ORIGINAL ARTICLE

Vitamin D Level in Unmarried Females with Polycystic Ovarian Syndrome

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ABSTRACT

Objective: To assess and compare the levels of vitamin D in the young unmarried patients of PCOS and healthy females of same age group in population of Rawalpindi.

Study Design: A descriptive cross sectional study.

Place and Duration of Study: The study was conducted in Gynecology and Obstetrics department of Railway Hospital, Rawalpindi in collaboration with Department of Biochemistry IIMC, Rawalpindi from October, 2014 to April, 2015.

Materials and Methods: A sample of 150 young, unmarried females of 16-25 years was enrolled with their written consent in this study. These females were divided into two groups, 50 apparently healthy females and 100 diagnosed patients of polycystic ovarian syndrome selected according to Rotterdam criteria. Vitamin D assay was measured by using 25OH Vitamin D Total Elisa Kit. Data was collected on pre-designed questionnaire. The Data was subjected to SPSS version 21 and analyzed using independent T-test.

Results: We had 56% controls and 56% PCOS patients suffering from severe vitamin D deficiency. Women with PCOS showed no significant differences in the vitamin D level (10.618+5.296 ng/ml in patients vs 11.846+7.898 ng/ml in controls, respectively, p=0.324). We also did not find significant association between severity of hypovitaminosis D and clinical symptoms of hyperandrogenism like hirsutism (P=0.669), acne (P=0.480) and alopecia (P=0.317).

Conclusion: Hypovitaminosis D is equally common among both PCOS patients and healthy females. There is no difference in the vitamin D level among PCOS and control subjects suggesting that there is no role of vitamin D in the pathogenesis of PCOS.

Key Words: *Polycystic Ovarian Syndrome, Hyperandrogenism, Vitamin D.*

Introduction

The Polycystic ovarian syndrome (PCOS) is the commonest heterogeneous endocrine disorder and major health problem among females in their reproductive life with the prevalence of 6-15% but it may high up to 20%, depending on used criteria.[1] Study among Asian people showed the prevalence of PCOS about 6.3% among females in their reproductive age (15-45 years) in Srilanka, 2-7.5% in females of China by using Rotterdam criteria.[2] A study from Pakistan in 2005 reported a prevalence of PCOS about 20.7%.[3] It was first reported in 1935 with the clinical manifestations including amenorrhea, hirsutism, obesity, acne and alopecia[4] and has been significantly associated with insulin resistance, hyperinsulinemia, diabetes mellitus type 2, hypertension, dyslipidemia and cardiovascular disease.[5] The PCOS cases don't exhibit the clear pathophysiology however some factors including insulin resistance, obesity, genetic, environmental, endocrine and metabolic factors may thought to be involved in the development of PCOS.[6] Under the 2003 Rotterdam diagnostic criteria for polycystic ovary syndrome, presence of any two features out of three (polycystic ovarian (PCO) morphology on ultrasound scan, clinical and biochemical hyperandrogenism and oligo-amenorrhoea) are the diagnostic tool for declaring polycystic ovary syndrome.[7]

In past few years vitamin D has got the popularity in the field of research due to its association with many diseases including reduced fertility, endometriosis and polycystic ovarian syndrome. Vitamin D is a fat soluble steroid hormone synthesized in the skin by

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ultra-violet radiations which convert the 7-dehydrocholesterol into cholecalciferol. Active form of vitamin D ($1,25(OH)_2D$) is formed by the two hydroxylation process occurring in liver and kidney in the presence of 25-hydroxylase and 1α-hydroxylase.[8] $1,25(OH)_2D$ is then bound with the VDR, a transcription factor, involved in the expression of up to 2000 gene.[9] Vitamin D has a significant role in bone metabolism and mineral homeostasis but also involved in cellular growth process, differentiation and metabolic modulations. Many studies have investigated the vitamin D status in PCOS patients but the contradictory results have made the role of vitamin D suspicious in pathogenesis of PCOS. Limited studies with modest sample sizes have shown the association between VDR (vitamin D receptor) polymorphisms and the development of a polycystic ovarian syndrome.[8]

Most studies have focused on Caucasian population and no such research has been done before in Pakistan. Our study aim was to find out the association between PCOS and vitamin D and to know whether a low vitamin D status can be observed in unmarried young female with PCOS. In addition we also investigated the association between clinical symptoms of hyperandrogenism and hypovitaminosis D.

