



Role of vitamin D levels on the ovarian function and androgen profile in adolescents with polycystic ovarian syndrome

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Background: The presence of the vitamin D receptor (VDR) has been found on the granulosa and theca cells of the ovary but its role in menstruation is not well understood. A few clinical studies in adults have shown that Vitamin D supplementation can lower androgen levels, lower anti-Mullerian hormone (AMH) levels, normalize the metabolic profile and regularize periods in women with polycystic ovarian syndrome (PCOS) whereas others have failed to show any effect on these parameters. Moreover, only a handful of studies have looked at adolescents. In this study, we hypothesized that vitamin D supplementation in adolescents with PCOS and vitamin D deficiency will regularize the periods and have a positive effect on the androgen profile.

Methods: We conducted a retrospective chart review of obese adolescent females 12–17 years of age with vitamin D deficiency and irregular menstruation or PCOS (n=17). Patients with obesity and vitamin D deficiency with regular menstruation were included as controls (n=7). Follow up data of at least 3 months after the initiation of vitamin D and when available, data every 3 months till a maximum of 30 months from the first visit was recorded.

Results: Thirteen/17 study subjects had regular periods at the end of the study. Of these 13 patients, 84.6% were treated with oral contraceptive pills (OCPs) with or without metformin, 7.6% with vitamin D alone and 7.6% with Metformin alone. Four/17 patients continued having irregular periods; 3 of these 4 were on Metformin and one was on vitamin D alone. Improvement in the vitamin D level occurred in 11/17 subjects during the study period. Among them, 82% of these had regular periods and all of them were on OCPs. The Free testosterone decreased significantly; but total testosterone, LH and LH/FSH ratio did not show any statistically significant improvement.

Conclusions: Our study did not support the role of vitamin D in regularizing menstruation or improving the androgen profile in adolescents with PCOS. However, a larger prospective study is needed to confirm these findings.

Keywords: Vitamin D deficiency; irregular menstruation; androgen profile

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Introduction

Vitamin D is a hormone that acts via the vitamin D receptor (VDR) which belongs to the steroid/thyroid nuclear receptor superfamily (1). The presence of VDR has been found on the granulosa cells and the theca cells of the ovary (2). Animal studies have shown impaired folliculogenesis and lower estrogen levels in VDR null mice (3), indicating a role of vitamin D in reproductive functions. In humans, activated vitamin D or 1,25 di-hydroxyvitamin D has been shown to stimulate estrogen and progesterone production in the ovarian cells (3). Additionally, Vitamin D has been shown to stimulate aromatase activity in human skin fibroblasts, and it has been postulated that a similar mechanism may be responsible in the ovary as well (3,4).

A few clinical studies in adults have shown that Vitamin D supplementation can lower androgen levels, lower AMH levels, normalize the metabolic profile and regularize periods in women with polycystic ovarian syndrome (PCOS) (5,6) whereas certain studies have failed to show any effect on these parameters (7). There is a paucity of studies done in the adolescent age group.

Based on these findings, we hypothesized that vitamin D supplementation in patients with PCOS and vitamin D deficiency will regularize the periods and have a positive effect on their androgens. The objective of this study was to evaluate the effect of hypovitaminosis D on the ovarian function of adolescents with PCOS as assessed by clinical improvement (regularization of menstruation) and/or biochemical improvement (changes in serum LH/FSH levels and the serum androgen profile) by comparing the data before and after supplementation with vitamin D.

Methods

Study design

We performed a retrospective chart review of adolescent females 12–17 years of age who came to the Bellevue Pediatric endocrinology clinic from January 1, 2016 to August 20, 2018.

Females with vitamin D deficiency and irregular menstruation 2 years after menarche/or a diagnosis of PCOS in their problem list were included in the study as cases. And patients with vitamin D deficiency and obesity with regular menstruation were included as controls. The Rotterdam criteria was used to diagnose PCOS (any 2 of the following features: Irregular menstruation/chronic anovulation; biochemical or clinical evidence of hyperandrogenism and

polycystic ovarian pathology). Vitamin D deficiency was defined as severe if the level was <10 ng/mL, mild-moderate for levels between 10–19 ng/mL and vitamin D insufficiency if the level was 20–29 ng/mL. A vitamin D level of 30 ng/mL or more was considered normal.

