Original Article

Effect of Vitamin D Supplementation on Glucose Metabolism in Polycystic Ovary Syndrome

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Abstract

Objective: To study the role of vitamin D supplementation on glucose metabolism in polycystic ovary syndrome.
Study Design: Prospective, quasi experimental study design.
Place and Duration of study: Railway General Hospital, Rawalpindi from December 2014 to December 2015.
Methodology: From all participants of the study the standard anthropometric measurements including weight, height, and hip and waist circumference were recorded. Basal blood samples for hormonal and metabolic determinants were taken in the morning after overnight fasting at 2nd to 5th day. Among all the participants of the study, a fasting 75g oral glucose tolerance test was carried out. After that the samples of blood for insulin, glucose and C-peptide were taken at 30, 60, and 120 minutes.
Results: 108 patients met the eligibility criteria of PCOS and received the treatment. The findings of this study showed that weekly supplementation of 20,000 IU vitamin D3 orally administered results in a significant improvement of glucose metabolism and menstrual pattern in a relatively large group of PCOS women. Moreover, triglyceride levels significantly decreased but total cholesterol and LDL cholesterol increased after vitamin D treatment.
Conclusion: Vitamin D treatment can be helpful in improving glucose metabolism and menstrual irregularities observed in PCOS patients.

Keywords: Glucose metabolism; Polycystic ovary syndrome; Supplementation; Vitamin D

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Introduction

Polycystic ovarian syndrome (PCOS) also known as Stein-Leventhal Syndrome was first described in 1935. It is one of the most widespread endocrine disorder affecting females of reproductive age group having an estimated occurrence of 12-21%.[1] It usually presents with obesity, hyperandrogenism, anovulation, hirsutism and infertility.[2] PCOS is also associated with an increased incidence of impairment in glucose tolerance, atherosclerosis, type 2 diabetes and dyslipidemia.[3] Despite various researches conducted, the underlying mechanism of PCOS and its etiology is still unknown, however, there is substantial evidence present which suggests that along with the genetic element to the syndrome, the insulin has an essential pathologic role in it.

Calcium and vitamin D are required in the maturation of oocyte. Women having polycystic ovary syndrome are frequently suffered from metabolic instability including vitamin D metabolism related to insulin resistance. It is evident from previous studies that in PCOS, the

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deficiency of vitamin D might be concerned with the insulin resistance pathogenesis and metabolic syndrome.[4,5] One plausible explanation involves the fact that the active vitamin D receptor (VDR) complex is responsible for regulating more than 300 genes, which also includes genes essential for the metabolism of lipid, glucose and regulation of blood pressure. Gene transcription is regulated by vitamin D through nuclear vitamin D receptors (VDR) which are dispersed across different tissues like skeleton, ovaries and parathyroid glands.[6] The PCOS pathogenesis is connected to the effect of VDRs (BsmI, ApaI, FokI, TaqI and Cdx2 polymorphisms) on levels of SHBG and LH,[7] level of testosterone,[9] insulin resistance and levels of serum insulin.[8,9]

Among south Asian, especially Pakistani women, the occurrence of PCOS is much higher (52%) compared to 20 - 25% among women of UK. There is a deficiency of local research which can explore the prevailing factors responsible for this disease. The high incidence has been associated with environmental or genetic factors and intermarriages.[10] The beneficial effect of vitamin D supplementation upon insulin resistance has been revealed by two small intervention studies. It is also evident that in menstrual dysfunction, there is an advantageous role of vitamin D supplements; however, the effects of vitamin D supplements on pregnancy are not clear from literature. These studies propose that Vitamin D supplements can be helpful in the management of PCOS; however, this area of study needs further investigation. Taking into consideration the high prevalence in our local population, we felt prompted to conduct this trial as no such trial has been previously reported from Pakistan.

This study aimed to study the role of vitamin D supplementation on glucose metabolism in polycystic ovary syndrome.

Methodology

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This study aimed to study the role of vitamin D supplementation on glucose metabolism in polycystic ovary syndrome.

This prospective, quasi experimental study was conducted at Railway General Hospital, Rawalpindi from December 2014 to December 2015. The study was approved by the Institutional Review Board (IRB). Initially 150 patients were recruited for the study out of

which 108 patients agreed to participate potentially, fulfilled the criteria and were selected as the subjects of the study. Patients with overt diabetes, thyroid disease pituitary disorders or hyperparathyroidism were excluded from the study. 108 females having PCOS diagnosis according to the Rotterdam Criteria[11] were selected. Out of the following conditions: oligo- and/or anovulation, biochemical and/or clinical signs of hyperandrogenism, polycystic ovaries, two of them were suggested to confirm the PCOS diagnosis. The oligo- or anovulation was confirmed with the existence of amenorrhea or oligomenorrhea. Hyperandrogenism was confirmed by the existence of hirsutism (Ferriman Gallwey-Score ≥6), alopecia or acne and/or increased levels of androgens. The morphology of polycystic ovaries was scanned by ultrasound. In each ovary the occurrence of ≥12 follicles (2-9 mm in diameter) defined the existence of polycystic ovaries.

