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Effect of vitamin D supplementation on serum VEGF levels in vitamin d deficient polycystic ovarian syndrome patients

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ABSTRACT

Aim: To estimate and compare serum VEGF levels before and after vitamin D supplementation in vitamin D deficient PCOS patients.

Materials and Methods: The present study was conducted in Department of Obstetrics and Gynaecology in collaboration with Department of Biochemistry at University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital (GTBH), Delhi from November 2017 to April 2019 (including enrollment of subjects, data analysis and thesis writing). Considering a standard deviation of 36.5 before supplementation and 42.7 after supplementation in VEGF levels in case of PCOS patients in study of Irani et al., to estimate an absolute difference of 31 units pg/ml decrease in VEGF levels, at α= 5% and power= 80%, a sample of 26 cases is required. Since there is a follow up of 3 months adding an attrition of 15%, the total sample size becomes 30 cases.

Result: Significant increase in serum vitamin D levels post supplementation was observed from pre supplementation 9.524±4.41 to post supplementation 32.07±12.40; p-value <0.001. We observed significant fall in angiogenesis marker, serum VEGF levels post vitamin D supplementation from 773.547±344.173 to 639.97±119.02, p value 0.004 which suggests negative correlation between serum Vitamin D and serum VEGF levels.

Conclusion: Vitamin D supplementation in PCOS patients who are vitamin D deficient, leads to significant fall in angiogenesis marker serum VEGF, which is manifested as improvement in clinical and biochemical parameters in these patients.

1. Introduction

PCOS is the most common endocrinopathy affecting reproductive age women with prevalence of 6-10%.[1] It's a heterogeneous disorder characterised by obesity, insulin resistance, adverse lipid profile and hyperandrogenism.[2] Important reproductive aspects associated with PCOS are polycystic ovaries, anovulation leading to oligomenorrhea or amenorrhea. Metabolic disorders include hyperinsulinemia, insulin resistance impaired pancreatic cell insulin secretion, increased risk of type 2 diabetes mellitus and hypertension.[3] It's also frequently associated with increased risk of depression, anxiety, endometrial carcinoma.[4]

2. Aims and Objectives

2.1. Aim

To estimate and compare serum VEGF levels before and after vitamin D supplementation in vitamin D deficient PCOS patients.

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2.2. Primary objective

Estimation and comparison of serum VEGF levels before and after vitamin D supplementation in vitamin D deficient PCOS patients.

2.3. Secondary objective

Estimation and comparison of serum VEGF levels before and after vitamin D supplementation in vitamin D deficient PCOS patients.

To study the effect of vitamin D therapy on various clinical parameters like hirsutism, acne status, BP, and intermenstrual interval.

To study the changes in insulin resistance and lipid profile of study subjects after vitamin D supplementation.

3. Materials and Methods

3.1. Study setting

The present study was conducted in Department of Obstetrics and Gynaecology in collaboration with Department of Biochemistry at University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital (GTBH), Delhi.

3.2. Duration

This study was conducted from November 2017 to April 2019 (including enrollment of subjects, data analysis and thesis writing).

3.3. Study design

Prospective interventional study.

3.4. Sample size

Considering a standard deviation of 36.5 before supplementation and 42.7 after supplementation in VEGF levels in cases of PCOS in study of Irani et al[5] to estimate an absolute difference of 31 units pg/ml decrease in VEGF levels, at α = 5% and power = 80%, a sample of 26 cases is required. Since there is a follow up of 3 months adding an attrition of 15%, the total sample size becomes 30 cases.

3.5. Ethical clearance and consent

Clearance from institutional ethical committee was obtained and a written informed consent was taken from all the participants.

3.6. Inclusion criteria

All the women between 16-45 years diagnosed as Vitamin D deficient PCOS.

3.7. Exclusion criteria

1. Women on any hormonal therapy in last 3 months
2. Women on vitamin D supplementation
3. Women with known hypersensitivity to vitamin D.
4. Patients on any chronic medication like metformin or lipid lowering drugs etc.
5. Patients with chronic medical illness like tuberculosis, diabetes mellitus, hypertension etc.