Exclusion criteria

Patients with irregular menstruation in the first 2 years after menarche, any patient already on vitamin D at the time of the initial evaluation, patients with any genetic syndrome, patients with any other chronic disease like hypothyroidism, hyperthyroidism, celiac disease, JIA, diabetes were excluded from the analysis.

All the patients were started on vitamin D supplementation after their first visit. They were started on 50,000 units weekly \times 6–8 weeks followed by 2,000 units daily if the level was <20 ng/mL or 2,000 units daily if the level was >20 ng/mL. We relied on the medical records to assess compliance.

Data collection

Patient charts were reviewed to gather axiological data including BMI and ethnicity. The present and past medical history, menstrual history, medication history, birth history, family history was reviewed from the chart notes at each visit. Biochemical data including vitamin D (immunoassay), total and free testosterone (LC/MS), FSH and LH (immunoassays) was collected and when available, ultrasound pelvis was reviewed for the size of ovaries.

Follow up data at least 3 months after the initiation of vitamin D was recorded. When available, the 6-, 9-, 12-, 15-, 24- and 30-month follow up data with respect to menstrual history, vitamin D levels, androgen and gonadotropin profile was reviewed.

Data analysis

Descriptive analysis was made. For within the group, comparisons from pre–post vitamin D supplementation, and between group comparisons between cases and controls the paired *t*-test were used. $P < 0.05$ are considered statistically significant.

Results

Twenty-four patients fulfilled the criteria to participate in the study. 17 patients qualified as cases and 7 patients were

	N	Cases (17)		P*	N	Controls (7)		P**	P^	
Age (years)	17	15±1.4			7	14.4±0.9 (0.3)		0.3		
Ethnicity	17	Hispanic 64.7% (11)			7	Hispanic 71.4% (5)				
		South Asian 5.9% (1)				South Asian 28.6% (2)				
		Caucasian 11.8% (2)								
		Other 17.6% (3)								
Family hx of T2DM	17	Yes: 70.6% (12)			7	yes: 71.4% (5)				
		No: 17.6% (3)				No: 28.6% (2)				
		Unknown: 11.8% (2)								
BMI		Before	After	0.9		Before	After	0.88	0.95	
		32.2±7.6	31.9±6.3			32.8±11	33.2±11.4			
Vit D baseline (ng/mL)	17	16.2±6.4			7	17±3.5		0.78		
<10		23.5% (4)				0				
10–19		59% (10)				86% (6)				
20–29		17.5% (3)				14%(1)				
Vit D at 3–9 months	10	26.24±10.2			5	28.8±5.68		0.6		
Vit D at 9–15 months	8	26.9±9.4			3	24.3±5.5		0.66		
Vit D at 15–30 months	7	21.4±7.7			5	21.8±10.9		0.94		
Normal vitamin D at any time	17	0	64.7% (11)		7	0	28.5% (2)			
Menstrual history at end of study period	17				7					
		Regular	0%			76.5% (13)	100%			
		Irregular	100%			23.5% (4)	0%			
Time since menarche (years)	17	3.5±1.5			7	3.14±1.7				
Testosterone total (ng/dL)	16	57.68±22.8	43.2±23.25	0.08	3	35.9±8.6	37.2±8.4 (n=2)	0.12	0.8	
Testosterone free (pg/mL)	16	5.1±3.9	1.9±1.16	0.03	3	2.4±0.8	2±1.1 (n=2)	0.25	0.66	
LH (μ/L)	17	12.7±6.7	9.8±3.8 (n=16)	0.13	2	10.3±2.5				
LH:FSH ratio		2.27±0.64	1.96±0.9	0.26						
Ovarian volume (cc)	14									
		>10	7 (50%)							
		<10	7 (50%)							

Figure 1 Demographic, clinical and lab characteristics of cases and controls before and after vitamin D supplementation. P*, P value (cases before and after); P**, P value cases *vs.* controls; P^, P value controls (before and after).

controls. The cases and controls were similar in regard to age, BMI, time since menarche, and family history of type 2 diabetes. Ethnic background of study patients included 64.7% Hispanic, 5.9% South Asian, 11.8% Caucasian and was unknown in 17.6%; controls included 71.4% Hispanic and 28.6% South Asian. All 24 patients were >2 years post-menarche (mean: 3.5±1.5 years for cases and 3.14±1.7 years for controls) (*Figure 1*).

