOS patient with no vitamin D deficiency received 100mg daily clomiphene for five days in each month plus CoQ10 200mg daily for 2 months. Fasting blood samples used to measure serum estradiol, antimullerian hormone (AMH), luteinizing hormone (L.H.), and follicle-stimulating hormone (FSH) to calculate L.H.:FSH ratio, mid-cycle transvaginal ultrasound for determination of ova size and endometrial thickness. Both Vitamin D3 supplementation and CoQ10 supplementation resulted in a significant increase in ova size ($P < 0.001$), while the increase in endometrial thickness was substantial only after vitamin D supplementation ($P < 0.001$). Meanwhile, serum estradiol levels increased in both study groups after intervention but still not significant ($P > 0.05$). In conclusion, receiving vitamin D and CoQ10 supplements by clomiphene resistance PCOS women improves serum hormone, including AMH and L.H.: FSH ratio, also improving ovulation outcome (ova size and endometrial thickness).

Keywords: Clomiphene Citrate Resistant; Polycystic Ovary Syndrome; Co-Q10; Vitamin D 3; Estradiol; Ovarian Functions; Endometrial Thickness

Introduction

On of the common endocrine disorder affect ibf women at reproductive age is the polycystic ovary syndrome (PCOS) is occurring in (4-12%) of women, which affects reproductive performance and the overall health including the oligomenorrhea and obesity along with polycystic ovaries [1]. The oligomenorrhea, menstrual

cycles last more than 35 days in eight cycles per year and consider as anovulatory processes; however, hyperandrogenemia with regular menstruation do not exclude chronic anovulation, especially in women with [2], since hirsutism considers the most common clinical sign of hyperandrogenemia and seen in approximately 60% of women with PCOS [3]. Clomiphene Citrate (CC) resistance, is a term which signifies persistence anovulation after standard CC therapy, in other words, failure to ovulate after receiving 150 mg of CC daily for five days per cycle, for at least three cycles [4,5]. Clomiphene resistance occurs approximately in (15 to 40%) of PCOS women and considers a significant challenge in gynecologic endocrinology[6].

In PCOS women, estrogen secretion is characterized by chronic secretion without the cyclic pattern associated with the anovulatory cycle. Serum estradiol (E2) levels may vary in PCOS. Still, serum estrone (E_1) levels are usually more significant than those of $E_2$and this chronic estrogen secretion increase risk of endometrial hyperplasia and possibly the development of endometrial carcinoma [7]. A result of the lack of ovulation in PCOS leads to continuous high levels of estrogen. Insufficient progesterone results in increased serum luteinizing hormone (L.H.) levels and alterations in Anti-Mullerian Hormone (AMH) secretion. The higher the antral follicles count, the higher AMH levels, and women with PCOS typically have high antral follicles [8].

Vitamin D deficiency is common in PCOS, about) 67-85% (of PCOS women presented with 25-hydroxy Vitamin D (25OHD) serum concentrations of <20ng/ml [9], vitamin D deficiency may strengthen symptoms of PCOS, in an observational studies lower 25OHD levels potentiate insulin resistance, ovulatory and menstrual irregularities, lower pregnancy success rate, hirsutism, hyperandrogenism, obesity, and elevated cardiovascular disease risk factors [10], hence improved vitamin D status through supplementation can be theorized to hold potential for improving ovulation induction therapy, live birth rates, and reducing the risk of pregnancy loss in women with PCOS, a population that is already at enhanced risk for pregnancy wastage [11].

Coenzyme Q10 (CoQ10) is produced in mitochondria which is critical for the normal function of the body, especially reproduction, to facilitate energy production for the production of eggs, also; in women, oxidative stress cause mutations leading to decreased pregnancy rates and egg production, also increase abortion rate, accordingly, antioxidant effects of CoQ10 protects eggs from free radicals and oxidative stress; hence, CoQ10 supplementation may improved fertility [12,13]. Xu., *et al.* (2018) found that Pretreatment with CoQ10 improves embryological parameters in poor ovarian reserve in IVF-ICSI cycles [14]. Endogenous CoQ10 increases until 20 years of age, and then it decreases throughout life. Deficiency can be caused by biosynthesis impairment, insufficient feeding, or excessive body utilization [15].