3.8. Statistical analysis

Whole data analysis was done by SPSS 20.0 software.At the end of the study data was compiled and outcome parameters were measured as follows:

1. Comparison of changes in various clinical and biochemical parameters in study subjects after vitamin D supplementation.
2. Comparison of change in the serum levels of vitamin D and VEGF in study subjects pre and post vitamin D supplementation.
3. Correlation of the serum levels of VEGF and vitamin D both pre and post supplementation.

Comparison of pre and post vitamin D supplementation of clinical parameters, biochemical parameters, serum vitamin D and serum VEGF levels was obtained by Paired t-test. Correlation of the serum levels of VEGF and vitamin D both pre and post supplementation was done by Pearson's correlation test. P value of <0.05 was considered significant.

Comparison before and after vitamin d supplementation

4. Results and Discussion

PCOS is the most common endocrinopathy affecting reproductive age women. Increased ovarian mass supported with new blood vessel proliferation in stroma and theca is a key feature in PCOS pathophysiology.[6,7] Various angiogenic factors have a role in neovascularisation in ovarian stroma, chief among them being VEGF.[8] Recent studies have suggested relation between VEGF and ovarian perifollicular and stromal blood flow.[9] It has been seen that VEGF is raised in women with OHSS, giving an idea that VEGF is an important mediator of inflammation and thus leading to increased proliferation of stroma and theca in ovaries of PCOS patients.[10–12]

Studies have suggested role of Vitamin D in pathology of PCOS.[13] Hypovitaminosis D may induce higher inflammatory response thereby leading to higher angiogenesis. Vitamin D deficiency related increased angiogenesis in PCOS is believed to be mediated by vascular endothelial growth factor (VEGF) dysregulation.[1] It has been shown that vitamin D supplementation decreases VEGF production by human lumbar annulus cells and cancer cells.[5]

Table 1: PCOS related clinical features

Clinical features	Pre Vit D therapy No. (%)	Post Vit D therapy No. (%)	p-value
Menstrual history			
Infrequent cycles	16 (61.53%)	12 (46.1%)	
Amenorrhea	7 (26.92%)	4 (15.3%)	
Regular cycles	3 (11.53%)	10 (38.4%)	
Hyperandrogenic features			
Acne	7 (26.925)	4 (15.38%)	
Pillsbury shelly kligman scoring(mean±SD)	0.38±0.68	0.307±.61	**0.004**
Hirsutism	11 (42.30%)	8 (30.7%)	
FG score (mean±SD)	9.88±5.218	8.65±3.417	**0.001**
Insulin resistance			
Acanthosis	6 (23.07%)	6 (23.07%)	
BMI (kg/m^2)			
18.5-22.9	4 (15.38%)	4 (15.38%)	
23-24.9	1 (3.8%)	9 (34.61%)	
≥25	21 (80.7%)	13 (50%)	
mean±SD	26.884±3.289	25.56±2.838	0.060
Blood pressure (mm/Hg)			
SBP (mean±SD)	122.07±7.494	120.23±5.225	0.97
DBP (mean±SD)	79.096±9.644	77.62±5.216	0.96

Table 2: Effect of vitamin D supplementation on biochemical parameters in vitamin D deficient PCOS patients

Biochemical parameters	Pre vit D mean ±SD	Post vit D mean±SD	p-value
Fasting blood sugar(mg/dl)	81.42±7.66	81.34±7.66	0.45
Postprandial blood sugar (mg/dl)	106.53±12.70	101.73±9.31	**0.003**
Serum fasting insulin (μIU/ml)	13.97±11.68	12.48±9.81	0.99
QUICKI	0.3455±.045	0.347±.044	0.8224
HOMA-IR	2.91±.5351	2.58±.460	0.070
Serum total testosterone (mIU/ml)	0.77±0.38	0.729±.338	**0.001**
Serum DHEAS(μg/dl)	309.176±71.8586	302.23±68.87	**0.002**
Lipid profile			
Total cholesterol (mg/dl)	188.23±41.139	174.96±37.43	1.00
HDL-C (mg/dl)	46.46±8.026	50.54±8.60	0.998
VLDL-C (mg/dl)	23.15±11.540	19.35±12.27	0.70
TG (mg/dl)	139.85±50.454	125.62±43.12	**0.010**

Paired t test

Most of the information available in literature are from foreign studies. To our best of knowledge no Indian study has studied quantification of serum VEGF in PCOS women after vitamin D supplementation.

