Original Research Article

Correlation of vitamin D levels with polycystic ovary syndrome: A cross-sectional study.

Abstract

Objective: To compare vitamin D status in, women with PCOS & fertile women without PCOS and its subsequent evaluation.

Introduction: PCOS is an endocrine disorder of women in reproductive age, characterised by obesity, hyperandrogenaemia and insulin resistance. Women with PCOS tend to be overweight and have increased risk of development of Type II Diabetes and cardiovascular disease. Exact Etiology of PCOS still remain an enigmatic dilemma however various studies conducted till date include diet and lifestyle modification as the key factor to promote health, BMI, reduced hyperinsulinemia and reduce the risk of development of PCOS.

Main aim of our study was to compare vitamin D status in women having PCOS, with fertile women in a tertiary care hospital in Uttarakhand.

Methodology: The conducted study was cross sectional, involving the enrolment of 100 women comprising of 50 women with PCOS and 50 fertile women without PCOS. Participants were selected from gynaecological OPD at Shri Mahant Indiresh Hospital, associated with Shri Guru Ram Rai Institute of Medical Sciences, Dehradun from July 2019 to January 2020. The diagnostic criteria of PCOS used was the Rotterdam criteria. The serum 25-hydroxy vitamin D and other metabolic markers were measured. Vitamin D deficiency was defined as serum 25 (OH) D concentrations less than 20 ng/ml measured on an instrument named as miniVidas (BioMerieux, Germany) based on ELFA (enzyme linked fluorescent assay).

Result: Serum 25-hydroxyvitamin D was significantly lower in women with PCOS compared to fertile controls ($p < 0.0001$), and the prevalence rates of 25(OH) D deficiency and insufficiency were higher in women with PCOS than in fertile women ($p < 0.0001$). The study results showed that the prevalence of 25 (OH) D deficiencies in PCOS women was significantly high. Serum 25 (OH) D concentrations were significantly negatively correlated with body mass index (BMI), waist-to-hip ratio (WHR), fasting insulin, total cholesterol and low-density lipoprotein cholesterol (LDL-C), ($P < 0.05$). In comparison, serum 25 (OH) D concentrations were significantly positively correlated with high-density lipoprotein cholesterol (HDL-C) ($P < 0.05$). Increased BMI and WHR, high levels of fasting insulin, total cholesterol and LDL-C were regarded as risk factors, but high level of HDL-C was considered to be protective factor of vitamin D deficiency in PCOS women.

Conclusion: The study demonstrated that women with PCOS have a significantly lower 25(OH) D compared to fertile controls. A compromised vitamin D status in PCOS women is associated with a higher prevalence and metabolic risk of PCOS in women.

Introduction

PCOS is an endocrine disorder of women in reproductive age and is the main cause of anovulatory infertility (1). The prevalence between 6 and 10% based on the National Institute of Health criteria and as high as 15% when the broader Rotterdam criteria are applied. (2,3). Characteristic feature of PCOS is presence of polycystic ovaries and menstrual dysfunction leading to infertility along with biochemical abnormality. PCOS manifest clinically as hirsutism, Hyperandrogenism and acne (4). The most prevalent characteristics of PCOS is Obesity, Hyperandrogenemia and insulin resistance (5). The prevalence of PCOS will rise in near future (6). The potential impact will be negative on population growth and cardiovascular morbidity and mortality, and consequently, it will lead to major public health concern. The development of PCOS is influenced by Vitamin D and hormonal modulation that further influences insulin metabolism and fertility regulation (7,8). Various studies have reported low levels of vitamin D in women with PCOS, with average 25-hydroxy vitamin D (25OHD) levels between 11 and 31 ng/ml (7,9,10,8,11-17). While few amongst these stated that vitamin D (25OHD) levels usually have values <20 ng/ml (67–85%).(9,18,19,8,13,14) The functioning of all systems of the body is disrupted by vitamin D deficiency, which increases the risk of chronic disease including physical diseases such as cancer, cardiovascular, autoimmune and infectious diseases; and psychological disorders such as depression and chronic pain (20). Vitamin D3 is obtained from the diet or synthesised endogenously through sunlight- induced photochemical conversion of cholesterol in the skin and subsequently hydroxylation in the liver and kidney. In the skin, 7-dehydrocholesterol undergoes ultraviolet photolysis to form vitamin D3. Vitamin D3 then undergoes two successive hydroxylation, the first of which takes place in the liver and is catalysed by vitamin D-25 hydroxylase to form 25OHD. The second hydroxylation step is regulated by parathyroid hormone (PTH) and mediated by 25-hydroxyvitamin D3 1a-hydroxylase and occurs predominately in the kidney. This second hydroxylation produces the final active metabolite of vitamin D3, which is 1,25-dihydroxyvitamin D3. 1, 25-dihydroxyvitaminD3 circulates bound to vitamin D-binding protein until it reaches its target tissue where it binds to vitamin D receptors to initiate its effect.

