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Original Research Article

Correlation of serum vitamin D level with various aspects of polycystic ovarian syndrome

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ABSTRACT

Background: Women with PCOS usually suffer from metabolic disturbances and IR, which might be associated with vitamin D metabolism. The objectives of this study are to determine the association of Vitamin-D with metabolic and endocrine parameters in PCOS women and to evaluate specific role of Vitamin-D in PCOS and to correlate the serum Vitamin-D level with various demographic, anthropometric, biochemical, metabolic and hormonal parameters in PCOS patient.

Methods: This case control study was carried out between July 2014 and June 2016 in the Department of Obstetrics and Gynaecology, IMS, BHU got ethical clearance from the institute. The study involved 192 patients with polycystic ovarian syndrome based on Rotterdam criteria and 200 healthy age matched women as controls. The data analysis was performed with the help of SPSS 16.0 version for windows.

Results: When study subjects are compared according to vitamin-D level (sufficiency, insufficiency and deficiency) there was significant difference for BMI (kg/m^2), Waist Hip ratio and TRG (mg/dl). Vitamin-D had significant negative correlation with BMI ($p=0.000$); Waist:Hip ratio ($p=0.012$); Triglyceride ($p=0.022$).

Conclusions: Our study found an association between the high prevalence of obesity and metabolic dysfunction in women with PCOS, and vitamin-D deficiency could be secondary to obesity in such patients. Thus, vitamin-D deficiency cannot be considered as an independent factor through the development of metabolic disorders in PCOS patients.

Keywords: PCOS, Vitamin D, Polycystic ovarian syndrome, Triglyceride, Waist hip ratio

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is the most commonly diagnosed female endocrine disorder, with a prevalence rate of nearly 5-10% among women of reproductive age.¹ Hyperandrogenism is believed to be the central pathophysiologic mechanism of PCOS that leads to various clinical and biochemical manifestations of PCOS like insulin resistance (IR), obesity, impaired glucose tolerance, hypertension, type 2 diabetes (T2DM), and metabolic syndrome (MS).²⁻⁴ Vitamin D is a fat soluble

vitamin that is involved in bone metabolism and calcium homeostasis.⁵ Vitamin D influences glucose and insulin metabolism, and low vitamin D status is a risk factor for impaired glucose tolerance, IR and T2DM.⁶ Women with PCOS usually suffer from metabolic disturbances and IR, which might be associated with vitamin D metabolism.⁷ It has been emerging over recent years that vitamin D may have a role in disorders outside the skeletal system including immune disorders, diabetes, hypertension, cardiovascular disease, infectious diseases, and cancer.^{8,9}

Keeping an eye over the above background we have designed this study to achieve the following objectives.

Objectives

Objectives of current study were to determine the association of Vitamin-D with metabolic and endocrine parameters in PCOS women and to evaluate specific role of Vitamin-D in PCOS and to correlate the serum Vitamin-D level with various demographic, anthropometric, biochemical, metabolic and hormonal parameters in PCOS patient.

METHODS

This case control study was carried out between July 2014 and June 2016 in the department of obstetrics and gynaecology, IMS, BHU, Varanasi. The study involved 192 patients attending outpatient department and diagnosed as polycystic ovarian syndrome based on rotterdam criteria and 200 healthy age matched women as controls.

Inclusion criteria

Inclusion criteria were; Rotterdam criteria (2003) was used to diagnose PCOS which requires any two of the following three features: Oligomenorrhoea and/or amenorrhoea (oligomenorrhoea ≥ 45 days or < 8 cycles per year and amenorrhoea > 3 months in a woman with previous periodic menses) for a period of 6 months. Clinical and/or biochemical hyperandrogenemia presence of acne, hirsutism (FG score ≥ 8) and alopecia. Polycystic ovaries on sonography (≥ 12 follicles in one or both ovaries, 2-9 mm in diameter and/or increased ovarian volume > 10 ml).

Exclusion criteria

Exclusion criteria were; patient with Cushing's syndrome, non-classical CAH, hyperprolactinemia, primary hypothyroidism, acromegaly, premature ovarian failure, virilizing adrenal or ovarian tumor, primary hypothalamic amenorrhoea, OCPs use within last 4 month and Adolescent patient who had not reached menarche or who had reached menarche within previous 6 month.