Taking all of these in consideration, we have hypothesized that vitamin D supplementation will lead to decrease in inflammation in ovaries, consequently decrease in ovarian neoangiogenesis and proliferation which would be manifested by a decrease in VEGF levels.

In the present study, 80 women with clinical diagnosis of PCOS like acne, obesity, oligomenorrhea, hirsutism etc. were screened from November 2017 to October 2018. Out of them 40 women who satisfied selection criteria were subjected to serum vitamin D levels estimation. 4 ml blood sample was taken and Vitamin D levels were estimated in 2 ml of sample. 30 women out of these 40 were found to be Vitamin D deficient and were enrolled in the study.

Detailed history taking, general examination was done and blood sample was taken for biochemical parameter assessment.

All women received vitamin D supplementation in form of cholecalciferol granules in sachet of 60,000 IU for 8 weeks followed by maintenance therapy. Post vitamin D supplementation, blood sample was collected for biochemical parameter assessment.

Out of 30 participants, 1 woman did not complete the study, 1 spontaneously conceived, 1 took alternative treatment like metformin during the study and 1 did not come for follow up.

Total 26 women completed the study who were evaluated after 8 weeks for biochemical and at fifth month for clinical parameters.

Serum vitamin D and serum VEGF levels along with various clinical and biochemical parameters were compared before and after vitamin D supplementation.

30 out of 40 women i.e. 75% were found to be vitamin D deficient. 26 out of 30 subjects completed the present study. Out of 26 subjects, 15 (57.69%) had severe VDD while 11 (42.30%) had mild VDD. This favours that vitamin D deficiency is 67-85% prevalent in PCOS women as observed in a study by Keshavarz et al[14] conducted in 2013 in Iran, wherein out of 73 recruited women 64 (79%) of them had serum vitamin D level<20 ng/ml. Hypovitaminosis D was observed in 93.8% of all subjects with mean serum vitamin D level of 7.30±4.45 ng/ml.

Pre vitamin D supplementation, 16 subjects had irregular cycles, while post supplementation 12 subjects had irregular cycles which showed 15% improvement in cyclicity of menses.(Table 1)

Similarly we observed an improvement in cycle regularisation in 10 subjects post supplementation in comparison to 3 subjects pre supplementation which is 27% improvement.(Table 1)

Improvement in intermenstural intervals after vitamin D supplementation was also observed by Irani et al[1] in their study which was a randomized placebo controlled trial on 53 subjects (80 ± 9 to 60 ± 6 days; p=0.04) .Similar results were observed by Tehrani et al[15] improvement in menstrual cycles in 13 out of 65 in metformin + calcium +vitamin D supplementation group and improvement in 4 subjects out of 20 in calcium+vitamin D supplementation group

We observed a significant improvement in acne status of subjects. Mean Pillsbury shelly kligman scoring change was from, 0.38±0.68 to 0.307±0.61 post supplementation. This difference in mean was statistically significant ,p value 0.004.(Table 1). Tehrani et al[15] conducted a study on 80 PCOS women improvement in acne was seen following vitamin D supplementation 50% improvement, in metformin + calcium +vitamin D supplementation group and 25% improvement in calcium+vitamin D supplementation group.

