



Estimation Of Serum Vitamin D Levels In Patients with Polycystic Ovarian Syndrome

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ABSTRACT

Introduction

Around 4 percent to 18 percent of women of reproductive age have PCOS (Polycystic ovary syndrome), the most prevalent endocrine syndrome in women. Due to its lipophilic properties as a lipid-soluble vitamin that is stored in adipose tissue, vitamin D insufficiency is more frequent in PCOS females.

Objectives

To estimate level of serum Vitamin D in PCOS patients.

Material and Methods

250 females who were between 18- 35 years, who attended the obstetrics and gynecology outpatient department and were admitted to the obstetrics and gynecology inpatient and outpatient department at "Swaroop Rani Nehru Hospital and Kamla Nehru Memorial Hospital", both of which are affiliated with Moti Lal Nehru Medical College in Prayagraj were examined for PCOS, 150 women were excluded & 100 women participated in the observational analytical study from August 2018 to July 2019. Vitamin D levels were measured by a two-step competitive binding enzymatic immunoassay. Statistical analysis was done using the Chi-square test.

Result

Out of 100 PCOS women enrolled in the study, maximum cases had deficient Serum Vitamin D (13.59 ± 4.02 ng/ml) i.e., 67(67%) and only 33(33%) had normal Serum Vitamin D (39.21 ± 6.51 ng/ml). ($p = 0.0001$ i.e., significant).

Conclusion

The study concludes that the serum Vitamin D level was markedly reduced in PCOS patients (67%) than those in the normal population. Thus serum Vitamin D can be evaluated as a reliable indicator for ovarian dysregulation in PCOS.

Keywords: PCOS, Serum Vitamin D, Ovarian dysregulation.

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INTRODUCTION

Around 4 percent to 18 percent of women of reproductive age have PCOS (Polycystic ovary syndrome), the most prevalent endocrine syndrome in women.¹ After excluding other endocrinopathies such as Cushing's disease, androgen-producing tumors, nonclassic adrenal hyperplasia, and drug-induced androgen excess, a conference sponsored by the NIH (National Institutes of Health) classified PCOS as HA (hyperandrogenism) with oligo-anovulation in 1990.² In 2003, the Rotterdam consensus added any two of the given characteristics to the diagnostic criteria: 1.) oligo-anovulation; 2.) biochemical/clinical hyperandrogenism; 3.) PCO (Polycystic ovaries), after excluding similar endocrinopathies³.

A history of menstrual periods longer than 38 days was used to define oligomenorrhea. A modified hirsutism score of seven or above, as well as an increased total testosterone level of $\geq 3.96 \text{ nmol/L}$, were used to define hyperandrogenism.⁴

Due to its lipophilic properties as a lipid-soluble vitamin that is stored in adipose tissue, vitamin D insufficiency is more frequent in PCOS females. Vitamin D may influence glucose metabolism by increasing insulin production and release through raising insulin receptor expression and suppressing pro-inflammatory cytokines, both of which may play a vital role in the development of insulin resistance. In addition to improving ovarian androgen synthesis and reducing SHBG production (sex hormone-binding globulin), insulin resistance increases hyperandrogenism.⁵

Aims and objectives

- To estimate the level of serum Vitamin-D in PCOS patients.

Material and methods

250 females who were between 18- 35 years, who attended the obstetrics and gynecology outpatient department and were admitted to the obstetrics and gynecology inpatient outpatient department at "Swaroop Rani Nehru Hospital and Kamla Nehru Memorial Hospital", both of which are affiliated with Moti Lal Nehru Medical College in Prayagraj were examined for PCOS, 150 women were excluded & 100 women participated in the observational analytical study from August 2018 to July 2019. A detailed clinical

history and examination was done at first visit of the patient. The history included age, food habit, life style, increase in body weight, contraceptive or any other drug intake. Menstrual & obstetric history were taken in detail. In examination special consideration was given to weight, height, BMI, obesity, waist and hip circumference ratio, hirsutism, acne, acanthosis nigricans. In routine investigations CBC and GBP were done. Serum Vitamin D levels & Ultrasonography of pelvis were done as specific investigations.

