Role of Vitamin D Supplementation Therapy on Ovulation and Insulin Resistance in Women with PCOS: A Randomized Controlled Trial

Original Article

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ABSTRACT

Introduction: Polycystic ovarian syndrome (PCOS) is the most prevalent worldwide female endocrine disorder, affecting nearly 5%-12% of reproductive-aged women. PCOS is the most common cause of anovulatory infertility and its foremost clinical symptoms include anovulation or oligo-ovulation, infertility, menstrual irregularity, polycystic ovaries and hyperandrogenism. PCOS is also common among infertile Arabian female population and it is associated with significant elevations in markers of metabolic syndrome, insulin resistance and cardiovascular risks. Unfortunately, PCOS is not a simple pathophysiologic process for which one treatment is sufficient to control all manifestations. Therefore, when choosing a treatment regimen, it should target specific manifestations and individualized patient goals.

Aim of the Work: The aim of this study is to assess the safety and the efficacy of vitamin D supplementation therapy on ovulation and metabolic changes in women with PCOS.

Study design: Prospective randomized controlled clinical trial.

Patients and Methods: The current study was conducted in the infertility clinics of Ain Shams University Maternity Hospital in the period between May 2015 and May 2017. It included 300 women diagnosed with polycystic ovary syndrome attending the infertility clinics of Ain Shams University Maternity Hospital.

Results: Being a classification criterion, 25OHD level was significantly lower in the vitamin D deficient subgroup compared to the normal vitamin D subgroup; whereas no significant differences were found between the vitamin D deficient group and the control group. In the same context, 25OHD level was statistically significantly lower in the control group compared to the normal vitamin D subgroup. Vitamin D deficient PCOS women tended to have higher degree of insulin resistance. Fasting glucose was statistically significantly higher in the vitamin D deficient subgroup compared to the normal vitamin D subgroup and the control group; and higher in the control group compared to the normal vitamin D subgroup. Fasting insulin level was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup; whereas no statistically significant differences were found between the former two groups. HOMA2-IR was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup; whereas no statistically significant differences were found between the vitamin D deficient subgroup and the control group. No statistically significant differences were found between the three groups in the various components of the lipid profile.

Conclusion: Results of the thesis showed that cumulative ovulation rate was significantly higher in the vitamin D deficient subgroup following vitamin D supplementation compared to the normal vitamin D subgroup and the control group with a rate ratio of 1.27 and 1.22, respectively. Number needed to treat was calculated to be 5.34 and 6.38 compared to normal vitamin D subgroup and control group respectively, i.e. 6.38 women are needed to be supplemented with vitamin D for one of them to benefit compared to control women. No significant differences were found between the three subgroups regarding the median ovulating dose of clomiphene citrate. Also, no significant differences in the cumulative clinical pregnancy rate between the ovulatory women of the three subgroups.

INTRODUCTION:

Polycystic ovarian syndrome (PCOS) is the most prevalent worldwide female endocrine disorder, affecting nearly 5%-12% of reproductive-aged women[1]. PCOS is the most common cause of anovulatory infertility and its foremost clinical symptoms include anovulation or oligo-ovulation, infertility, menstrual irregularity, polycystic ovaries and hyperandrogenism[2].

PCOS is also common among infertile Arabian female population and it is associated with significant elevations in markers of metabolic syndrome, insulin resistance and cardiovascular risks[3]. Unfortunately, PCOS is not a simple pathophysiologic process for which one treatment is sufficient to control all manifestations. Therefore, when choosing a treatment regimen, it should target specific manifestations and individualized patient goals[4].

There are many considerations to explain the underlying bases of PCOS, particularly the well-known roles of hypothalamic-pituitary gonadal dysfunctions, metabolic abnormalities and the genetic factors. However, the definite pathogenesis and real underlying etiologies of PCOS remain uncertain and ripe with opportunities for further research[5]. Moreover, there is variation in the prevalence and phenotypes of PCOS in many ethnic/racial groups[6].