The clinical and biochemical characteristics of the cases and controls before and after vitamin D supplementation

are shown in *Figure 1*. At baseline, all the cases had irregular menstruation and all the controls had regular periods. Thirteen/17 study patients had regular periods at the end of the study.

The mean (± SD) vitamin D level of the study patients at baseline was 16.2±6.4 ng/mL and that of controls was 17±3.5 ng/mL (P value: 0.78); 23.5% (4/17) of the cases presented with severe vitamin D deficiency, 59% (10/17) had mild-moderate vitamin D deficiency and 17.5% (3/17) had vitamin D insufficiency. None of the controls

Table 1 Menstrual pattern of study patients before and after vitamin D supplementation

Variable	N	Before	N	After
Menstrual history	17	100% irregular		Regular Irregular
At the end of the study			17	76.5% [13] 23.5% [4]
Prior to/without OCPs			17	11.8% [2] 88.2% [15]
Patients with regular periods on vitamin D			13	
Metformin				7.7% [1]
Metformin + OCP				30.8% [4]
OCP				53.8% [7]
No intervention				7.7% [1]
Patients with irregular periods on vitamin D			4	
Metformin				75% [3]
OCP/OCP + metformin				0
No intervention				25% [1]

OCP, oral contraceptive pill.

presented with vitamin D levels <10; 86% (6/7) had levels between 10–19 ng/mL and 14% (1/7) had levels between 20–29 ng/mL.

Out of the 17 study patients, 10 had vitamin D levels re-measured between 3–6 months, 8 patients had a repeat level done between 9–15 months and 7 between 15–30 months. Among the controls, 5 patients had a repeat Vitamin D level done between 3–6 months, 3 patients had a repeat level between 9–15 months and 5 patients had a repeat level between 15–30 months. All the patients had their vitamin D levels repeated at least once in the 30-month follow up period. There was no statistical difference between the vitamin D levels of the cases and controls at baseline, 3–9, 9–15 and 15–30 months after vitamin D supplementation. Sixty-five percent (11/17) cases and 28.5% of the controls (2/7) normalized their vitamin D levels at some point in the follow up period.

The mean initial total and free testosterone concentrations for the cases (n=16) were 57.68±22.8 ng/dL and 5.1±3.9 pg/mL respectively and that for the controls (n=3) was 35.9±8.6 ng/dL and 2.4±0.8 pg/mL respectively. There was no statistical difference between the initial total and free testosterone levels of the cases and controls (P value: 0.12 and 0.25 respectively). After vitamin D supplementation, the free testosterone concentration showed a significant decline from 5.1±3.9 to 1.9±1.16 pg/mL (P value: 0.03). The total testosterone level decreased to 43.2±23.25 ng/dL but failed to reach statistical significance (P value: 0.08).

Since the controls had a normal Total and free testosterone concentration at baseline, repeat levels were not performed in a majority.

The mean initial LH level (n=17) of the study patients was 12.7±6.7 µ/L which decreased to 9.8±3.8 µ/L during the follow up period but was not statistically significant (P value: 0.13). The mean LH:FSH ratio also decreased from 2.27±0.64 to 1.96±0.9, but was also not statistically significant (P value: 0.26). Fourteen/17 study patients had a baseline pelvic ultrasound done. 50% of these patients had an ovarian volume >10 cc on either side.

Table 1 shows the menstrual pattern of the patients in the study group. 76.5% (13/17) of the patients regularized their periods during the 30-month follow up period whereas 4 patients continued to have irregular periods. All the patients were on vitamin D supplementation. Among the 13 patients with regular periods, 1 patient was also on metformin, 7 patients on oral contraceptive pills (OCPs), 4 on both metformin and OCPs and 1 required no additional intervention.

Eleven of the thirteen patients (65%) with regular periods required OCPs. Of these eleven patients, 3 were started right at diagnosis, and the remaining 8 were started 6–27 months (mean: 12.75±8.2 months) after their initial visit. None of these 8 patients could get regular periods on vitamin D alone or on vitamin D + metformin.

Of the 11 study patients with a normal vitamin D level during the follow up period, 9 patients had regular periods.

All 9 of these patients needed to be started on OCPs to regularize the periods.

There were only three patients who did not require metformin or OCPs. Only one patient's periods regularized but the vitamin D level remained <20 , in the other two patients the periods remained irregular. The vitamin D level increased to 34 ng/mL in one of them but remained in the mild-moderate deficiency range in the other.