They have taken together these facts. This study was designed to explore further the potential benefit of both supplements (Vitamin D3and CoQ10) in improving the quality of ova and endometrial thickness and their effectiveness in enhancing hormonal markers in PCOS women with clomiphene citrate resistance.

Materials and Methods

Patients

This prospective comparative open-label study of a total of 35 patients diagnosed CC resistant PCOS enrolled in the study. Patients ages of 18 and 40 and wanted to become pregnant, from private clinic's gynecologic and obstetric were enrolled. The patients were under the care of a gynecologist and were diagnosed using Rotterdam standards and treated according to practice guidelines. Resistant to clomiphene citrate (CC) monotherapy defined as persistent anovulation or ovulate with a very thin endometrium <5 mm, on the day of HCG administration when the patient previously take induction therapy with clomiphene citrate alone for five days at least two or three cycles. Vitamin D deficient (< 20 ng/ml) PCOS patients were enrolled in one group, and PCOS patients with no vitamin D deficiency enrolled in other group. The PCOS patients had oligomenorrhea for more than one month, progesterone (Allylestrenol) 5mg tablet twice daily for ten days used to attained menstrual bleeding, thereafter enrolled in the study. Patients with other gynecological and hormonal disorders are omitted from study.

The study received approval from the institution's scientific ethics committee and the general health directorate's approval. Patient informed permission was obtained after a thorough clarification of the study's intent, ensuring the accuracy of the data collected. This study is designed to evaluate combining oral vitamin D3 tablet or oral Co-enzyme Q10 with clomiphene citrate tablet on ovulation induction through ova size and endometrial thickness in

CC-resistance PCOS patients, in addition to their effect on serum estradiol, L.H.:FSH ratio, and AMH. Group 1 patients included 21 patients treated with clomiphene citrate tablets 50mg twice daily after the meal for five days monthly with a 10000IU daily dose of vitamin D3 oral tablets for two months. Group 2 include 14 patients received the same clomiphene citrate dosing plus 200mg daily dose of CO- Q10 capsule l for two months.

Transvaginal ultrasound at day ten and day 12of the cycle was performed to all patients to determine the number and size of the dominant follicle (≥18 mm) in addition to endometrial thickness. When at least one strand measuring at least (18 mm) was detected, Pregnyl 5000-10,000 IU (HCG) injection was released. Patients were recommended to have sexual activity for the next 24-36 hours after receiving HCG injection. Patients' blood samples were taken after the two months for post-treatment assessment.

Method

Serum estradiol determination using ELISA estradiol kit by fully automated ELISA apparatus [16], serum L.H. and FSH determination using the MaglumiFSH (L.H.) assay, a sandwich chemiluminescence immunoassay [17,18] then L.H.: FSH ratio calculation was done. The Ultra-Sensitive Antimullerian Hormone/Mullerian Inhibiting Substance (AMH/MIS) ELISA used to measure Serum Antimullerian Hormone (AMH) using sandwich immunoassay [19]. Transvaginal ultrasound, an examination of the female pelvis, was done for all patients in the study on days 10 and 12 of the cycle to follow up the ovulation (ova size and endometrial thickness) using an ultrasound machine radiologist [20]. And used for ova size and endometrial thickness determination to decide the time of human chorionic gonadotropin (hCG)administration when mature follicles are seen; that average endometrium thickness was 8mm mature follicle size from 16-22 mm [21,22]. The blood samples were first phenotyped for ABO blood groups (A, B, AB, and O) using commercially available antisera (Biotest, Germany) utilizing a slide agglutination test [23].