Post vitamin D therapy, significant improvement in hirsutism was observed. Mean FG score pre supplementation 9.88±5.218 to post supplementation 8.65±3.417, with p-value 0.001 which is also statistically significant (Table 1) our study results were consistent with the study conducted by Irani et al[5] in 2017, following vitamin D supplementation, significant decrease in Ferriman gallwey hirsutism score (9.8±1.5 to 8.1±1.5 p<0.001) was seen. Improvement in hirsutism post vitamin D supplementation was also observed by Tehrani et al.[15]

Post vitamin D supplementation mean SBP and mean DBP values were lower, however these changes are not significant in our study. This is consistent with results obtained by Raja Khan et al,[16] change in mean SBP (117.46 ±10.00 to 118.53 ±6.97) and mean DBP change was (79.08 ±8.28 to78.97 ±5.27) which was not significant, however vitamin D supplementation was seen to have protective effect on diastolic blood pressure, similar to our study . In a study by Pal L berry[17] et al, significant lowering in BP parameters was seen in participants with baseline BP ≥ 120/80 mmHg (n = 8) and in those with baseline serum 25OHD ≤20 ng/ml (n = 9).

The change in mean FBS post vitamin D supplementation was from 81.42±7.66 to 81.34±7.66 mg/dl; p-value =0.45 which was not statistically significant. We observed a significant fall in PPBS post supplementation, 106.53±12.70 to 101.73±9.31 mg/dl; p-value 0.003 which was statistically significant. (Table 2)

We observed a fall in parameters of insulin resistance. Fasting serum insulin mean pre supplementation 13.97±11.68 to 12.48±9.81 μIU/ml post supplementation with p-value=0.99. Mean QUICKI pre supplementation was 0.3455±0.045 to 0.347±0.44 post supplementation p value being 0.822 and mean HOMA IR values pre vitamin D supplementation 2.91±0.535 to 2.58±0.460 post supplementation with p-value =0.07, but these changes were not statistically significant which may be due to small sample size.(Table 2)

Considering HOMA IR > 2.5 as measure of insulin resistance, the post vitamin D HOMA IR mean was 2.58 which is although an improvement from pre vitamin D HOMA IR of 2.91 but it is not statistically significant.

Vitamin D supplementation did not have any significant effect on glycemic picture of the study subjects. Mean fasting plasma glucose change from pre to post vitamin D supplementation (87.9±7.22 to 90±7 mg/dl; p=0.43), mean fasting insulin (17.3±15.28 to 10.3±5.92; p<0.01), mean HOMA IR (3.8±3.40 to 2.3±1.32; p=0.03).

Similar to this in study by Raja Khan et al[16] using a high dose of vitamin-D for 12 weeks, there was no significant differences in QUICKI and other measures of insulin sensitivity but trends towards lower two hour insulin and lower 2- hr glucose was observed which is consistent with results of present study. In this study, mean fasting plasma glucose change from pre to post vitamin D supplementation was (84.92±9.46 to 83.82±8.02 mg/dl; p=0.7) , post prandial plasma glucose change was (122.08±36.29 to 110.73±24.84 mg/dl; p=11.66) mean fasting insulin (26.31±9.60 to 38.09±37.60 μIU/ml ; p=13.04), mean HOMA IR (5.47±1.82 to 7.79±7.37; p=2.57) ,meanQUICKI (0.302±0.014 to 0.296±0.022; p=0.008)

Contrary to our results, Pal L Berry et al[17] observed parameters of glucose homeostasis and IR remained unchanged (p > 0.05).

A RCT carried out by Bonakdaran et al[18] among 51 PCOS patients, did report a decrease in HOMA IR after 3 months of vitamin D supplementation, but this decrease was not significant due to small sample size

In the only study, by Seligmolu et al[19] where vitamin D supplementation resulted in decrease in IR, the results were of borderline significance. In this study they gave single oral dose of 300,000IU of vitamin D to 11 PCOS women 3 weeks after administration HOMA IR decreased significantly (p=0.043). However, it would be difficult to compare results of our study with these studies as the dose and duration of vitamin D supplementation is quite variable among the different studies.