Overall, 11/13 of the study patients with regular periods required OCPs \pm metformin, 1 patient was on metformin and only one patient's periods regularized with vitamin D supplementation alone but without normalization of their vitamin D.

Discussion

Numerous studies have tried to examine the vitamin D status of women with PCOS *vs.* obese controls. Some of these studies showed significantly lower baseline vitamin D levels in women with PCOS (8-12) whereas others have failed to show any such correlation (13-17). Wehr *et al.* demonstrated that adult women with PCOS have lower vitamin D levels compared to obese controls (9). Whereas, a study published by Kim *et al.* in 2014 failed to show any difference in the vitamin D levels of adult women with PCOS and obese controls (13). Two studies done in adolescents also failed to show a statistically significant difference between the vitamin D levels of patients with PCOS *vs.* controls (15,17). Like the latter three studies, our study failed to show any difference between the baseline vitamin D levels of adolescents with PCOS and Obese controls.

The impact of intervention with vitamin D supplementation in PCOS subjects has also been studied with mixed results. A randomized double-blind placebo controlled study done in India in 50 women with PCOS (18-45 years of age) in which they were given 12,000 units of vitamin D weekly for 12 weeks, showed that vitamin D supplementation alone was able to increase the percentage of patients with regular periods from 20% to 48% and was able to reduce the number of antral follicles by 40% (6). Similar findings were seen by Wahr *et al.* in 2011, where 23 out of 46 patients (18-46 years of age) with irregular menstruation were able to achieve regularization of the menses after vitamin D supplementation for 24 weeks (18). In our study, most of the patients were not on Vitamin D alone and required Metformin and/or OCPs to regularize menses. Only one patient on vitamin D alone managed to achieve regular periods. The vitamin D level in this patient increased

from 6 to 16 ng/mL, but failed to normalize over a follow up period of 30 months. Another patient required Metformin to regularize the menses, however this patient's vitamin D level remained <10 ng/mL, indicating that vitamin D did not have an impact on the regulation of menses in these two patients. In the other patients, since all of them were also on OCPs/ Metformin, it is difficult to interpret the independent effect of vitamin D supplementation on menstruation.

Pal *et al.* published a before-after study in 12 obese women (19-39 years) with PCOS and found that vitamin D supplementation can lower the total testosterone and androstenedione levels (5). In our study, we saw a significant decrease in the free testosterone concentrations after vitamin D supplementation, however, since a lot of our patients were also on OCPs/metformin, it is hard to interpret the independent effect of vitamin D on the androgen profile; as OCPs are potent suppressors of ovarian androgen production. No change was noticed in the total testosterone, LH or the LH:FSH ratio after vitamin D supplementation. Our findings are similar to the findings in two meta-analyses published by Azadi-Yazdi *et al.* in 2017 and He *et al.* in 2015. Azadi-Yazdi reviewed the clinical trials looking at the effect of vitamin D supplementation on the androgenic profiles and found that the effect was not significant in randomized case-control studies (7). He *et al.* also failed to show an improvement in the hormonal profile of PCOS patients after supplementation with vitamin D (19).

A major limitation of our study is that it is a retrospective chart review, we had to rely on the medical records and were unable to gather all the data. Another limitation is that the sample size of our cohort is small which increases the risk of a type B error. Compliance with vitamin D is likely questionable. Many our patients were either on Metformin or OCPs or both, confounding our results. We were unable to analyze the androgen profiles prior to starting OCPs as they were not repeated in most of the patients. We did not have follow up pelvic ultrasounds to analyze a change in the size/distribution of the ovarian follicles after vitamin D supplementation. We did not have anti-Mullerian hormone (AMH) levels for our patients which strongly correlates with the number of ovarian follicles seen on ultrasound and can be used as a surrogate to evaluate for PCOS in adolescents (20,21). A decline in the AMH level with vitamin D may indicate an effect of vitamin D on ovarian function.

Conclusions

Thus, in conclusion, our study was unable to demonstrate

an impact of vitamin D supplementation on regularizing menstruation or improving the androgen profile in adolescent girls with PCOS. A larger prospective study needs to be performed to evaluate the effect of vitamin D on the ovarian function.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <https://gpm.amegroups.com/article/view/10.21037/gpm.2019.10.01/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional IRB of New York University School of Medicine (IRB number i18-01023). Since it is a retrospective chart review, the requirement for informed consent was waived.

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