During the course of this study, all women continued their routine life style, sunlight exposure, dietary intake and physical activity. From all participants of the study the standard anthropometric measurements including weight, height, and hip and waist circumference were recorded. Basal blood samples for hormonal [free testosterone, cortisol, PTH, total testosterone, free (fT3), TSH, fT4, SHBG, 17αOH-progesterone] and metabolic (total cholesterol, insulin, glucose, HDL cholesterol, C-peptide, triglycerides, LDL-cholesterol) determinants were taken in morning after overnight fasting at 2nd to 5th day in women having menstrual bleeding, while in amenorrheic women it was measured randomly. Among all the participants of the study, a fasting 75g oral glucose tolerance test was carried out. After that the samples of blood for insulin, glucose and C-peptide were taken at 30, 60, and 120 minutes. The insulin resistance was measured by using the homeostatic model assessment-insulin resistance (HOMA-IR). HOMA-IR was measured as the multiplication of the value of fasting plasma insulin (μU/ml) and the value of fasting plasma glucose (mg/dl), and then was divided by 405.[12] All women received 20,000 IU orally administered cholecalciferol each week for 12 weeks (equivalent to 2857 IU/day). All anthropometric measurements and laboratory investigations explained for the pretreatment evaluations were repeated after week 12. The presence or absence of menstrual bleeding of all respondents was recorded on daily basis in a menstrual cycle diary which was distributed among them at the time medication was given.

Statistical Methods:

Baseline characteristics are presented as Means ± SD for continuous variables. Paired student's t-test was used to compare means in continuous variables at baseline to mean values at 12 weeks. Statistical analyses were performed by SPSS 17.0 and a p-value <0.05 was considered statistically significant.

Results

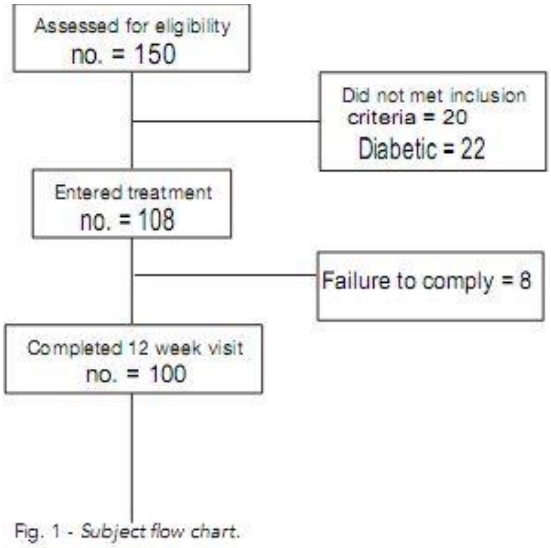

Fig. 1 - *Subject flow chart.*

Baseline Characteristics

150 PCOS patients were screened out of which 108 met the eligibility criteria and received the treatment. The study protocol involved measuring baseline parameters and repeating them after 12 weeks of weekly Vitamin D therapy. 100 out of 108 patients completed the study till 12-weeks follow-up. The rest failed to comply. Table I depicts the common clinical findings found in our cohort.

Table I: Frequency of clinical findings found among PCOS women

Clinical Findings	Percentage (%)
Obesity	45
Infertility	68
Deranged Androgen levels	22
Hypothyroidism	8
Deranged Prolactin levels	20
Vitamin D deficiency (< 30 ng/ml)	82

Changes with drug treatment

At baseline 82% of our study subjects had 25(OH)D

levels below 20 ng/ml, which indicates an insufficient vitamin D status. We observed a significant increase of 25(OH)D levels from 29±12 ng/ml to 53.3±17.9 ng/ml after 12 weeks of weekly oral administration of 20,000 IU cholecalciferol (p<0.001). PTH levels significantly decreased and 1,25-dihydroxyvitamin D levels increased after 12 weeks of vitamin D treatment. We observed no significant changes in BMI, waist circumference, hip circumference and blood pressure after 12 weeks of vitamin D treatment. No clinically significant adverse event was reported during the study. (Table II).