Statistical analysis

The SPSS version 21 was used for categorical variables presented as number and percente. Continuous variables were presented as (Means ± S.D.).The categorical variables were linked using the Fisher exact test. To equate the means of two classes, the independent samples t-test was used. For paired reading, the paired t-test was used to compare ways. Significant was described as a p-value of less than 0.05.

Results

Socio-demographic and disease characteristics of PCOS patients on vitamin D3 and Co-Enzyme Q10 supplements

The patient demographic and Disease characteristics of 35 female patients are allocated as 21 patients in group 1 (58.5%) and 14 patients in group 2 (41.5%), Table (1). The mean age was 24.57 ± 4.16years for group 1 patients and 22.36 ± 3.48 years for group 2 patients. The body mass index for group 1 patients and group 2 patients was 27.16 ± 4.66 kg/m2 and 26.79 ± 4.42kg/m2. Vitamin D level mean was 11.34 ± 3.41 and 36.68 ± 17.75 for patients in groups 1 and 2, respectively. Positive family history was recorded in (37.5%) of patients in group 1 and (29.4%) of patients in group 2. The disease history for patients in group 1 and 2 were as follows 19% versus 28.6% for less than 2 years duration, 71.4% versus 64.3% for 2-10 years duration, and 9.6% versus 7.1% for more than 10years duration, there is no significant differences between study groups regarding age, BMI, family history and duration of symptom while a significant difference was seen between study groups regarding vitamin D level.

The ABO blood group phenotypes for patients in group 1 where patients carrying O phenotype represents (47.6%), A phenotype patients (23.8%), and the B and A.B. phenotypes means (14.3%) for each phenotype, meanwhile, in group 2, higher percent (35.7%) were A phenotype and (28.6%) were O phenotypes while (21.4%) and (14.3%) of patients was B and A.B. phenotypes consequently, no significant difference between study groups regarding ABO blood group.

Among PCOS phenotype patients in group 1 and 2 were 66.7% versus 28.6% have all Rotterdam criteria (ovulatory dysfunction +PCOS on US+ androgen excess), and 23.8% versus71.4% have (ovulatory dysfunction +PCOS on the U.S.) criteria, while (PCOS on U.S. + androgen excess) was seen only in two patients in group 1. No significant difference was found between both groups concerning all baseline socio-demographic and Disease characteristics data ($P > 0.05$) except for Rotterdam criteria and vitamin D level that show a significant difference between both study groups ($P \leq 0.05$).

Study variables	Study groups		P-value
	Group 1 (n = 21)	Group 2 (n = 14)	
Age (years)	(24.57 ± 4.16)	(22.36 ± 3.48)	0..098^{NS}
	n %	n %	
BMI (kg/m^2)	27.16 ± 4.66	26.79 ± 4.42	0.816^{NS}
Vitamin D level (Baseline)	11.34± 3.41	36.68 ± 17.75	*<0.001**
Family history of PCOS			
Yes	9 (42.9)	4 (28.6)	0.488^{NS}f
No	12 (57.1)	10 (71.4)	
Duration of symptoms			
<2 year	4 (19.0)	4(28.6)	
(2-10) years	15 (71.4)	9 (64.3)	0.800^{NS}
>10 years	2 (9.6)	1 (7.1)	
Rotterdamcriteria			
Phenotype (A) Ovulatory dysfunction +PCOS on US+ androgen excess	14(66.7)	4(28.6)	
Phenotype (B) Ovulatory dysfunction +androgen excess	0(0.0)	0(0.0)	0.017*
Phenotype (C) PCOS on US + androgen excess	2(9.5)	0(0.0)	
Phenotype (D) Ovulatory dysfunction +PCOS on US	5(23.8)	10 (71.4)	
Blood group phenotypes			
O	10(47.6)	4(28.6)	
A	5(23.8)	5(35.7)	0.699^{NS}
B	3(14.3)	3(21.4)	
AB	3(14.3)	2(14.3)	

Table 1: Baseline socio-demographic and Disease characteristics of patients.