Recently, a special emphasis has recently been directed to the potential role of vitamin D and some regulatory peptides (e.g. adipokines and follistatin) and their associated metabolic roles and genetic factors in the development of PCOS and its related co-morbidities. In this concept, the discovering of vitamin D receptors (VDRs), which are nuclear receptors present in nearly every tissue in the body and regulates the expression of about 229 genes of the whole human genome, has revolutionized the importance of vitamin D and provided new insights into its functions[7].

Aim of the Work: The aim of the current study was to assess the safety and the efficacy of vitamin D supplementation therapy on ovulation and metabolic changes in women with PCOS.

PATIENTS AND METHODS

Study Design:

Prospective randomized controlled clinical trial. The current study was conducted in the infertility clinics of Ain Shams University Maternity Hospital in the period between May 2015 and May 2017. It included 300 women diagnosed with polycystic ovary syndrome attending the infertility clinics of Ain Shams University Maternity Hospital.

Inclusion criteria:

Age 20-35 years of age, Polycystic ovary syndrome,

diagnosed according to the revised 2003 consensus on diagnostic criteria of polycystic ovary syndrome by the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group by the presence of two of the following three diagnostic features: Oligo- or anovulation, Clinical hyperandrogenism [defined by the presence of hirsutism (assessed by a modified Ferriman-Gallwey score ≥ 8 (Yildiz *et al.*, 2010)), acne or androgenic alopecia (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003)] and/or biochemical signs of hyperandrogenism [defined by elevated free androgen index (RCOG Green-top guideline no. 33, 2014)], Polycystic ovaries [defined as presence of 12 or more follicles in each ovary measuring 2 – 9 mm in diameter, and/or increased ovarian volume > 10 mL) in the early follicular phase (cycle days 3 – 5)] in regularly menstruating women. Oligo-/amenorrhoeic women were scanned either at random or between days 3 and 5 after a progestin-induced withdrawal bleeding (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003).

Exclusion criteria:

Age less than 18 or more than 40 years, Diagnosed diabetes mellitus according to American Diabetes Association criteria: HBA1C ≥6.5%, fasting plasma glucose ≥126 mg/dl, 2-h plasma glucose ≥200 mg/dl during an OGTT using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water or a random plasma glucose ≥200 mg/dl in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis (American Diabetes Association, 2010), Any endocrinological disorder, e.g. hypo/hyperthyroidism, Cushing's disease, Any chronic medical disorder, e.g. renal disease, chronic liver disease, hypertension or autoimmune disorders, etc., Insulin sensitizing therapy, e.g. metformin or dipeptidyl peptidase 4 (DPP-4) inhibitors as sitagliptin, etc., Drugs affecting insulin resistance, e.g. steroids, Women with other concomitant cause of infertility (e.g. abnormal semen analysis, tubal abnormalities, endometriosis, etc.) or pelvic pathology (e.g. endometrial polyp, hyperplasia, ovarian cyst, etc.), Vitamin D or calcium supplementation, Oral contraceptive pill therapy or other hormonal treatment within last three cycles or Mental condition rendering the patients unable to understand the nature, scope and possible consequences of the study.

After enrollment, an informed written consent was taken from all participants before recruitment in the study.

Patients fulfilling the inclusion criteria were randomized to two groups.

Vitamin D Group: This group included 150 women with PCOS seeking fertility. This group was further stratified according to their vitamin D status into 2 subgroups by a cutoff level of serum 25-hydroxyvitamin-D of 20 ng/mL[8]:

- Vitamin D deficient subgroup included PCOS women with serum 25(OH)D < 20 ng/mL, who received supplemental vitamin D3 for 8 weeks, followed by induction of ovulation with clomiphene citrate.

- Normal vitamin D subgroup included PCOS women with serum 25(OH)D ≥ 20 ng/mL, who started induction of ovulation with clomiphene citrate directly, without vitamin D supplementation.