Metabolic Changes

We observed no significant changes in Fasting and stimulated glucose levels after 12 weeks of vitamin D treatment, however, C peptide levels showed a significant decrease at the end of 12 weeks. Moreover, we observed a significant decrease in triglyceride levels, whereas total cholesterol and LDL cholesterol levels significantly increased after vitamin D treatment. (Table II).

Endocrine Parameters

Endocrine parameters including testosterone, SHBG and free testosterone remained unchanged after 12 weeks of vitamin D treatment. We observed a significant reduction of estradiol levels after 12 weeks of vitamin D treatment however FAI after 12 weeks showed a statistically insignificant reduction (p value = 0.09) (Table II).

Menstrual regularity

At baseline, 13 out of 108 women (12.0%) reported regular menses whereas 95 out of 108 women (88%) had oligo- or amenorrhea. After 12 weeks of vitamin D treatment, out of 95 PCOS women who previously had menstrual disturbances 38 (40.0%) reported normalization or improvement in the regularity of menses. (Table II).

Discussion

This 12-week trial showed that weekly supplementation of 20,000 IU vitamin D3 orally administered results in a significant improvement of glucose metabolism and menstrual pattern in a relatively large group of PCOS women. Moreover, triglyceride levels significantly decreased but total cholesterol and LDL cholesterol increased after vitamin D treatment. To begin with, 82% of our study population had vitamin D deficiency as compared to 61.5% as reported by Wehr et al[13] which is a considerable difference suggesting widespread Vitamin D insufficiency in our population. In line with epidemiological findings, first experimental studies show that vitamin D supplementation may reduce various chronic diseases and mortality.[14-16] Moreover, there is evidence suggesting that the deficiency of vitamin D has influence on insulin response and postprandial glycemia, and these processes can be optimized by supplementation. A recent study by Wehr et al[13] established a significant association between low level 25(OH)D and high level of stimulated glucose and fasting, HOMA-IR, AUC gluc, stimulated insulin and fasting. Likewise, the study conducted by Hahn et al. revealed that there is an association between low level 25(OH)D and insulin resistance among PCOS women.[17] Contrary to these findings, an experimental study with administration of alfacalcidol revealed useful effect of vitamin D upon first phase secretion of serum lipids and insulin among PCOS women.[18] Another small study comprising of 11 obese insulin resistant PCOS women showed significant decrease of HOMA-IR upon vitamin D supplementation,)[19] whereas our data indicates a significant decrease in glucose and C-peptide levels without a change in HOMA-IR. However, our study participants had a lower baseline BMI and HOMA-IR to begin with.

The role of vitamin D supplementation in lipid metabolism is ambiguous. We observed an improvement of triglyceride levels, similar to the findings of a pilot study which showed that after treatment with vitamin D, the improvements of triglycerides and HDL was observed among women having PCOS.[18] These results might be accredited to the decreasing effect of vitamin D on the concentration of serum PTH. As the elevation in the concentration of PTH is accompanied by a reduction in post-heparin lipolytic activity of plasma [20], a decrease in serum PTH in the current study may have reduced serum triglycerides through increased peripheral removal. Contrary to this, total and LDL cholesterol levels decreased, which has also been reported during a study investigating the effect of vitamin D during weight loss.[16] However, data from the Women's Health Initiative shows that Vitamin D supplementation does not affect lipid profiles when studied over 5 years.[21] Besides, vitamin D has a significant role in reproductive processes, as indicated by improvement of menstrual pattern in about 40% of women having PCOS. This is in line with the results of a study conducted by Thys-Jacobs et al, which reported that menstrual cycle can be normalized in

Table II: Endocrine parameters, anthropometric parameters, oral glucose tolerance test, lipids and menstrual regularity at baseline vs. 12 weeks