The Inclusion Criteria:

- Women with Oligo or amenorrhea
- Women with infertility,
- Women with acne, hirsutism and obesity
- Women who have a family history of diabetes

Exclusion Criteria: The following medicines should not have been taken by study participants women in the previous three months: -

- Insulin sensitizers,
- Antiepileptic medicines
- Fertility medicines
- Drugs causing alteration of lipid metabolism

Blood sample collection and preparation:

Serum (gel and no gel) and plasma (lithium heparin) are the recommended samples. All blood samples were collected observing standard precautions for venipuncture. Serum samples were allowed to clot completely before centrifugation in an upright position. Tubes were kept stoppered at all times. Serum or plasma were separated from contact with cells as soon as possible. Stored samples were tightly stoppered at room temperature (15 to 30°C) for no longer than 72 hours. Thaw samples not more than 3 times. Ensure residual fibrin and cellular material have been removed prior to analysis. Grossly lipemic or hemolyzed samples should be discarded.

Estimation of serum Vitamin D

The Vitamin D estimation was done using a two-step competitive binding immunoenzymatic assay. In the initial incubation, sample was added to a reaction vessel with a DBP releasing agent and paramagnetic particles coated with sheep monoclonal anti-25(OH) Vitamin D antibody. 25(OH) vitamin D after releasing from DBP binds to the immobilized monoclonal anti-25(OH) vitamin-D on the solid phase. Subsequently, a 25(OH) vitamin-D analogue-alkaline

phosphatase conjugate was added which competes for binding to the immobilized monoclonal anti-25(OH) vitamin D. After a second incubation, materials bound to the solid phase were held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos*530 was added to the vessel and the light generated by the reaction was measured with a luminometer. The light production is inversely proportional to the concentration of 25 (OH) vitamin-D in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Reporting results

The ideal levels of serum 25(OH) Vitamin D are now a topic of discussion. The Clinical Guidelines

Subcommittee of the Endocrine Society Task Force provided the following recommendations for optimal serum range of 25(OH) Vit. D in 2011: Upper Safety Limit- > 100 ng/ml

Sufficient- 30-100 ng/ml

Insufficient- 20- 30 ng/ml

Deficient- <20 ng/ml

Correlation of serum Vitamin-D with age and history were evaluated and analysed. The t-test for continuous variables and chi-square test for categorical variables were applied and correlation between these factors for evaluation of PCOS patient was seen. For all statistical analysis p-value <0.05 was considered as significant.

Observation

The no of participants in our study were 100.

Table- 1 Distribution of cases according to age group

Age in years	No of case	Percentage	Mean age (yrs)±SD
15-20	11	11%	25.2±3.36
21-25	35	35%	
26-30	50	50%	
31-35	4	4%	

The Youngest patient in our study was of 18 years and oldest one was of 32 years of age (range 18-32 years). Out of the 100 cases, most of cases i.e. 50(50%) were from 26-30 years, 35(35%) cases

were from 21-25 years age group, 11(11%) cases were from 15-20 year age group, and 4(4%) cases from the 31-35 year age group.

Table-2 Distribution of cases according to BMI

BMI(KG/M ²)	No of case	Percentage	Mean value±SD (kg/m ²)
18.5-24.9	39	39%	25.38±3.77
25- 29.9	57	57%	
30- 34.9	4	4%	
Total	100	100 %	

Table 2 Shows that out of 100 cases, maximum cases i.e. 57 (57%) were overweight (BMI 25-

29.9), 39 (39%) had normal BMI (18.5-24.9) , and 4 (4%) were obese (BMI 30-34.9).

Table -3 Distribution of cases based on Serum Vitamin D level

Serum Vitamin D Level (ng/mL)	No of patients	Percentage	Mean Value±SD (ng/mL)	p-value
Deficient (< 20)	67	67%	13.59±4.02	0.0001
Sufficient (30 - 100)	33	33%	39.21±6.51	
Total	100	100%		

Out of 100 cases of PCOS, maximum cases i.e., 67 (67%) had deficient Serum Vitamin D (Mean Value 13.59±4.02 ng/ml), and 33(33%) had normal Serum Vitamin D (Mean Value 39.21±6.51ng/ml). p value=0.0001 i.e., significant. Therefore a

statistically considerable difference was noticed between the serum Vitamin D levels in Deficient serum Vitamin D cases and sufficient serum Vitamin D cases. Mean value of Serum Vitamin D was 22.05±13.08ng/ml