Control Group:

This group included 150 women with PCOS seeking fertility. This group started induction of ovulation with clomiphene citrate without prior vitamin D supplementation.

Random allocation sequence generation

A computer generated list via MedCalc® Software, version 13.2.2 was used, assigning each participant number to either study groups.

Allocation Concealment

Assignment was done by sequentially numbered, otherwise identical, sealed envelopes (SNOSE), each containing a 2-inch by 2-inch paper with a written code designating the assigned group. These papers were placed in a folded sheet of aluminum foil fitted inside the envelope. Effort was taken to assure the absence of any detectable differences in size or weight between intervention and control envelopes.

Envelopes were chosen to be opaque and lined inside with carbon paper and were opened sequentially only after writing the subject's tracking information on the envelope so that the carbon paper served as an audit trial.

Drugs, dosage and regimen

Vitamin D supplementation: Oral solution of cholecalciferol containing 100 IU/drop (Vidrop®, Medical Union Pharmaceuticals, Egypt).

Regimen: According to the Endocrine Society guidelines, 6000 IU of vitamin D3 were given daily to patients with vitamin D deficiency for 8 weeks to achieve a serum level of 25(OH)D above 30ng/mL,

followed by a maintenance dose of 2000 IU daily. Patients with BMI >30 Kg/m2 were given a higher dosage of 10,000 IU daily followed by a maintenance dose of 6,000 IU daily.

Calcium supplementation: 500 mg tablets of calcium carbonate (Calcimate®, Memphis, Egypt).

Regimen: 1000 mg of calcium carbonate were given daily with vitamin D supplementation.

Induction of ovulation with clomiphene citrate: 50 mg tablets of clomiphene citrate (Clomid® 50mg, Hoechst Marion Russel, Cairo, Egypt).

Regimen: 50 mg daily starting dose of clomiphene citrate was given for 5 days starting on day 2 of spontaneous or progestin-induced menstrual bleeding for maximum 6 cycles with 50 mg dose-increments in subsequent cycles in patients who failed to ovulate, up to a maximum daily dose of 150 mg (The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2007). Folliculometric follow-up was done every other day starting at the 10th day of the induction cycle. When at least one follicle reaches 18 mm, ovulation is triggered by 10,000 IU of human chorionic gonadotropin (Choriomon®, IBSA, Switzerland) intramuscularly with timed intercourse 24-36 hours thereafter.

Primary outcome: Evidence of ovulation as detected by size of dominant follicle by transvaginal ultrasound (TV U/SS) and cycle day 21 serum progesterone level (more than 10 ng/ml).

Secondary outcomes: Change in insulin resistance as measured by fasting glucose/insulin ratio (ratio becomes more than 4.5), Change in plasma concentration levels of adipokines (ghrelin, resistin and adiponectin), follistatin and activins in participants diagnosed with PCOS after vitamin D supplementation therapy and Resumption of regular cycles.

RESULTS

No statistically significant differences were found between the three groups regarding the age, type and duration of infertility. However, BMI differed significantly between the three groups, being statistically significantly higher in the 25OHD deficient subgroup compared to the other two groups.

Table 1: Comparison between the three subgroups regarding basic demographic and clinical characteristics.

	Vitamin D Group		Control Group	P
	25OHD Deficient subgroup	Normal 25OHD subgroup		
Age (Yrs)	28.4 ± 3.2	27.9 ± 4.7	28.0 ± 3.9	0.69
BMI (Kg/m²)	26.7 ± 3.7	25.8 ± 1.9	25.9 ± 2.8	0.01
Type of infertility				
1ʳʸ	66 (70.9%)	38 (66.6%)	104 (69.3%)	0.85
2ʳʸ	27 (29.03%)	19 (33.3%)	46 (30.6%)	
Duration of infertility (Yrs)	5.2 ± 1.3	5.8 ± 1.8	5.6 ± 1.7	0.06