The difference between vitamin D levels among PCOS women and healthy women, is still very controversial and debatable along with the relationship between vitamin D and metabolic factors in PCOS women. Certain studies have shown that PCOS women had lower serum 25 (OH) D concentrations than healthy women, and they suffered from vitamin D deficiency.

Vitamin D deficiency and PCOS are associated with metabolic disorders, but little is known about vitamin D status in women with PCOS in this part of Uttarakhand. Therefore, this study aims to investigate vitamin D status and analyse the relationship between vitamin D deficiency and metabolic risk factors in women with PCOS.

Methodology

It was an observational, cross-sectional study conducted from July 2019 to January 2020 at Shri Mahant Indiresh Hospital, Dehra Dun. The sample size was n= 100, out of which 50 were control and 50 patients belonged to PCOS group. Owing to financial constraints and bearing of total costs by the author, the sample size was a limitation of this study. All women fulfilling the inclusion criteria such as Oligo/amenorrhea, Signs of Hyperandrogenism-hirsutism, severe acne, male pattern baldness, Signs of Hyperinsulinemia-obesity, acanthosis nigricans, History of PCOS in mother/sibling were included after informed consent. The diagnosis was made on the basis of Biochemical Tests, Physical Examination and Ovarian

Ultrasonography. Women with thyroid disease, congenital adrenal hyperplasia or autoimmune diseases were excluded. Non probability sampling technique was used for the purpose of data collection. All the young women of age (18-40), and those with polycystic ovary syndrome for more than or equal to one year duration were included. The data was collected in self- structured validated questionnaire. PCOS women can be diagnosed by two of the three criteria; Oligo-ovulation or anovulation, clinical or biochemical evidence of hyperandrogenism and polycystic ovaries (12 follicles of size 5-7mm). Vitamin D deficiency was defined as 25- hydroxyvitamin D [25OHD] level < 20 ng/ml. The protocol was approved by the Institutional Ethical committee of the hospital.

Statistical Analysis

Data in this study was analyzed using SPSS version 19.0. The continuous variables are presented as mean ± SD, which were performed by Student's t-test or variance analysis. The categorical parameters are displayed as numbers (%), which were analyzed by chi-square test. Linear regression analysis was used to analyze the correlation of 25 (OH) D concentrations with metabolic parameters. Post-stratification was done by applying Chi- square test. $P \leq 0.05$ was taken as significant.

Result

The numerical range for age in our study was from 18 to 40 years with mean age of control 24.02 ± 4.3 and PCOS group 23.16±5.0 years, mean duration of PCO was 2.5+1.2 years and mean BMI for control 24.48±5.72 and PCOS 25.90±5.16. Overall, 29(38.16%) control and 47(61.84%) PCOS group of women had vitamin D deficiency which was statistically significant (p=0.0001). (Table1) When outcome variable was stratified with respect to age, BMI, waist hip ratio, systolic and diastolic blood pressure, it showed no significant difference as p-values were 0.3577, 0.1942, 0.4663, 0.1547 and 0.20980.752 respectively. Table 2 shows the clinical and biochemical profiles of the subjects. Women with PCOS showed increased serum levels of FSH, LH, TSH and T3 compared with matched controls, but they showed no differences in the levels of T4. There was marked difference in Vitamin D levels or prevalence of vitamin D deficiency (< 20 ng/ mL) (Table 2). In addition, we found correlations between serum vitamin D level and clinical or metabolic profiles in both PCOS patients and controls (Table 2). Vitamin D insufficiency was observed in the majority of the subjects (61.84% of patients and 38.16% of the controls Table 2).