We observed an improvement in lipid profile of subjects post vitamin D therapy. Changes in mean values pre to post vitamin D supplementation were, of total cholesterol from 188.23±41.139 to 174.96±37.43 mg/dl; p-value=1.00, of mean HDL-C from 46.46±8.026 to 50.54±8.60 mg/dl; p-value 0.998 of mean VLDL-C from 23.15±11.54 to 19.35±12.27 mg/dl; p-value 0.70, but they were not statistically significant. A significant fall was observed in serum triglycerides level from pre vitamin D supplementation 139.85±50.454 to 125.62±43.12 mg/dl post supplementation with p value-0.01 (Table 2)

Similar observations were made by Irani et al.[5] mean value of total cholesterol (172±31 to 158±20 mg/dl; p-value=0.08), mean HDL-C from 42±3 to 43±6 mg/dl; p-value 0.36 mean serum triglyceride from 116±102 to 81±42 mg/dl; p-value< 0.01 which although was an improvement, but they were not statistically significant.

In study by Raja Khan et al,[16] where high dose vitamin D supplementation was given, the results were, mean value changes from pre supplementation to post supplementation, of total cholesterol from 172±42.70 to 177.18±37.17 mg/dl; p-value=1.69), of mean HDL-C from 45.54±17.60 to 45.73±18.40 mg/dl ;p-value 0.7 of mean serum triglyceride from 139.08±71.61 to 127.73±58.09 mg/dl; p-value< 2.21)which is an overall improvement in lipid profile same as our current study.

In the present study, biochemical parameters of hyperandrogenism like serum total testosterone and serum DHEAS were reduced post vitamin D therapy with mean values of 0.72±0.338 and 302.23±68.875respectively. This change in serum testosterone and serum DHEAS values post vitamin D supplementation was statistically significant (p value 0.001 and 0.002 respectively).(Table 2)

This is similar to the study by Pal L Berry et al[17] in 2012, which demonstrated positive effects of vitamin D and calcium supplementation on metabolic and hormonal milieu in PCOS patients. It showed improved serum 25OHD (p<0.001) and reductions in total testosterone (p = 0.036) and rostenedione (p=0.090) levels were noted following 3-month supplementation, compared to baseline.

A direct effect of vitamin D on steroid genesis pathway has been proposed to explain the observed fall in circulating androgens.

We observed a significant rise in serum vitamin D levels in study subjects after vitamin D supplementation. The mean serum vitamin D values raised from 9.524±4.41 pre supplementation to 32.07±12.40 ng/ml post supplementation, the p-value being <0.001 which was statistically significant. (Table 3).

Irani et al[5] also reported significant change in serum vitamin D levels post supplementation. They observed pre supplementation mean was 16.3±0.9 to post supplementation mean 43.2±2.4 ng/ml with p value <0.01.

Similar results were observed in study by Raja Khan et al[16] post supplementation (19.95±9.47 to 67.36±28.62 ng/ml; p<0.001) from pre vitamin D therapy 7.7±6.05 to post vitamin D serum vitamin D mean 31.5±13.88 ng/ml; with p<0.001, where there was significant rise in serum vitamin D post supplementation

Similarly we also observed fall in serum VEGF levels post vitamin D supplementation from pre supplementation mean of 773.547±344.173 to 639.97±119.02 pg/ml which is statistically significant reduction(p-value 0.004).(Table 3)

This result is consistent with the findings of Irani et al[5] where they found significant decrease in serum VEGF levels from pre vitamin D supplementation 1106±36.5 to post vitamin D supplementation 965.3±42.7 pg/ml with p-value<0.001

A similar study by Irani et al[5] in showed decrease in bioavailability of TGF-β1which is one of the angiogenesis marker in PCOS similar to VEGF. There was significant decrease in TGF-β1to sENG ratio from pre supplementation 6.7±0.4 to post supplementation 5.9±0.4 and p-value=.04 thus proving that vitamin D is anti–inflammatory as it decreases angiogenic markers in serum of PCOS patients.[20]

After supplementation 23 out of 26 subjects achieved normal serum vitamin D levels and 3 subjects in severe vitamin D deficient category, remained mild vitamin D deficient who were kept on maintenance therapy.