300 PCOS women were recruited and randomized to either vitamin D group (in which vitamin D level was assessed and supplemented if deficient) or control group. Among the 150 women assigned to the vitamin D group, 93 were documented to have vitamin D deficiency ; whereas the rest (57 women) were found to have normal vitamin D levels. Analysis of the effect of vitamin D deficiency on the clinical characteristics of PCOS women was done. No statistically significant differences were found between the three groups regarding BMI, frequency of menstrual irregularity and modified Ferriman-Gallwey hirsutism score. Analysis of the effect of vitamin D deficiency on the biochemical characteristics of PCOS women was also done. Being a classification criterion, 25OHD level was significantly lower in the vitamin D deficient subgroup compared to the normal vitamin D subgroup ; whereas no significant differences were found between the vitamin D deficient group and the control group. In the same context, 25OHD level was statistically significantly lower in the control group compared to the normal vitamin D subgroup (Teble 1).

Table 2: Comparison between the three subgroups regarding clinical characteristics.

	Vitamin D Group		Control Group [n= 150]	P	Post-hoc analysis		
	25OHD Deficient subgroup [n= 93]	Normal 25OHD subgroup [n= 57]			25OHD Deficient subgroup		Normal 25OHD vs Control Group
					Vs Normal 25OHD Subgroup	Vs Control Group	
BMI (Kg/m²)	26.7 ± 3.7	25.8 ± 1.9	25.9 ± 2.8	0.12	–	–	–
Menstrual irregularity	75 (80.6%)	39 (68.4%)	118 (78.6%)	0.19	–	–	–
mFG hirsutism score	10.3 ± 6.1	10.1 ± 6.1	10.6 ± 4.9	0.82	–	–	–

Vitamin D deficient PCOS women tended to have higher degree of insulin resistance. Fasting glucose was statistically significantly higher in the vitamin D deficient subgroup compared to the normal vitamin D subgroup and the control group; and higher in the control group compared to the normal vitamin D subgroup. Fasting insulin level was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup ; whereas no statistically significant differences were found between the former two groups (Table 2)

HOMA2-IR was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup ; whereas no statistically significant differences were found between the vitamin D deficient subgroup and the control group. No statistically significant differences were found between the three groups in the various components of the lipid profile.

Induction of ovulation using clomiphene citrate was initiated in 69 vitamin D deficient women, 57 women with normal vitamin D levels and 150 control women. During the six-cycle-course of induction of ovulation, 9 women from the vitamin D deficient subgroup, 5 women from the normal vitamin D subgroup and 43 women from the control group were excluded from the study due to loss during follow up or because the participant opted for switching to adjuvant IUI, gonadotrophin induction of ovulation or IVF/ICSI as depicted in the CONSORT flow chart.

Cumulative ovulation rate was significantly higher in the vitamin D deficient subgroup following vitamin D supplementation compared to the normal vitamin D subgroup and the control group with a rate ratio of 1.27 and 1.22, respectively. Number needed to treat was calculated to be 5.34 and 6.38 compared to normal vitamin D subgroup and control group, respectively, i.e. 6.38 women are needed to be supplemented with vitamin D for one of them to benefit compared to control women. No significant differences were found between the three subgroups regarding the median ovulating dose of clomiphene citrate. Also, there were no significant differences in the cumulative clinical pregnancy rate between the ovulatory women of the three subgroups (Table 3).

Table 3: Comparison between the three subgroups regarding basal biochemical characteristics.