Inclusion criteria for control

Regular menstrual cycle, Absence of hirsutism, alopecia and acne, Absence of polycystic ovary on sonography and Normal hormonal parameters including TSH, testosterone, prolactin, LH, FSH, LH:FSH ratio.

Clinical variables, such as body weight, height, waist circumference (WC), and blood pressure were assessed in all subjects. Serum levels of total Testosterone, free Testosterone and sex hormone-binding globulin (SHBG) were measured in all PCOS patients and controls whose blood samples were taken during the follicular phase of the menstrual cycle. The FAI was calculated as total

testosterone/SHBG $\times 100$. In all subjects, after a 12 hours overnight fast serum insulin, glucose, and lipid parameters were measured and the homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as glucose (mg/dl) \times insulin (μ U/ml)/405. We used blood samples for vitamin D determination and subjected to centrifugation, storage of serum at -40°C and this was followed by Vitamin D evaluation by using Enzyme-Linked Immunosorbent Assay (ELISA) Kit for vitamin D. Vitamin D insufficiency was defined as a 25-(OH) D3 concentration of < 30 ng/ml, and frank vitamin D deficiency was defined as a 25(OH) D3 concentration of < 20 ng/ml.

Statistical analysis

The data analysis was performed with the help of SPSS 16.0 version for windows. Data were entered in Microsoft Excel software and then transferred to SPSS 16.0 version for analysis. Numerical data were presented in the form of mean + standard deviation and quantitative data were shown in the form of number and percentage. Student-t-test was used to compare the significant difference in mean values between cases and control groups. Parametric Oneway analysis of variance test was applied to find out the significant difference in mean values if groups were more than two. If this test showed the significant difference, then posthoc test (Student Newman Kuel test) was applied to find pairwise difference. Chi square test was used to test the significant association between the qualitative variables. Odds ratio and 95% CI were also calculated to find out the significant risk factor for the disease. Linear correlation coefficient was calculated to see the amount and direction of relationship between quantitative variables. To estimate the value of dependent variable on the basis of known values of independent variable, if correlation was significant, linear regression was used.

RESULTS

In the present study a total of 192 PCOS patients based on Rotterdam criteria (2003) were included and compared with healthy age matched control. Vitamin D evaluation was done in all cases and controls. Comparative study of vitamin D level was done with clinical, biochemical, hormonal and sonographic parameter of PCOS patients. The (Table 1) shows Demographic, sonographic, clinical parameters in study group. Out of total 192 patients 40.1% belongs to lower middle socioeconomic status and 59.9% belong to upper middleclass. About 48.4% patients have BMI > 25 and 51.6 % are having BMI < 25 . Oligomenorrhoea was the most common presenting complaint (61.9%) followed by primary infertility (59.9%), acne (53.6%), hirsutism (49.5%), secondary amenorrhoea (25.5%). 9% patients had normal menstrual cycles. 9.3% patients had regular cycle. The (Table 2) shows the anthropometric characteristic of the study population. Among study subject (PCOS cases & controls) significant difference was found for BMI and Waist hip

ratio ($p < 0.001$) whereas there was no significant difference as far as age is concerned ($p = 0.401$).

Table 1: Demographic, sonographic, clinical parameters in study group.

Parameters	PCOS (N=192)		Control (N=200)	
	N	%	N	%
Socioeconomic status				
Lower middle	77	40.1	89	44.5
Upper middle	115	59.9	111	55.5
BMI				
<25 kg/m ²	99	51.6	182	91.0
>25 kg/m ²	93	48.4	18	9.0
Marital status				
Married	140	72.9	113	56.5
Unmarried	52	27.1	87	43.5
Menstrual pattern				
Amenorrhea	49	25.52	0	0.0
Oligomenorrhea	119	61.97	0	0.0
Menometrorrhagia	6	3.12	0	0.0
Regular	18	9.3	200	100
Parity				
Primary infertility	115	59.9	86	43.0
One abortion	8	4.2	0	0.0
Two abortion	10	5.2	0	0.0
Normal	59	30.7	114	57.0
USG				
Normal	53	27.6	85	42.5
PCOD	139	72.4	0	0.0
Others	0	0.0	115	57.5
Hirsutism				
Absent	97	50.5	200	100
Present	95	49.5	0	0.0
Acne				
Absent	89	46.4	177	88.5
Present	103	53.6	23	11.5