Basic Characteristic of the Studied Groups

Table 1 illustrates the baseline characteristics of women in the two groups.

	Control N=50	PCOS Group N=50	P-value
Age(year)	24.02±4.3	23.16±5.0	0.3577
BMI	24.48±5.72	25.90±5.16	0.1942
Waist circumference	102.86±15.35	94.58±12.78	0.0042

Hip circumference	0.95±0.06	0.94±0.06	0.0031
Waist hip ratio	0.95±0.06	0.94±0.06	0.4663
Blood Pressure Systolic Diastolic	 122.02±7.41 84.8±5.79	 124.28±8.3 83.2±6.8	 0.1547 0.2098

Clinical and Biochemical profiles of the subjects

Table 2: illustrates the clinical and biochemical profiles of women in the two groups.

	Control N=50	PCOS Group N=50	P-value
FSH	4.44±5.22	4.51±5.84	0.4586
LH	2.89±7.22	3.6±7.76	0.9231
TSH	1.76±2.19	1.79±2.56	0.1192
T_3	81.0±89.26	84.2±89.12	0.2220
T_4	5.98±6.21	5.98±7.63	0.0729
Triglyceride	130.31±17.89	124.13±29.25	0.2059
Total Cholesterol	166.08±25.05	151.29±30.76	0.0098
LDL	83.78±28.03	75.62±23.88	0.1207
Vitamin D_3	21.09±18.07	11.91±10.57	0.0001

Table 3: illustrate the Vitamin D Outcome in PCOS patients and Control group

Variable	Control N=50	Case N=50	p- value*
Vitamin D3	18.2 (6-66.5) 21.9±13.4	11.2 (5.23-31.2) 11.9 ±4.7	<0.0001
Sufficient (>=20.00) Low Vitamin D3 (<20.00)	21 (42) 29 (58)	3 (6) 47 (94)	1 (ref) 11.3 (3.1-41.4)#

Data expressed as Mean±Sd and Median (Min-Max) , f(%) . *Ranksum test, # Odd's ratio (95%CI)

It was observed that the mean vitamin D3 level among cases was less as compared to controls, and it was found statistically significant. The prevalence of vitamin D deficiency

was high in the study population (76%) however the odds of having disease was 11 times more in Vitamin D deficient as compared to normal (Table 3).

No significant difference was found when comparing other baseline characteristics between the two groups (P > 0.05).

Vitamin D Status between PCOS Women and Controls

The serum 25 (OH) D concentrations were significantly lower in PCOS women than in controls (11.91± 10.57 vs. 21.09 ± 18.07 ng/mL, P < 0.001). In addition, the serum 25 (OH) D concentration deficiencies were significantly high in women with PCOS than in controls (61.84% vs. 38.16%, P < 0.0001). Furthermore, the prevalence of normal 25 (OH) D statuses in women with PCOS was significantly lower than that in controls (12.5% vs. 87.5%, P < 0.0001). Table 2 shows the Clinical and Biochemical profile of patients in two groups. There were statistically significant differences in HDL and Abdomen girth among the two groups (P < 0.05). No significant difference was found when comparing FSH, LH, TSH, T3, and T4, total cholesterol, LDL and triglycerides among two groups (P > 0.05).

