

Title page

Title:

EFFECT OF VITAMIN D SUPPLEMENTATION ON INSULIN SENSITIVITY AND ANDROGEN LEVEL IN VITAMIN D DEFICIENT PCOS PATIENTS

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Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/ have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

ABSTRACT

Objectives: There is limited evidence that giving vitamin D supplementation had beneficial effect on insulin resistance and dysfunction of menstrual cycle in females with PCOS. Thus, the aim of present study is evaluate the effect of vitamin D supplementation on insulin sensitivity and androgen level in Iraqi females with PCOS.

Material And Method: A randomized blinded clinical trial design , studied 60 Iraqi females with PCOS referring to the women' s counseling , outpatients at Maternity and Pediatrics teaching hospital in AL-Qadisiyah city , Iraq and private clinic . Non-probabilistic sampling involved women aged from 18 to 45year established on inclusion criteria. the patient's basic data have been recorded. Then we measured vitamin D ,testosterone level and impaired glucose tolerance test to all females. After the diagnosis of vitamin d deficiency has been done vitamin d was administering at 50000 once weekly orally for 8weeks. All data were re-measured after two months.

Result : Sixty patients with poly cystic syndrome enrolled in study, age range from 18-39 years, mean age 27.48 ± 5.95 years. With metabolic parameter at start of study ,Vitamin D level 16.1 ± 5.6 ,impaired glucose test reading 8.8 ± 0.7 and testosterone level 4.5 ± 0.64 . after 2 month of supplementation there were 83.7% of patients reach the normal level of vitamin D and 16.3% still had low level of vitamin D ($p \leq 0.002$) between two groups. In regarded to impaired glucose tolerance test after 2 month of supplementation, there were 51.6% of patients still had impaired test while 48.4% reach the normal reading ($p \leq 0.001$).

Conclusions: Women with PCOS have statically significant low level of vitamin D in mean ,impaired glucose tolerance test in mean and high level of testosterone. and there were Inverse Correlations between vitamin D with IGTT and testosterone.

Key Word: PCOS, Vitamin D, IGTT

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common ovarian disorder associated with disturbances of reproductive , hyperinsulinemia and androgen excess in women [1] . Definition and diagnosis of PCOS is based on criteria including clinical evidence of hyper androgenism ,ovarian dysfunction such as oligo-ovulation, and the exclusion of other causes of hyperandrogenism such as adrenal hyperplasia, hyperprolactinemia, and thyroid disorders [2]. PCOS is associated with insulin resistance, hypertension, central lipidemia and central venous dysfunction, all of which are risk factors for metabolic syndrome, type 2 diabetes, and coronary artery disease [3].

Metabolic disturbances are common in PCOS women: 30–40% have glucose tolerance disorder, 60–80% are resistant to insulin, and 10% have type 2 diabetes in their thirties or forties. Evidence suggests the pivotal role of insulin resistance in PCOS pathogenicity [4]. Decrease level of vitamin D is common in women with PCOS [5]. Vitamin D Data from more than 1,000 participants: "Vitamin D deficiency in PCOS women was associated with a reduced likelihood of these women becoming pregnant and delivering babies, regardless of BMI, race, age, markers of metabolic functioning, or fertility treatment"[6]. Vitamin D has been associated with reducing androgen levels in women with PCOS. A review of six clinical trials with 183 women with PCOS revealed that vitamin D supplementation significantly reduced total testosterone levels [7].

MATERIALS AND METHODS:

Study Design: After having permission from Ethics Committee of AL – Qadisiyah university of medical sciences , this study was conducted as a randomized blinded clinical trial design from March 2018 TO July 2018 we studied 60 Iraqi females with polycystic ovary syndrome referring to the women' s counseling, outpatients at Maternity and Pediatrics teaching hospital in AL- Qadisiyah city ,Iraq. In our study, the aim of the project was explained to all females , and if they agreed, informed consent was obtained .

Inclusion Criteria

1. the age of women range from 18 to45 years.
2. serum Vitamin D below 30 ng/ml .
- 3.women should not being pregnant or lactating.
- 4.Rotterdam criteria for PCOS diagnosis have been use [2] so patient should present with two out of the following three manifestation :hyperandrogenism with a clinical or laboratory diagnosis , ovulatory dysfunction and polycystic ovary.[8]
5. all women which included in this study have testosterone level between 3.5and 5nmol/L (normal level of testosterone in females between 0.5 to 3.5 nmol/L)
6. screening for impaired glucose tolerance test , women with two hour plasma glucose level of 140 t0 199mg /dl (7.8 to 11.0 mmol/L) was involved in this study .

Exclusion Criteria:* drugs which affect metabolic parameters such as metformin, Corticosteroid three months prior to the experiment, calcium and multivitamin six months before the study were not be used.