The (Table 3) shows Comparison of clinical and biochemical characteristic of study population. Among study subject significant difference found for LH level, FSH level, LH:FSH, prolactin, Testosterone, fasting blood glucose level, Fasting insulin Glucose insulin ratio, HOMA IR, Cholesterol level, Triglyceride level, HDL level, LDL level, ACNE, Hirsutism and Vitamin D level ($p < 0.001$). Mean vitamin d level in PCOS patients is (23.54 ± 5.663 ng/ml) while in control it was (34.67 ± 6.819 ng/ml) $p < 0.001$. 77.6 % OF PCOS patients had Vitamin D level < 30 ng/dl and $p \leq 0.001$ whereas 22.4% have > 30 ng/ml. While 30% of patients in control group are having Vitamin D < 30 ng/ml. When these pcos patients are again divided on basis of sufficiency and deficiency (Table 4). When study subjects are compared according to vitamin D level (sufficiency, insufficiency and deficiency) level there is significant difference were found for BMI (kg/m²), Waist Hip ratio and TRG (mg/dl) (Table 5). Among cases, vitamin D had significant

negative correlation with BMI ($p = 0.000$); Waist:Hip ratio ($p = 0.012$); Triglyceride ($p = 0.022$); This has been depicted in (Table 6). The scattered diagram showing parameters having negative linear correlation with serum Vitamin D in PCOS patients are depicted as (Figure 1-3).

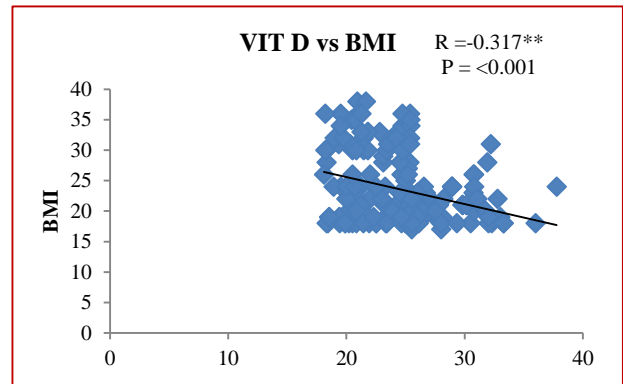


Figure 1: Correlation of serum vitamin D level in PCOS patients and BMI (Vitamin D was negatively correlated with BMI ($r = -0.317$) however $p = 0.000$ was significant).

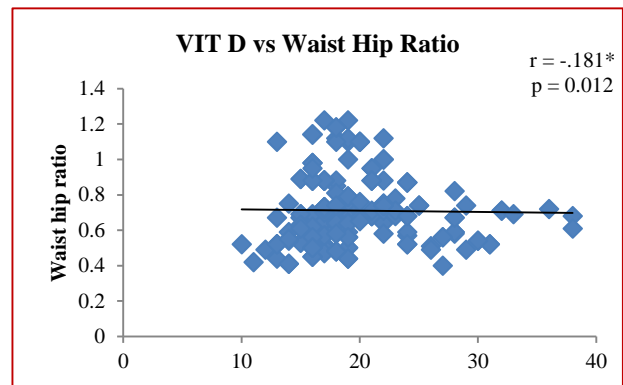


Figure 2: Correlation of serum vitamin D level in PCOS patients and waist hip ratio (vitamin D was negatively correlated with waist hip ratio ($r = 0.181$; $p = 0.012$)).