Discussion:

In women with PCOS has low vitamin D levels that are associated with obesity, metabolic and endocrine disturbances, vitamin D supplementation might improve menstrual frequency and metabolic disturbances in those women.[21] Vitamin D deficiency is still considered a problem of the past by health care professionals and the public. Populations at risk include infants, children, pregnant and postmenopausal women.
This study focuses on the serum levels of vitamin D and the prevalence of vitamin D deficiency in patients with PCOS. The result of the study clearly shows significant differences in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between women with PCOS and control group. Additionally, we found correlations between serum vitamin D and hormonal or metabolic profiles in either PCOS patients or controls. Our findings suggest that there is role of vitamin D in the pathogenesis of PCOS. Similar to our study Wehr et al. [20] reported lower serum vitamin D levels in a large number of women with PCOS (n = 545) compared to controls (n = 145) (25.7 vs. 32.0 ng/mL, respectively), a substantial number of studies suggest that serum vitamin D levels are similar in women with and without PCOS [12,17,30]. In previous studies, average serum vitamin D levels in women with PCOS were reported to be between 11 ng/mL and 31 ng/mL, with the majority having mean values < 20 ng/mL [12,13,15-17,20,22,29, 31-33]. In our study, the mean 25-(OH) D3 level in women with PCOS was also < 20 ng/mL (11.91 ± 10.57 ng/mL), and vitamin D deficiency (lower than 20 ng/mL) was observed in 61.84% of patients. However, vitamin D deficiency is also common in the control group, with 60% of adults having values lower than 20 ng/mL. The control subjects in the current study also showed a high prevalence of vitamin D deficiency (38.16%), with a mean level of 21.09 ng/ mL. Many studies have investigated an association between vitamin D status and hormonal or metabolic features in PCOS. PCOS women with low vitamin D level is thought to be at risk of metabolic factors such as insulin resistance, high total cholesterol, blood pressure, glucose, C-reactive protein, triglycerides, and low high-density lipoprotein (HDL) cholesterol [14,17]. In addition, vitamin D replacement therapy may have a beneficial effect on insulin resistance or fasting and on stimulated glucose and triglycerides levels in women with PCOS [16, 33]. Furthermore, several studies have identified relationships between low vitamin D status and measures of hyperandrogenism such as SHBG, the degree of hirsutism, FAI, total T and

dehydroepiandrosterone sulphate [13, 14, 17, and 29]. Although our study found significant differences in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between PCOS women and matched controls, the results need to be interpreted with caution. First, vitamin D deficiency may be a universal phenomenon across PCOS patients and controls. Second, inverse associations between obesity (BMI, body fat and waist measurements) and serum vitamin D levels have been reported in many studies [12-14, 17, 22, and 29]. As vitamin D is fat soluble, a higher proportion of vitamin D may be sequestered in adipose tissue in obese individuals, which might lower serum levels. In our study, patients and controls were matched by BMI, thus there were no differences in BMI, WC, or body and visceral fat masses between the two groups. Third, a potential limitation of the present study is the modest sample size in the PCOS group, which precludes drawing strong conclusions. Finally, we did not evaluate the presence of other potential confounding factors, such as outdoor times or dietary patterns which could affect the serum vitamin D levels. In summary, we found significant difference in the absolute level of serum vitamin D or prevalence of vitamin D deficiency between women with PCOS and matched controls. The women with PCOS are at 11.34% at risk of developing vitamin D deficiency. Additionally, we did not find any correlation between serum vitamin D and hormonal or metabolic profiles in either PCOS patients or controls. Although our findings suggest that the role of vitamin D in the pathogenesis of PCOS is clear, vitamin D deficiency is a common finding among PCOS patients and controls. Finally, the potential relationship between vitamin D and PCOS requires further investigation, since vitamin D deficiency has been continuously proposed to increase the risk of insulin resistance and T2DM, which is also a core pathophysiology of PCOS.

Conclusion:

The study demonstrated that women with PCOS have a significantly lower 25(OH) D compared to fertile controls. A compromised vitamin D status in PCOS women is associated with a higher prevalence and risk of metabolic disorders. The prevalence of vitamin D deficiency was high in the study population (76%) however the odds of having disease was 11 times more in Vitamin D deficient individuals as compared to fertile women.

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