Data presented as mean ± S.D., Number (n), Percentage (%),No significant differences (NS) (P > 0.05),*: significant difference (P ≤ 0.05),

f: Fisher exact test used for statistical analysis of (family history).

Independent -sample t-test is used for statistical analysis of (age, BMI, vitamin D).

Chi-square test is used for statistical analysis of (blood group, Rotterdam criteria, duration of symptoms).

Effect of vitamin D3 and co-enzyme Q10 supplements on serum estradiol level (E2), antimullerian hormone (AMH), and L.H.:FSH ratio

In the pretreatment mean estradiol level, no significant difference was noticedbetween group 1 and group 2 patients (P > 0.05), post-treatment increase in estradiol in both study groups was non-significant when compared to pretreatment level (P > 0.05),also no significant difference between both study groups after treatment (P > 0.05). In contrast, the pretreatment means AMH shows

a significant difference between group 1 and group 2 patients (P ≤ 0.05), and post-treatment, the difference was non-significant. Still, both study group post-treatment improvement was significant (P ≤ 0.05), L.H.: FSH ratio means no significant difference was noticed between group 1 and group 2 pre and post-treatment. In contrast, the decrease in the ratio in both study group after the intervention was significant (P ≤ 0.05), (Table 2).

Study variable	Study group		P-value
	Group 1	Group 2	
E2 (pg/ml)	n=21	n=14	
Pre treatment	89.13 ± 41.43	81.91 ± 21.40	0.36^{NS}
Post treatment	122.54 ± 88.83	83.95 ± 25.34	0.072 ^{NS}
P-value	0.122 ^{NS}	0.826 ^{NS}	
Anti-mullerian hormone AMH (ng/ml)			
Pre treatment	4.75 ± 2.76	4.08 ± 3.10	0.020*
Post treatment	3.51 ± 1.56	2.96 ± 1.88	0.067 ^{NS}
P-value	0.003*	0.022*	
LH/FSH ratio			
Pre treatment	1.11 ± 0.40	1.35 ± 0.55	0.185^{NS}
Post treatment	0.86 ± 0.31	0.80 ± 0.24	0.519^{NS}
P-value	0.019*	0.001*	

Table 2: Effect of Vitamin D3 and Co-Enzyme Q10 Supplements on Serum Hormonal Level.

Data presented as mean ± S.D., Number of patients (n).

NS: No significant differences (P > 0.05), *: significant difference (P ≤ 0.05).

Effect of vitamin D3 and co-enzyme Q10 supplements on ova size and endometrial thickness (E.T.)

The mean ova size of patients in group 1 and group 2 showed no significant difference Pretreatment (P > 0.05); nevertheless, a significant increase in the mean ova size in both groups 1and group 2 was produced post-treatment with both supplements when compared to pretreatment level (P < 0.05) after two months of intervention, (Table 3). Moreover, the mean endometrial thickness in patients between group 1 and group 2 was not different pretreatment (P > 0.05); however, a significant increase in endometrial thickness was revealed in group 1 patients when compared to pretreatment level (P < 0.05) after two months of intervention, meanwhile the increase was non-significant in group 2 patients after intervention (P > 0.05).

Study variable	Study group		P-value
	Group 1	Group 2	
Ova size (mm)	n = 21	n = 14	
Pre treatment	11.06 ± 4.9	12.66 ± 4.82	0.311^{NS}
Post treatment	17.15 ± 4.12	19.18 ± 3.87	0.155^{NS}
P-value	<0.001*	0.001*	
Endometrial thickness (mm)			
Pre treatment	6.76 ± 1.48	7.48 ± 1.74	0.214 ^{NS}
Post treatment	8.36 ± 1.66	7.86 ± 2.27	0.488 ^{NS}
P-value	<0.001*	0.526 ^{NS}	

Table 3: Effect of vitamin D3 and coenzyme Q10 supplements on Ova size and Endometrial Thickness.

Data presented as mean ± S.D., Number of patients (n).

NS: No significant differences (P > 0.05), *(P ≤ 0.05) is considered a significant difference.