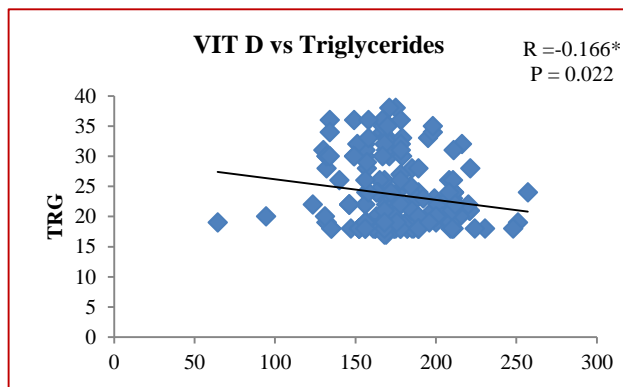


Figure 3: Correlation of serum vitamin D level in PCOS patients and serum triglycerides (Vitamin D was negatively correlated with triglyceride ($r = -0.166$; $p = 0.000$)).

Table 2: Anthropometric parameters of study population (Mean±SD).

Parameters	PCOS, Mean±SD, N=192	Control, Mean±SD, N=200	T value	P value
Age (years)	24.31±3.997	24.00±3.087	0.840	0.401
BMI (kg/m ²)	24.658±4.0407	21.323±2.3907	9.990	0.000
Waist hip ratio	0.734±0.169	0.626±0.0932	7.915	0.000

Table 3: Comparison of clinical and biochemical indices between two groups.

Parameters	PCOS, Mean±SD, N=192	Control, Mean±SD, N=200	T value	P value
LH (mIU/ml)	16.302±7.642	8.0175±1.7957	14.910	0.000
FSH (mIU/ml)	5.5673±2.264	6.4809±2.6819	-3.637	0.000
LH_FSH	3.0618±1.131	1.39138±.4995	19.031	0.000
PRL (ng/ml)	16.009±15.261	12.897±3.7667	2.797	0.005
TSH (µIU/ml)	2.9847±3.204	2.8317±1.048	0.641	0.522
Testosterone (ng/dl)	121.100±6.773	83.075±14.5436	32.958	0.000
Fasting blood glucose (mg/dl)	103.460±16.836	93.811±7.2562	7.419	0.000
Fasting insulin (µU/ml)	24.879±3.776	16.509±3.3665	23.184	0.000
Glucose insulin ratio	4.3839±0.416	5.9180±1.3151	-15.438	0.000
HOMA IR	1.6419±0.853	1.8493±0.095	-3.413	0.001
CHOL (mg/dl)	237.274±24.574	170.495±16.947	31.427	0.000
TRG (mg/dl)	177.488±27.623	94.086±18.250	35.402	0.000
HDL (mg/dl)	52.013±9.275	53.610±6.1879	-2.013	0.045
LDL (mg/dl)	152.561±12.799	66.050±17.182	56.351	0.000
USG	0.72±0.448	1.15±0.991	-5.445	0.000
Acne	0.54±0.500	0.12±0.320	9.982	0.000
Hirsutism	0.49±0.501	0.00±0.000	13.960	0.000
Vitamin D (ng/ml)	23.54±5.663	34.67±6.819	-17.547	0.000

Table 4: Distribution of vitamin D in study population.

Vitamin D	PCOS (N=192)		Control (N=200)	
	N	%	N	%
<30 ng/dl	149	77.6	60	30.0
>30 ng/dl	43	22.4	140	70.0
Total	192	100	200	100

$\chi^2=89.189$; $p<0.001$

DISCUSSION

Vitamin D is a fat-soluble vitamin that is involved in bone metabolism and calcium homeostasis.⁵ It has been emerging over recent years that vitamin D may have a role in disorders outside the skeletal system including immune disorders, diabetes, hypertension, cardiovascular disease, infectious diseases, and cancer.^{8,9} Vitamin D deficiency is very prevalent worldwide, as people adopt sedentary indoor lifestyles and use sunscreen and protective clothing to reduce skin cancer risk.¹⁰ Serum 25-hydroxyvitamin D (25[OH]D) concentration is widely accepted as the functional indicator of vitamin D status in the body. At present, there is no general consensus on the minimum serum level of 25(OH)D that is optimal for health. A concentration of 25(OH)D less than 50 nmol/l (20 ng/ml) is generally considered as vitamin D deficiency, whereas serum 25(OH)D concentrations of 50 to 74 nmol/l (20-30 ng/ml) are considered as vitamin D insufficiency.¹¹ Serum 25(OH)D concentrations between 30 and 50 nmol/l (12-20 ng/ml) are frequently associated with biochemical