TABLE- 4 Relationship between Serum Vitamin D and age in PCOS

No of cases	AGE (in years)					p-value
	15-20	21-25	26-30	31-35	Total	
No. of cases with Deficient Vitamin D	10(10%)	26(26%)	29(29%)	2(2%)	67(67%)	0.045
No. of cases with Sufficient Vit-D	1(1%)	9(9%)	21(21%)	2(2%)	33(33%)	
Mean Vit-D Level (ng/ml)	16.63 ±4.009 (n=11)	20.04 ±10.05 (n=35)	24.15 ±19.03 (n=50)	28.23 ±5.65 (n=4)		

The correlation between serum Vitamin D and PCOS patient age is seen in Table 4. A maximum of 29 (29%) of the 67 (67%) patients with deficient serum Vitamin D belonged to the age bracket of 26 to 30 years, 26 (26%) to 21-25 years, 10 (10%) to 15-20 years, and 2 (2%), to 31-

35 years. A maximum of 21 (21%) of the 33 (33%) patients with sufficient serum Vitamin D belonged to the age bracket of 26–30 years, 9 (9%), to 21–25 years, 2 (2%), to 31-35 years, and 1 (1%), to 15-20 years.

DISCUSSION

100 females who were between 18- 35 years, who attended the obstetrics and gynecology outpatient department and were admitted to the obstetrics and gynecology inpatient outpatient department at "Swaroop Rani Nehru Hospital and Kamla Nehru Memorial Hospital", both of which are affiliated with Moti Lal Nehru Medical

College in Prayagraj, participated in the observational analytical study from August 2018 to July 2019. The study was undertaken to evaluate polycystic ovarian syndrome by hormonal assessment of serum Vitamin D. Our study discovered that most of the patients with Polycystic ovarian syndrome had deficient serum

vitamin D levels. Maximum cases i.e., 67 (67 %) in our study had deficient Serum Vitamin D (Mean Value 13.59 ± 4.02 ng/ml) as compared to 33 (33%) who had normal Serum Vitamin D (Mean Value 39.21 ± 6.51 ng/ml) $p=0.0001$ i.e., significant. Hence, a statistically considerable difference in serum Vitamin D level in patients with deficient serum Vitamin D and those with sufficient serum Vitamin D was discovered. Our results were nearly comparable to those of the results presented by Wehr E et al. (2011)⁶, which reported a vitamin D deficiency in 72.8% of cases. In the study by Kim J J et al (2014)⁷ and Sidabutar E et al (2016)⁸ 57.9% and 39.13% cases respectively were vitamin D deficient. In this research, the overall Mean serum Vitamin D value was 22.05 ± 13.08 ng/ml which was contrary to study by Wehr E et al (2011)⁶, Kim J J et al (2014)⁷ and Sidabutar E et al (2016)⁸ where mean values were 28.0 ± 11 ng/ml, 19.6 ± 6.6 ng/ml and 25.25 ± 7.55 ng/ml respectively.

On correlating the age with serum vitamin D levels in PCOS patients, it was found that the mean serum Vitamin D level in patients in the 20–30-year age range in the current research was 22.04 ± 4.9 ng/dl, which is significant ($p=0.0458$).

Thus age and serum Vitamin D levels in PCOS patients have a significant association. A much lower mean was reported by Arslan E et al (2019)⁹ and Kadhim H H et al (2019)¹⁰ where the Mean serum Vitamin D levels in 20–30 years age group were 8.5 ± 6.7 and 11.11 ± 3.1 ng/dl respectively.

This study also discovered a negative correlation between BMI and serum Vitamin D levels, as 61 % patients in our study were obese or overweight and most of i.e. 67 % patients had deficient serum Vitamin D levels. The mean BMI of the study was 25.38 ± 3.77 kg/m². Almost similar to studies by Agarwal N et al (2015)¹¹ and Swetha S et al (2015)¹² where mean BMI was 24.62 ± 4.93 kg/m² and 24.9 ± 2.5 kg/m² respectively. But in contrast to study by Szczuko M et al (2017)¹³ where mean BMI was higher i.e. 29.16 ± 5.8 kg/m².

CONCLUSION

The study concludes that the serum Vitamin D levels were markedly reduced in PCOS patients (67%) than those in the normal population. Thus serum Vitamin D can be evaluated as a reliable indicator for ovarian dysregulation in PCOS.

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