Materials and Methods

A descriptive cross sectional study was conducted in Gynecology and Obstetrics department of Railway Hospital, Rawalpindi in collaboration with Department of Biochemistry IIMC, Rawalpindi over a period of 6 months after obtaining approval for research proposal. We enrolled 150 young, unmarried females of 16-25 years from OPD of Gynecology and Obstetrics department of Railway Hospital by using non-probability purposive sampling technique with their written consent after explaining the purpose of study. These females were divided into two groups, 50 apparently healthy females had normal menstrual cycles and none of them had clinical symptoms of hyperandrogenism and 100 newly diagnosed patient of polycystic ovarian syndrome selected according to Rotterdam criteria. Accordingly, the presence of two features out of three i.e polycystic ovarian (PCO) morphology on ultrasound scan (presence of 12 or more follicles measuring 2-9 mm in diameter per ovary or ovarian volume above 10cc), clinical/biochemical hyperandrogenism (hirsutism, acne or alopecia and /or elevated androgens levels) and ovulatory dysfunction (oligomenorrhea or amnorrhea) were existed. The females taking drugs(oral contraceptive, hypoglycemic drugs, vitamin D supplementation) and with other medical causes including Cushing syndrome, androgen secreting tumor, thyroid disorders, and prolectinemia were excluded from the research. Ferriman-Gallwey scoring system was used to evaluate the hair growth at seven sites: upper lip, chin/ face, chest, back, abdomen, arms and thighs. A score above 8 was indicative of hirsutism. Body weight in kilogram and height in meters was measured and BMI was calculated as weight (kg) divided by the square of the height (m). Subjects were categorized as normal weight (BMI between 19.5 to 25), overweight (BMI between 25 to 29.9) and obese (BMI > 30).[10] 3ml venous blood was drawn in vaccum clot activator tubes from both groups and centrifuged at 2500-3000rpm for 15 minutes for serum separation. Serum samples were stored at -20°C[11] until further analysis at Biochemistry Research Laboratory IIMC. The vitamin D assay was measured by Enzyme Linked Immunosorbent Assay by using 25OH Vitamin D Total Elisa Kit: Cat#KAP1917; LOT#131106 (DIAsource, S.A,Belgium). Hypovitaminosis was defined as plasma level of 25(OH) D less than 30ng/ml, graded as severe vitamin D deficiency for plasma level of 25(OH) D <10ng/ml, mild vitamin D deficiency for plasma level of 25(OH) D 10-19.9ng/ml and vitamin D insufficiency for plasma level of 25(OH) D 29.9ng/m.[12] Statistical analysis was done in SPSS version 21.0. Results were compared by applying independent T test and Chi-square. The level of significance was set as $P<0.05$.

Results

Vitamin D levels were tested in both the groups, cases and controls and it is found that 56% controls and 56% PCOS patients have severe vitamin D deficiency (>10ng/ml), 30% controls and 38% PCOS women have vitamin D deficiency (10-19.9ng/ml). Only 1 (2%) woman in control group has sufficient level of vitamin D (>30ng/ml). The mean vitamin D level is bit higher in controls (11.846±7.898) as compared to PCOS patients (10.618±5.296), but this is not statistically significantly (P-value > 0.05)

different in both groups as given in table I. According to the results it is found that there is significant (p-value < 0.05) difference in BMI of both groups. The mean BMI in PCOS patients (27.094±4.396) is significantly (p-value < 0.05) greater in comparison to control group (20.739±3.452). The association of

Table I: Comparison of mean Vitamin D level in 50 controls and 100 PCOS patients

	Group	N	Mean	Std. Deviation	P-Value
Vitamin D level (ng/ml)	Control	50	11.846	7.898	0.324
	PCOS	100	10.618	5.296	

Table II: Association of BMI with vitamin D status in 100 PCOS patients

Body Mass Index	Vitamin D status			Total	P-value
	Severe Vit D deficiency	Vit D deficiency	Vit D Insufficiency		
Normal weight	13	5	2	20	0.322
Over weight	10	5	2	17	
Obese	33	28	2	63	
Total	56	38	6	100	

BMI and vitamin D status in PCOS patients is found insignificant (p-value > 0.05). There is no significant relationship between body mass index and vitamin D level in patients of PCOS as elaborated in table II. The distribution of clinical symptoms presented by the PCOS patients show that majority of PCOS subjects has oligomenorrhea (59%), hirsutism (51%), polymenorrhea and irregular menstruation (9%), acne (8%), alopecia (7%) and amenorrhea (5%) but study does not find any significant association between severity of hypovitaminosis D with clinical symptoms of hyperandrogenism like hirsutism (P-value>0.05), acne (P-value>0.05) and alopecia (P-value>0.05).