Discussion

Many of the PCOS patients in this sample were resistant to clomiphene citrate and wanted to get pregnant, and they were between the ages of 18 and 34, as in the majority of prior trials [24-27]. Most PCOS patients in the current study were overweight and obese. This finding was consistent with that of previous studies where more than half of enrolled patients were obese [28,29], weight gain occur in (61%) and (76%) of PCOS women [30]. Hyperinsulinemia, insulin resistance, and hyperandrogenemia affect adipocyte activity and distribution by inhibiting adipocyte differentiation, which regulates lipolysis and lipogenesis [31].

Positive family history of PCOS in the present study was between (30%-40%) of patients. Similarly (45% - 60.8%) of PCOS patients have a positive family history as reported previously [32], and this is due to the fact of a hereditary component of PCOS and family

association of reproductive and metabolic abnormalities causing increased risk of among first-degree relatives of PCOS women [33]. Another study found a 5 - 6 fold increase among first-degree relatives compared with PCOS prevalence in the general population [34]. In this study, most PCOS patients enrolled have duration of symptoms of a (2-10) years which is the leading cause of CC resistance or increase symptoms.

PCOS phenotype distribution in this study population fulfilled two out of three Rotterdam criteria giving a PCOS phenotype A [ovulatory dysfunction+polycystic ovary on US+androgen excess] reported in 18 women (14 of them in group 1 and 4 of them in group 2), PCOS phenotype B [ovulatory dysfunction and clinical and/or biochemical hyperandrogenism] was not observed in the present study patients, PCOS phenotype C [polycystic ovary on the U.S. and clinical and/or biochemical hyperandrogenism] only presented in 2 women in group 1, PCOS phenotype D [ovulatory dysfunction and polycystic ovary on the U.S.] repotted in 15 women (5 of them in group 1 and 10 of them in group 2).This finding showed that a higher percentage of enrolled patients carry PCOSphenotype A; this was inconsistent with previous studies [35,36]; meanwhile, other studies showed a higher percent of phenotype C (37-39%), as different phenotypes were less prevalence [37-40]. Most PCO women enrolled carry blood group O group phenotype, the finding in line with that of previous studies [41-43].

Estrogen has the potential to directly scavenge free radicals, enhance the function of certain naturally occurring antioxidant enzymes (particularly glutathione peroxidase), and function as a chain-breaking antioxidant in vivo when present in high concentrations [44]. On the other hand, anovulatory women have a comparatively steady-state estradiol level. They frequently have oligo- or amenorrhea since estrogen levels peak twice in the cycle, once in the late follicular phase, and to a lesser extent in the luteal phase [45]. Serum estradiol was tested during the early follicular phase in this study, and the baseline was varied but still within the normal range for serum estradiol during the follicular phase. After the intervention, serum estradiol increased in both classes, particularly after vitamin D supplementation. There was no previous research to interpret the impact of vitamin D or CoQ10 on serum estradiol levels. Still, the ambiguous effect could be due to the time of testing estradiol and the limited sample size of patients after excluding the patients who became pregnant in the study, since the risk of extreme elevation of serum estradiol during pregnancy, which was observed in the current study, could be due to the time of testing estradiol and the small sample size of patients after excluding the patients who became pregnant in the [46].

In PCOS women, an alteration in gonadotropin-releasing hormone secretion, causing an increase in luteinizing hormone (L.H.) secretion with normal follicle-stimulating hormone (FSH) secretion, has been observed and widely accepted as specific endocrine profiles [47], this profile gives rise to an abnormal LH/FSH ratio in many patients, and a valuable diagnosis biomarker of PCOS [48]. Hence, the L.H.: FSH ratio of (1: 2) when presented (< 1) it produce good ovulation and more mature follicle, according to the Wiser., *et al*. whom reported that the pregnancy rate in women with L.H.: FSH ratio of (> 1.5) was significantly lower (16.7%) than in those with a balance of (0.5-1.5) (40.4%), despite the nearly normal levels of both hormones [49]. In the present study, the L.H.: FSH ratio was significantly reduced ($P < 0.05$) after both vitamin D3 and CoQ10 therapy compared to the pretreatment level. However, the effect of alfacalcidol alone and combined with metformin in PCOS women produced no significant change in LH/FSH ratio after six months of therapy in the previous study [50].