	Baseline (n = 108)	12 Weeks (n = 100)	Baseline vs. 12 weeks
	Mean ± SD	Mean ± SD	p-value
Endocrine Parameters			
25(OH)D (ng/ml)	29.0 ± 12	53.3 ± 17.9	<0.001
1,25-Vit D (pmol/l)	123 ± 40.3	140.0 ± 50.6	<0.001
PTH (pg/ml)	39.9 ± 42.3	31.1 ± 12.7	<0.001
Free testosterone (pg/ml)	2.83 ± 0.0	2.69 ± 0.89	0.47
SHBG (nmol/l)	48.6 ± 33.2	53.7 ± 44.42	0.30
Testosterone (ng/l)	0.64 ± 0.22	0.62 ± 0.21	0.15
FAI	6.5 ± 4.3	5.8 ± 3.4	0.09
Estradiol (pg/ml)	63.7 ± 52.9	49.6 ± 30.3	0.02
Calcium (mmol/l)	2.36 ± 0.07	2.34 ± 0.06	0.12
Anthropometric Parameters			
Weight (kg)	71 ± 17	69 ± 19	0.79
BMI (kg/m^2)	24.4 ± 6.6	26.7 ± 6.6	0.69
WC (cm)	82 ± 16	83 ± 15	0.34
HC (cm)	106 ± 12	107 ± 11	0.60
WHR	0.78 ± 0.09	0.77 ± 0.07	0.74
BPsys (mmHg)	125 ± 20	120 ± 23	0.48
BPdias (mmHg)	85 ± 15	82 ± 17	0.95
Oral Glucose Tolerance Test			
Fasting glucose (mg/dl)	87 ± 7	88 ± 7	0.73
Glucose 30 min (mg/dl)	137 ± 25	149 ± 21	0.48
Glucose 1h (mg/dl)	130 ± 35	13 ± 35	0.18
Glucose 2h (mg/dl)	105 ± 26	99 ± 24	0.13
AUCgluc	114.8 ± 17.4	112.3 ± 17.5	0.47
Fasting insulin (µU/ml)	7.2 ± 6.5	7.15 ± 5.72	0.82
Insulin 30 min (µU/ml)	64.7 ± 55.4	55.5 ± 45.02	0.18
Insulin 1h (µU/ml)	67.3 ± 49	67.51 ± 48.19	0.9
Insulin 2h (µU/ml)	46.6 ± 39.3	47.64 ± 45.52	0.76
HOMA-IR	1.58 ± 1.49	1.59 ± 1.36	0.61
HOMA-β	101.0 ± 77.5	103.0 ± 73.8	0.69
C-Peptide fasting (ng/ml)	5.5 ± 3	3.78 ± 2.84	<0.001
C-Peptide 30 min (ng/ml)	12.9 ± 7.3	9.96 ± 4.78	<0.001
C-Peptide 1h (ng/ml)	13.8 ± 3.6	11.12 ± 3.5	<0.001
C-Peptide 2h (ng/ml)	13 ± 3.5	9.09 ± 3.91	<0.001
Lipids			
Cholestrol(mg/dl)	172 ± 20	18 ± 37	0.01
HDL (mg/dl)	70 ± 19	68 ± 21	0.82
LDL (mg/dl)	99 ± 27	111 ± 25	0.01
Triglycerides (mg/dl)	91 ± 53	83 ± 19	0.21
Menstrual Regularity			
Regular menses	13/108 (11.5%)	38/95 (40.0%)	nd

women with PCOS with vitamin D supplementation.[22]

There has been a significant association reported between Vitamin D deficiency and impaired insulin secretion and glucose tolerance [23,24], and IR and compensatory hyperinsulinemia were a constant finding in women with PCOS. According to previous literature, nearly 30% of the PCOS women had adequate levels of vitamin D.[17] It has been reported that the stimulation of insulin secretion, different effects on insulin sensitivity on receptor levels and suppression of PTH are some possible effects of vitamin D on metabolism of glucose. The underlying mechanism of this metabolism is not clear yet and it possibly involves both indirect and direct effects of vitamin D. The occurrence of vitamin D binding globulins (VDBG) and VDR in pancreatic islets can explain the possible role of vitamin

D in insulin resistance and its association with type II diabetes. Our study findings depicted a significant decline in C peptide levels however, HOMA IR and HOMA-beta didn't showed statistically significant decline.In addition, our study also showed significant decline in estradiol levels after 12 weeks of vitamin D treatment, confirming data from a study including 101 young volunteer women taking vitamin D supplements.[25] Our study subjects also showed insignificant changes in SHBG, free and total testosterone levels which is in line with the findings of Selimoglu et al.[19] Though FAI showed a decline, that too was statistically insignificant (p value = 0.09)

Conclusion

Vitamin D treatment can be helpful in improving glucose metabolism and menstrual irregularities observed in PCOS patients. Further large intervention trials are needed to confirm our findings and to evaluate the role of vitamin D in glucose metabolism and female reproduction in PCOS women.

Study Limitations: *The main limitation in this study was the lack of a placebo group and so the results should not be generalized. Moreover, 25(OH)D levels were high to begin with which might attenuate the effects of vitamin D replacement. Further, we did not perform structured interviews regarding food intake and earlier fat intake in a 24-h time might have influenced lipid parameters and might explain the relatively great variation of triglycerides at baseline.*

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