| | Vitamin D Group | | Control Group [n= 150] | P | Post-hoc analysis | | |
| | 25OHD Deficient subgroup [n= 93] | Normal 25OHD subgroup [n= 57] | | | 25OHD Deficient subgroup | | Normal 25OHD vs Control Group |
					Vs Normal 25OHD Subgroup	Vs Control Group	
25OHD (ng/mL)	13.7 ± 3.6	27.5 ± 1.9	14.5 ± 5.1	<0.001	<0.001	0.40	<0.001
Glycemic profile							
Fasting glucose (mg/dL)	96.7 ± 8.5	89.3 ± 8.5	93.6 ± 7.7	<0.001	<0.001	0.02	0.002
Fasting insulin (⌈IU/mL)	28.5 ± 3.7	20.6 ± 4.6	28.4 ± 3.4	<0.001	<0.001	0.98	<0.001
HOMA2-IR	3.2 ± 0.8	2.6 ± 0.5	3.5 ± 0.7	<0.001	<0.001	0.08	<0.001
Lipid profile							
HDL (mg/dL)	47.1 ± 6.4	48.1 ± 8.5	47.9 ± 8.4	0.73	–	–	–
LDL (mg/dL)	85.3 ± 21.5	87.6 ± 25.8	88.8 ± 28.7	0.66	–	–	–
VLDL (mg/dL)	18.9 ± 10.8	22.4 ± 7.9	21.8 ± 9.8	0.07	–	–	–
Triglycerides (mg/dL)	99.3 ± 56.7	112.0 ± 39.3	108.9 ± 48.9	0.28	–	–	–
Total cholesterol (mg/dL)	154.6 ± 21.6	154.1 ± 30.4	153.5 ± 32.1	0.96	–	–	–

DISCUSSION

A growing body of literature suggests the mechanistic implications of vitamin D deficiency for insulin resistance, inflammation, dyslipidemia and obesity, i.e. clinical and metabolic phenomena commonly encountered in PCOS[9].

According to the results of our study, vitamin D deficient PCOS women tended to have higher degree of insulin resistance. Fasting glucose was statistically significantly higher in the vitamin D deficient subgroup compared to the normal vitamin D subgroup and the control group. Fasting insulin level was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup. Consequently, HOMA2-IR was statistically significantly higher in the vitamin D deficient subgroup and the control group compared to the normal vitamin D subgroup.

Following vitamin D supplementation in vitamin D deficient-PCOS, glycemic profile showed a decline in tendency to insulin resistance noted before vitamin D3 supplementation. Fasting glucose levels statistically significantly declined following vitamin D3 supplementation compared to the pre-supplementation levels and the control PCOS women, to reach a level that didn't differ significantly compared to the normal 25OHD PCOS women. Fasting insulin levels also declined significantly in vitamin D deficient-PCOS women following vitamin D3 supplementation, achieving a level that is statistically significantly lower when compared to both the normal 25OHD and control PCOS women. Consequently, following vitamin D3 supplementation in vitamin D-deficient PCOS women, HOMA2-IR declined significantly to a value that is significantly lower than that estimated in the normal 25OHD and control groups.

At least in theory, vitamin D has an established regulatory effect on the intracellular and extracellular calcium levels that is essential for insulin-mediated intracellular processes, and may have impact on insulin secretion and insulin sensitivity[10]. Another hypothesis involves the stimulatory effect of vitamin D on the expression of insulin receptors[11]. In addition, vitamin D affects the immune system and can cause a higher inflammatory response associated with insulin resistance[12]. Also, vitamin D can affect insulin resistance through the renin-angiotensin-aldosterone system[13].

Wehr *et al*.[14] demonstrated that vitamin D treatment at a dosage of 20 000 IU weekly for 24 weeks improved glucose metabolism and menstrual frequency in PCOS women.

On the contrary, Ardabili *et al*.[15] observed no significant change in fasting serum insulin and glucose concentrations, insulin sensitivity and homeostasis model assessment of insulin resistance following supplementation with 50,000 IU vitamin D3 for 2 months among patients with PCOS. However, a major drawback of the latter study is the relatively low dose of vitamin D3 treatment which is much lower than the current proposed guidelines[8].