*women who suffer from a diseases which are chronic for example chronic kidney disease, liver cirrhosis, pancreatitis, nephrotic syndrome , tumors and Diabetes mellitus, Cushing's syndrome ,hyperprolactenemia ,congenital adrenal hyperplasia(adult type) , and androgen secretion tumors being excluded from this study .

Measurement Methods

Calculation of Body mass index(BMI):By measuring body weight in kilograms using a digital scale and dividing it in to squared height in meter (Kg/m²).Quantitative test of total 25 (OH)D₂/D₃ level in human serum /plasma was measured by ICHROMA DEVICE using immunofluorescence method using vitamin D kit. Testosterone level was measured by using Fluorescence immunoassay method (FIA).IGTT is done to all females included in this study and women who had blood glucose level between 7.8 to 11.0 mmol/L was included in our study .

Clinical Assessment :include determination of hirsutism using modified Ferriman-Galley scoring system (mfG)**[9]**. It should be noted that all trails were conducted at single private laboratories to increase reliability and minimize the variance between laboratories .after the diagnosis of vitamin d deficiency has been done vitamin d was administering at 50000 unit orally once weekly for 8weeks for each women in this study.All data were also re-measured two months after the start of the treatment.

RESULT

Sixty patients with poly cystic syndrome enrolled in this study age range from 18-39 years, mean age 27.48±5.95 years as in table 1. In beginning of study level of vitamin D was 16.11 ±5.6, after 2 month became 35.9 ±4.3 which is significant difference (p value = 0.002) as in table 2.

Table 1: age distribution.

Age (Mean ±Std. Deviation)	27.48 ±5.95
Minimum	18
Maximum	39

Table 2: level of serum vitamin D through the study.

	No.	Vitamin D	p-value
At baseline	60	16.11 ±5.6	0.002
After 2 months	50	35.9 ±4.3	

In regarded to impaired glucose tolerance test after 2 month of supplementation there were 51.6% of patients still had impaired test while 48.4% reach the normal reading (p value = 0.001), as in table 3. On another hand 75% of patients reach the normal level of testosterone and 25% had abnormal level (p value = 0.001), as in table 4 .

Table 3: show IGTT after 2 months.

		No. (%)	Mean \pm SD	p-value
IGTT after 2 months	Impaired	31 (51.6)	8.2\pm0.4	0.001
	Normal	29 (48.4)	6.4\pm 0.8	
	Total	60		

Table 4: show testosterone level after 2 months.

		No. (%)	Mean \pm SD	p-value
Testosterone after 2 months	Normal	45 (75)	2.2\pm0.4	0.001
	Abnormal	15 (25)	4.02\pm0.8	
	Total	60		

In table 5 show Inverse Correlations between vitamin D with IGT and testosterone, which mean an increase in vitamin D level after supplementation lead to decrease in serum level of testosterone (p value =0.02) and also decrease in reading of impaired tolerance test(p value = 0.04).

Table 5: show correlation between vitamin D and IGT and testosterone level.

	r	p-value
IGTT	-0.39	0.02
Testosterone	-0.15	0.04

DISCUSSION

Our result indicated women with PCOS have significant low level of vitamin D in mean 16.11 ± 5.6 , IGTT in mean 8.8 ± 0.8 , and high level of testosterone 4.5 ± 0.6 . [10,11] These indicate that low vitamin D levels are associated with insulin resistance in women with PCOS [12]. Our data suggest a relationship of vitamin D and BMI in PCOS women, which is in agreement with many studies. [13,14,15]. Other studies suggest that low vitamin D levels are related to impaired glucose clearance, insulin secretion, and insulin resistance [16,17].

The IGTT level at baseline was 8.8 ± 0.9 and after treatment, 48.4% became normal reading (6.4 ± 0.8), t-test showed a significant difference between the level of IGTT before and after treatment ($P < 0.05$).

On the other hand, the level of testosterone were 4.5 ± 0.6 before treatment, while 75% after treatment decrease to normal level (2.2 ± 0.4) ($P < 0.05$). a significant fall in serum testosterone was observed in 2 months in comparison with the baseline in the same group. A similar result has also been described in a study by Pal et al., [18]. Several factors may be able to explain the conflicting results, including the

different characteristics of the research subjects, the length of study and the various vitamin D forms used for supplementation[19,20,21].

CONCLUSION : Women with PCOS have statically significant low level of vitamin D in mean ,impaired glucose tolerance test in mean and high level of testosterone. After vitamin D supplementation for 2 month 42 out of 54 PCOS women previously affected by menstrual disturbances reported improvement of menstrual frequency. Marked reduce in the level of IGTT after treatment with vitamin D. Furthermore, there is an improvement in symptom of hyperandrogenism.

Conflicts Of Interest: There are no conflicts of interest.

Author Contribution:

***Saba M. Swadi AL-Thuwaynee:** contributing to the conception, study design, and data interpretation.

**** Amaal Raad Ahmed:** contributing to sample collection, writing the manuscript, and statistical analysis.

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