Discussion

PCOS is one of the most common female endocrine disorder with the prevalence of 5-10% in females of reproductive age causing infertility, metabolic and psychological disturbances leading decreased quality of life.[13] Vitamin D deficiency has been shown to be associated with the development of PCOS through the gene transcription.[14]

According to the result of our study the mean value of vitamin D is bit higher in controls (11.846+7.898 ng/ml) as compared to PCOS patients (10.618+5.296 ng/ml) but the difference is not statistically significant. So our study does not find any association between serum vitamin D and PCOS. Our findings are similar to the findings observed by Kim et al. who found no differences in the absolute level of serum vitamin D between PCOS patients (19.6+6.6 ng/ml) and matched controls (20.1+7.4 ng/ml) and p= 0.696. Panidis et al. also reported that vitamin D levels were similar in women with and without PCOS.[14] These results are also in line with our study. Wehr et al. reported lower serum vitamin D level in women with PCOS (n=545) compared to control (n=145) (25.7 vs 32.0 ng/ml, respectively).[15] Observational study conducted in Italy reported lower serum vitamin D level in 90 PCOS women (32.4nmol/l=10.18ng/ml) than in 40 controls(73.7nmol/l=23.17ng/ml).[11] The study carried out by Mahmoudi et al. even found a significantly higher serum vitamin D level in PCOS women (29.3ng/ml) than in controls (19.4ng/ml) with similar age and BMI.[14] Although there is discrepancy in the literature about the vitamin D levels between women with and without PCOS but it is clear that vitamin D deficiency is a common finding among controls and PCOS patients. Many studies have been carried out to investigate the association between vitamin D status and BMI. Our findings reveal that the mean BMI in PCOS patients (27.094+4.369) is significantly (p-value < 0.05) greater in comparison to control group (20.739+3.452). This result is in the line with the results of the other studies.[5,16,17] Our results shows insignificant association between BMI and vitamin D status among PCOS patients demonstrating that 33 obese and 13 normal weight PCOS patients are severe vitamin D deficient. The descriptive cross sectional study conducted by Faraj et al in 2014 also found no significant association between vitamin D and BMI.[18] Another study conducted in Iran by Firouzabadi et al. on 100 infertile PCOS women also found no significant association between BMI and serum vitamin D level before and after the treatment with calcium and vitamin D supplements.[19] Although

Wehr et al. found the strong correlation between BMI and vitamin D concentration in women with PCOS in 2009.[20,21] Although hypovitaminosis D is common in PCOS but we don't not find significant association between severity of hypovitaminosis D and clinical symptoms of hyperandrogenism like hirsutism (P=0.669), acne (P=0.480) and alopecia (P=0.317). The results of our study are in agreement with the result of study conducted in young females (16-20 years old) in 2014.[22] One small uncontrolled study (n=13) has shown the improvement of acne vulgaris in the affected females when treated with vitamin D supplements but study found no improvements in hirsutism and alopecia.[14] Our result are in contrast with the finding of Wehr et al who found negative correlation between hirsutism and vitamin D level.[20] It seems that this difference may be due to the age and presence of metabolic features in PCOS patients. Further work is needed in this field because studies show that 60% to 80% females with PCOS present with hyperandrogenism and hirsutism is the most common feature presented by 70% of PCOS patients.[17] A population based cohort analysis study in 2014 alarmed that PCOS or hirsutism alone cause persistently high depression and anxiety in women.[23]

The limitations in our study were small sample size, the lack of adjustments for confounders that may play a role in causing vitamin D deficiency and secondly the metabolic features of PCOS were not evaluated for comparison with vitamin D level.

To the best of our knowledge we are the first investigating the relationship between vitamin D deficiency and PCOS in Pakistan. To prove our findings new therapeutic approaches and large intervention trials are required.

Conclusion

Vitamin D deficiency is becoming a major public health problem worldwide in all age groups. Studies from Africa, Australia, Brazil, Middle East, Mongolia and New Zealand also indicate a high risk for vitamin D deficiency in both adults and children. On the basis of this findings vitamin D deficiency or insufficiency is present in 1 billion people around the world.

This study found no difference in the vitamin D level among PCOS and control subjects. Hypovitaminosis is common in both PCOS and healthy females suggesting that vitamin D has no role in the pathogenesis of PCOS. Our results are in agreement with the previous data supporting an association of BMI with PCOS but don't support the significant association of BMI with severity of hypovitaminosis D.

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