According to the assessment of hormonal profile aberrations in PCOS women in our study, no statistically significant differences were found between the vitamin D deficient- PCOS women and those with normal vitamin D status regarding gonadotropic, estrogenic and androgenic hormonal profiles. This might be explained by the clinical and biochemical nature of the diagnostic criteria of PCOS that identify a common phenotypic and/or hormonal abnormality, rather than a specific molecular defect. Following vitamin D3 supplementation in vitamin D deficient-PCOS women, both LH and

E2 levels significantly declined ; whereas FSH levels significantly increased. In the same context, after vitamin D3 supplementation in vitamin D deficient-PCOS women, marked improvement in the androgenic hormonal profile was noted, evidenced by statistically significant reduction in serum total testosterone level and increase in SHBG level yielding an overall decline in free androgen index. However, there were no statistically significant differences in modified Ferriman-Gallwey hirsutism score between vitamin D deficient-PCOS women following vitamin D3 supplementation compared to basal values and mean scores of normal 25OHD and control PCOS women. However, the latter finding should be interpreted in caution due to the relative short duration of the study to have a pronounced effect on the hair growth cycle.

Associations between serum 25OHD with hyperandrogenemia and hyperandrogenism were described and reductions in total testosterone were achieved with vitamin D supplementation, albeit inconsistently (14), as depicted by Azadi-Yazdi et al.[16] in their 2017 meta-analysis of six clinical trials including 183 PCOS women. However, these conclusions must be interpreted in caution. Among these studies, only one study[17] used a combination of vitamin D and calcium supplementation for intervention. Moreover, various doses of vitamin D were administered for intervention in the included studies. Studies also had small sample sizes and short duration of follow-up, e.g. the study conducted by Wehr et al.[14] could include only 52 participants for the intervention.

In our study, adiponectin level was significantly lower in the vitamin D deficient subgroup compared to the normal vitamin D subgroup, which subsequently showed statistically significant elevation following vitamin D3 supplementation in vitamin D deficient subgroup, with post-supplementation levels comparable to the normal 25OHD subgroup. Leptin levels were statistically significantly higher in the vitamin D deficient subgroup compared to the normal vitamin D subgroup and showed statistically significant decline following vitamin D3 supplementation in vitamin D deficient subgroup; with post-supplementation levels that is statistically significantly lower than the control group and higher than the normal 25OHD subgroup.

Data concerning the relation between vitamin D deficiency and alteration in adipokine profile in PCOS women are sparse. According to the 2018 study of Kensara[18], 25OHD levels of PCOS women were significantly correlated positively with adiponectin levels. In the 2017 randomized placebo-controlled clinical trial of Abootorabi et al.[19], statistically significant increase in adiponectin levels was noted following the supplementation of PCOS women with 50,000 IU of oral vitamin D3 once weekly for 8 weeks.

Several mechanisms have been suggested to explain the potential effects of vitamin D on adiponectin[20]. Vitamin D may affect adiponectin through renin-angiotensinogen system. Increased activity of renin–angiotensinogen system is associated with an increased angiotensin production, which leads to the production of dysfunctional adipocytes and finally, decreased adiponectin production. Vitamin D may involve in increasing serum adiponectin by down-regulating and decreasing angiotensin production[21]. Insulin resistance and glucose intolerance are inflammatory conditions that are associated with decreased production of adiponectin and increased activity of inflammatory cytokines as well as TNF-α and interleukin-1. TNF-α reduces adiponectin synthesis and vitamin D may be associated with increased serum adiponectin through decreasing gene expression[22].

It's likely that leptin aberrations in PCOS and their relation to vitamin D deficiency are related to insulin resistance associated with PCOS. Insulin has been shown to increase leptin mRNA in adipocytes, suggesting its possible role in stimulating leptin secretion. Elevated leptin in hyperinsulinemic PCOS women may be considered a secondary consequence of insulin-stimulated synthesis of leptin. Leptin on the other hand, inhibits insulin-mediated promotion of gonadotropin-stimulated steroidogenesis[23].

In this study, response of PCOS women with varying vitamin D status to induction of ovulation with clomiphene citrate was studied. Cumulative ovulation rate was estimated to be 86.56%, 67.85%, 70.89% in vitamin D deficient PCOS women, those with normal vitamin D status and control PCOS women, respectively. Vitamin D supplementation in vitamin D deficient PCOS women was noted to improve cumulative ovulation rate statistically significantly with a rate ratio of 1.27 (95%CI: 1.04 – 1.56) compared to those with normal vitamin D status.