We did not observe any symptoms or signs of vitamin D toxicity like nausea, gastrointestinal disturbances or skin manifestations in any of our subjects after vitamin D supplementation.

A negative correlation of -0.8925 was observed between serum VEGF and serum vitamin D values post vitamin D supplementation which means lower the serum vitamin D values higher would be serum VEGF and in turn higher would be neoangiogenesis in ovaries of such PCOS women.(Table 4)

By squaring the correlation and then multiplying by 100, we can determine what percentage of the variability is shared. Hence serum VEGF before supplementation shares about 37.94% of its variability with vitamin D before supplementation.

And serum VEGF after supplementation shares about 79.65% of its variability with Vitamin D after supplementation.

Table 3: Comparison of vitamin D supplementation effect on serum vitamin D and serum VEGF levels pre and post vitamin D therapy

	Serum vitamin D level Pre therapy (ng/ml)	**Serum vitamin D level Post therapy (ng/ml)**	**Serum VEGF levelPre therapy (pg/ml)**	**Serum VEGF levelPost therapy (pg/ml)**
mean ± SD of study population (n=26)	9.524±4.41	32.07±12.40	773.547±344.173	639.97±119.02
p value of study population (n=26)	**<0.001**		**0.004**	
Severe vit D def (mean±SD) Range **(n=15)**	6.233±1.957 (1.362 - 9.413)	24.158±5.17 (14.431 - 31.68)	884.029±422.246 (2378.53-694.572)	709.683±103.179 (989.067-609.82)
Mild vit D def (mean±SD) Range **(n=11)**	14.01±2.23 (10.25 - 17.094)	42.86±11.20 (32.78 - 66.85)	622.889±57.023 (691.951-531.974)	549.002±68.798 (608.154-393.133)

Paired t-test

Table 4: Correlation between VEGF and Vitamin D

VEGF and Vitamin D before therapy	**VEGF and Vitamin D after therapy**
-0.6160	-0.8925

Pearson's correlation test

5. Conclusion

PCOS is a heterogeneous disorder characterised by obesity, insulin resistance, adverse lipid profile, hyperandrogenism. Important reproductive aspect associated with PCOS are polycystic ovaries, anovulation leading to oligo or amenorrhea. One of the important aspects of PCOS pathophysiology is inflammation and neoangiogenesis in the PCOS ovaries. Vitamin D is anti-inflammatory. It reduces angiogenesis and excessive proliferation in ovaries of PCOS women.

1. Vitamin D supplementation improves clinical parameters in PCOS women manifested by, improvement in acne, hirsutism, improvement in menstrual cycle regularity. Trends towards lower BMI, lower SBP and lower DBP was observed
2. We observed improvement in biochemical parameters like significant fall in PPBS, serum testosterone and serum DHEAS level.
3. Improvement in parameters of insulin resistance like HOMA IR, QUICKI and serum fasting insulin post vitamin D supplementation. Improved lipid profile after vitamin D therapy. However the values were not significant due to small sample size.
4. Improvement in lipid profile post vitamin D supplementation, with a significant reduction in hypertriglyceridemia was observed.
5. Significant increase in serum vitamin D levels post supplementation was observed from pre supplementation 9.524±4.41 to post supplementation 32.07±12.40; p-value <0.001.
6. We observed significant fall in angiogenesis marker, serum VEGF levels post vitamin D supplementation from 773.547±344.173 to 639.97±119.02 p value 0.004 which suggests negative correlation between serum Vitamin D and serum VEGF levels.

So we propose that vitamin D supplementation is beneficial in PCOS women as it decreases angiogenesis and inflammation in ovaries as evident by lower serum VEGF levels in our study along with improvement in clinical and biochemical picture.

There are very few studies in literature that have measured change in serum VEGF levels in PCOS patients after vitamin D supplementation. And to the best of our knowledge no such Indian study has been done.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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