abnormalities such as raised parathyroid hormone (PTH) levels, but not clinical symptoms.¹² Investigators in the field of vitamin D research generally agree that a serum 25(OH)D level of at least 50 to 80 nmol/l (20-32 ng/ml) is required for optimal bone health.¹³ In the last National diet and nutrition survey (NDNS) conducted in the United Kingdom, vitamin D deficiency (25[OH]D level <50 nmol/l) was very common and identified in 54% of women aged 19 to 64, whereas severe vitamin D deficiency (25[OH]D level <25 nmol/l) was detected in 15% of those women. Several studies have explored the association of vitamin D with IR and metabolic features in PCOS.^{1,14} However these studies used indirect measures of IR such as oral glucose tolerance tests, homeostasis model assessment (HOMA), and quantitative insulin sensitivity check index. There has only been one study to date examining the relationship between vitamin D and IR in PCOS using hyperinsulinemic euglycemic clamps (gold standard); however, this study did not have a control population.

Table 5: Demographic, anthropometric, biochemical, hormonal profile of PCOS patients according to the distribution of vitamin D.

Parameters	Vitamin D, <20 ng/ml, Mean±SD	Vitamin D, 21-29 ng/ml, Mean±SD	Vitamin D, >30 ng/ml, Mean±SD	P value
Age (years)	23.56±3.734	25.19±4.309	24.28±3.744	0.046
BMI (kg/m ²)	25.211±3.9471	25.638±4.1618	22.058±2.7976	<0.001
Waist hip ratio	0.742±0.1828	0.771±0.1697	0.664±0.1163	0.004
LH (mIU/ml)	15.291±7.0509	16.852±7.2851	17.305±9.0922	0.288
FSH (mIU/ml)	5.394±2.2463	5.504±2.1090	5.992±2.5246	0.363
LH FSH ratio	2.886±0.9442	3.282±1.3908	3.036±0.9368	0.102
PRL (ng/ml)	14.164±7.9311	17.415±12.9433	17.188±25.6619	0.368
TSH (µIU/ml)	2.727±1.3164	3.097±1.7277	3.283±6.1943	0.617
Testosterone (ng/dl)	122.307±6.8515	119.930±6.9648	120.737±6.0544	0.094
Fasting blood glucose (mg/dl)	104.604±16.4246	104.234±13.6688	100.093±21.5438	0.329
Fasting insulin (µU/ml)	24.921±3.6535	25.341±4.0757	24.064±3.4402	0.219
Glucose insulin ratio	4.448±0.3971	4.365±0.3909	4.295±0.4765	0.135
HOMA IR	1.742±0.9683	1.618±0.7963	1.493±0.6944	0.292
CHOL (mg/dl)	233.341±25.5455	240.820±26.7445	238.903±17.5494	0.159
TRG (mg/dl)	176.446±29.5847	186.040±26.7656	165.706±19.9888	0.001
HDL (mg/dl)	52.750±10.0819	51.986±9.1256	50.687±7.8989	0.503
LDL (mg/dl)	151.244±13.6222	153.718±13.3454	153.157±10.0878	0.474

Table 6: Correlations of vitamin D and other parameters in PCOS patients.

Parameters	Vitamin D	
Vitamin D	R value	1
	P value	-
Age	R value	0.006
	P value	0.938
BMI	R value	-0.317**
	P value	0.000
Waist hip ratio	R value	-0.181*
	P value	0.012
LH	R value	0.121
	P value	0.093
FSH	R value	0.115
	P value	0.111
LH FSH ratio	R value	0.053
	P value	0.468
PRL	R value	0.047
	P value	0.514
TSH	R value	0.038
	P value	0.602
Testosterone	R value	-0.069
	P value	0.340
Fasting blood sugar	R value	-0.110
	P value	0.130
Fasting insulin	R value	-0.108
	P value	0.135
Glucose insulin ratio	R value	-0.116
	P value	0.110
HOMA IR	R value	-0.086
	P value	0.238
Cholesterol	R value	0.087
	P value	0.230

Continued.