Excessive ovarian development of anti-mullerian hormone (AMH), secreted by an excessive amount of developing follicles, has long been thought to be a key feature of PCOS, with growing data in the last decade supporting AMH's function in the syndrome's pathogenesis [48]. The baseline AMH was elevated in both groups, with a higher value in the hypovitaminosis PCOS patients. After two months of vitamin D3 and Co Q10 supplementation, the level significantly decreased to a near-normal level. Irani., *et al*. found that giving 50000IU vitamin D3 to PCOS women deficient in vitamin D once a week for eight weeks reduced and normalized AMH levels relative to placebo, resulting in better fertility outcomes [51]. Moreover, The results mirrored the existing findings, with mean values changing from 4.882 ng/ml to 3.792 ng/ml. Following the action, the proportion of women with stabilized AMH levels (4 ng/ml) increased from 20% to 80%, indicating that endogenous vitamin D levels have a critical regularizing impact on ovarian reserves in PCOS patients with vitamin D deficiency [52]. Despite the lack of previous studies to interpret the effect of CoQ10 supplementation on AMH levels in PCOS patients, one of the possible mechanisms of

CoQ10 in clomiphene-citrate-resistant PCOS patients involves cellular ATP formation.

Reduces oxidative stress in the ovary, which reduces the synthesis of pro-inflammatory cytokines and maintains plasma membrane integrity. Tissue depletion of CoQ10, a micronutrient, or an antiapoptotic pathway implicated in follicular cohort atresia may indirectly affect AMH levels.

Ova size in the present study was increased significantly after both interventions, and this improvement probably due to decreasing oxidative stress after use of antioxidants such as vitamin D and CoQ10 supplementations, and since oxidative stress considered as a critical signal in the initiation of apoptosis in antral follicles and granulosa cells of antral hairs by diverse stimuli, such as exposure to radiation, exogenous toxicants, or gonadotropin withdrawal, so that antioxidants protect against these stimuli [53]. This improvement in ova size in the present study led to improved overall ovulation stimulation as reported in the previous study where the overall ovulation stimulation was in 75% of PCOS patients; meanwhile, comprehensive pregnancy was 15% equal in both groups [54]. No previous studies had explored the effect of vitamin D or CoQ10 on ova size to the best search.

Finally, the endometrial thickness in the present study was increased significantly after vitamin D supplementation, as in agreement with studies by Rasekhjahromi Athar., *et al*. and Asadi., *et al*. [55,56]; meanwhile, no effect of CoQ10 on endometrial thickness was observed. Controversy data was reported previously regarding the effect of CoQ10 on endometrial thickness, Lakshmi., *et al*.'s finding stated that endometrial thickness in clomiphene citrate plus CoQ10 was significantly lowered than in clomiphene citrate only group [25]. Conversely, the study by Refaeey., *et al*. revealed that endometrial thickness considerably higher in the clomiphene citrate plus CoQ10 group than in the clomiphene citrate only group [24]. Nevertheless, following the overall results of the present study, clinical pregnancy occurs equally in 3 PCOS patients after receiving vitamin D and Co Q10 supplements in each study group.

Conclusion

Based on the findings of this report, oral vitamin D and CoQ10 supplementation improves reproductive outcomes while also improving hormonal profiles in clomiphene citrate resistance PCOS patients (ova size and endometrial thickness); a more extensive study is needed to see how vitamin D3 and CoQ10 supplementation affects metabolic status and other hormonal profiles including SHBG, TSH, and prolactin in PCOS patients, particularly after vitamin D status is restored.

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Conflict of Interest

The authors report no conflicts of interest in this work.

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