In the 2016 secondary analysis of the PPCOS I (Pregnancy in Polycystic Ovary Syndrome) multicentre randomized controlled trial, Pal and his colleagues[17] assessed serum 25OHD levels in the stored samples of the 626 participants in the PPCOS I trial and correlated them with the ovulatory response to induction of ovulation with clomiphene citrate alone or in combination with metformin. Vitamin D deficient women were significantly less likely to achieve OV compared to those with 25OHD levels ≥20ng/ml ($P = 0.006$). The probability of achieving ovulation varied directly with vitamin D status (68%, 77% and 78% in those with vitamin D deficiency, insufficiency and normal status, $P = 0.05$)[17].

In this study, no statistically significant differences were found in cumulative clinical pregnancy rate between ovulatory women receiving clomiphine citrate with corrected vitamin D deficiency, normal vitamin D status or control women with undetermined vitamin D status. Although vitamin D supplementation in vitamin D deficient PCOS women seems to improve chances of success of clomiphene citrate induction of ovulation, it doesn't increase the portion of ovulatory women who successfully conceive.

According to the study of Pal *et al*.[17], serum 25OHD was significantly higher in women achieving live birth compared to those failing to attain live birth (25.34 ± 10.39 vs 23.16 ± 9.71 respectively, P = 0.046). Each 1 ng/ml increase in 25OHD increased the likelihood of live birth by 2%. However, this association was apparent at serum 25OHD levels that are well beyond the threshold of 30ng/ml that is currently deemed as a target "normal" level. Highest likelihood for live birth was evident in women with serum 25OHD level >45 ng/ml ; in contrast, 25OHD levels <20 ng/ml (<50 nmol/L) were predictive of a dampened ovulatory response to ovulation induction strategies. Based upon these observations, Pat *et al*.[17] proposed that circulating 25OHD level of 45 ng/ml or higher should be considered as "optimal" for women attempting to conceive ; thus, introducing the concepts of distinct "reproductive thresholds" of vitamin D below which ovulatory response to induction of ovulation is blunted (Lower Reproductive Threshold-LRT <20 ng/ml) and beyond which (Upper Reproductive Threshold–URT > 45 ng/ml) likelihood of live birth may be optimized and propensity for pregnancy loss is reduced. Notably, the observed URT is higher than 25OHD levels of 20 ng/ml and 30 ng/ml that are identified by the Institute of Medicine (24) and the Endocrine Society[8], respectively, to reflect normal vitamin D status. However, a major limitation of the above conclusions of Pal *et al*.[17] is the small numbers of participants in their study with 25OHD levels above the specified thresholds for the defined outcomes, i.e. only 10/540 (2%) had 25OHD level >45 ng/ml (associated with LB) ; thus, the possibility of alpha errors being reflected in the observed associations is plausible.

It is noted from our results that, unfortunately, none of our participants achieved that proposed upper reproductive threshold; as we followed the Endocrine Society guidelines[8] adopting the 30 ng/mL threshold for normal vitamin D status. This limitation hinders proper assessment of the role of vitamin D supplementation on pregnancy rates.

In conclusion, vitamin D seems to be a contributing etiopathogenic factor in anovulatory, metabolic and hormonal aberrations in women with PCOS that might have a potential adjuvant role to standard induction of ovulation regimens.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Anagnostis P, Karras S and Goulis DG (2013): Vitamin D in human reproduction: a narrative review. Int J Clin Pract; 67(3): 225-35.

2. Guo Q, Kumar TR, Woodruff T, Hadsell LA, DeMayo FJ and Matzuk MM (1998): Overexpression of Mouse Follistatin Causes Reproductive Defects in Transgenic Mice. Mol Endocrinol; 12(1): 96-106.