Parameters		Vitamin D
Triglyceride	R value	-0.166*
	P value	0.022
HDL	R value	-0.127
	P value	0.079
LDL	R value	0.072
	P value	0.321

This study suggested that vitamin D levels in PCOS are primarily related to adiposity.¹⁵ In our study, we found a significantly increased waist hip ratio in cases (0.734 ± 0.169) as compared to controls (0.626 ± 0.0932) ($p=0.000$) signifying that obesity is an important component of PCOS and may lead to reproductive as well as metabolic consequences. A recent randomized controlled trial shows that supplementation with vitamin D improved IR, suggesting that vitamin D might have a protective role in the development of IR in women with PCOS).¹⁶ Women with PCOS often demonstrate clustering of cardiovascular risk factors and may have an increased risk of cardiovascular disease.¹⁷ It has been suggested that vitamin D may have a protective effect on some cardiovascular risk factors.¹⁸ In our study we found that level of vitamin D in PCOS patients is lower as compared to control group. The mean level of Vitamin D in PCOS was (23.54 ± 5.66 ng/ml) and in control (34.67 ± 6.81 ng/ml). In our study 77.6% of patients show vitamin D level less than 30ng/ml out of which 41.7% are vitamin D deficient and 35.9% show vitamin D insufficiency

Yildizhan et al found that serum 25-OH-VD mean levels were 56.31% lower in the PCOS patients when compared to control.¹⁹ Li et al also reported lower vitamin D levels, in women with PCOS when compared with women without PCOS.²⁰ Similarly Wehr et al also reported lower levels in women with PCOS (N=545) compared to the control women (N=145; 25.7 vs. 32.0 ng/ml, respectively, However Mahmoudi et al found that women with PCOS had a significantly higher vitamin D level (29.3 ng/ml in PCOS women vs. 19.4 ng/ml in control women).²⁰ Although there is still no definite consensus on the significance of serum vitamin D levels in patients with PCOS and those without PCOS, an inverse correlation between serum 25OHD concentrations and metabolic disturbances was reported in PCOS patients.²¹ Accordingly, previous studies revealed that women with PCOS frequently had associated vitamin D deficiency.²² Whereas others indicated that the lower vitamin D level found in women with PCOS was not significant compared to patients without PCOS.¹⁹ Moreover, multiple studies have shown that vitamin D3 supplementation in vitamin D deficiency led to improvements in several laboratory and clinical parameters of PCOS.^{23,24} It has also been demonstrated by previous studies that vitamin D deficiency is associated with other features of metabolic syndrome including glucose intolerance, hypertension, dyslipidemia, and chronic inflammation.²⁵ These metabolic risk factors are commonly found in women with PCOS, but a role for vitamin D deficiency in PCOS and

metabolic syndrome is not entirely clear.²⁶ In our study on applying linear regression analysis decreased vitamin D levels in PCOS patients were found to be most significantly correlated with BMI, Waist Hip Ratio and serum Triglyceride levels. But at the same time, we must mention that vitamin D deficiency can occur secondary to obesity, which has got a quite high prevalence in the said population. Hence this issue needs further future evaluation in the form of large well designed randomised controlled trials.

CONCLUSION

The decrease in of Vitamin D levels were directly correlated with BMI, waist hip ratio and triglyceride levels in PCOS patient. So clinical strategies aimed at reducing obesity may prevent development of atherosclerosis leading to various cardiovascular disease and several menstrual disturbances and infertility in PCOS. Based on our findings, we anticipate that the contribution of vitamin D to the development of PCOS deserves further investigations. In conclusion, our study found an association between the high prevalence of obesity and metabolic dysfunction in women with PCOS, and vitamin D deficiency is also occurred secondary to obesity in such patients. Thus, vitamin D deficiency cannot be considered as an independent factor through the development of metabolic disorders in PCOS patients.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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