3. Legro RS, Finegood D and Dunaif A (1998): A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab; 83(8): 2694-8.

4. Mattu HS and Randeva HS (2013): Role of adipokines in cardiovascular disease. J Endocrinol; 216(1): T17-36.

5. Sang Q, Zhang S, Zou S, Wang H, Feng R, Li Q, Jin L, He L, Xing Q and Wang L (2013): Quantitative analysis of follistatin (FST) promoter methylation in peripheral blood of patients with polycystic ovary syndrome. Reprod Biomed On; 26(2): 157-63.

6. Thomson RL, Spedding S and Buckley JD (2012): Vitamin D in the Aetiology and Management of Polycystic Ovary Syndrome. Clin Endocrinol; 77(3): 343-50.

7. Wang Y, Zhu J and DeLuca HF (2012): Where is the vitamin D receptor? Arch Biochem Biophys; 523(1):123-33.

8. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM (2011): Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab.; 96(7): 1911–30.

9. Krul-Poel YH, Snackey C, Louwers Y, Lips P, Lambalk CB, Laven JS, Simsek S. (2013): The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. Eur J Endocrinol.; 169(6):853–65.

10. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. (2007): The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab.; 92: 2017–2029.

11. Maestro B, Molero S, Bajo S, Davila N, Calle C. (2002): Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin D(3). Cell Biochem Funct; 20: 227–232.

12. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S, Kimmig R, Mann K, Janssen OE. (2006): Low serum 25-hydroxyvitamin D concentrations are associated with insulin

resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes; 114: 577–583.

13. Rammos G, Tseke P, Ziakka S. (2008): Vitamin D, the renin-angiotensin system, and insulin resistance. Int Urol Nephrol; 40:419-26.

14. Wehr E, Pilz S, Boehm BO, Marz W, Obermayer-Pietsch B. (2010): Association of vitamin D status with serum androgen levels in men. Clin Endocrinol (Oxf).; 73:243–248.

15. Ardabili HR, Gargari BP, Farzadi L. (2012): Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. Nutr Res 2012; 32: 195–201.

16. Azadi-Yazdi M, Nadjarzadeh A, Khosravi-Boroujeni H, Salehi-Abargouei A. (2017): The effect of vitamin D supplementation on the androgenic profile in patients with polycystic ovary syndrome: A systematic review and meta-analysis of clinical trials. Hormone and Metabolic Research.; 49(03): 174-9.

17. Pal L, Berry A, Coraluzzi L, Kustan E, Danton C, Shaw J, *et al*. (2012): Therapeutic implications of vitamin D and calcium in overweight women with polycystic ovary syndrome. Gynecol Endocrinol.; 28(12):965–968.

18. Kensara OA. (2018): Prevalence of hypovitaminosis D, and its association with hypoadiponectinemia and hyper-follistatinemia, in Saudi women with naïve polycystic ovary syndrome. Journal of clinical and translational endocrinology; 12:20-5.

19. Abootorabi S, Ayremlou P, Behroozi-Lak T, Nourisaeidlou S. The effect of vitamin D supplementation on insulin resistance, visceral fat and adiponectin in vitamin D deficient women with polycystic ovary syndrome: a randomized placebo-controlled trial. Gynecological Endocrinology. 2018 Jun 3;34 (6):489-94.

20. Baziar N, Jafarian K, Shadman Z, *et al*. (2014): Effect of therapeutic dose of vitamin D on serum adiponectin and glycemia in vitamin D-insufficient or deficient type 2 diabetic patients. Iran Red Crescent Med J; 16:e21458.

21. Vaidya A, Williams JS, Forman JP. (2012): The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS. Obesity; 20:186–91.

22. Liu E, Meigs JB, Pittas AG, *et al*. (2009): Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. J Nutr;139:329–34.

23. Vidal H, Auboeuf D, De Vos P, Staels B, Riou JP, Auwerx J, *et al*. (1996): The expression of ob gene is not actually regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. J Clin Invest.; 98:251–5.

24. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. (2011): The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab.; 96(1):53–8..