CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

The effect of correction of serum level of vitamin D on hyperandrogenism in women with polycystic ovary syndrome and hypovitaminosis D

A. M. Maysara[1], A. T. Nassar[2], H. K. Jubran[3]

[1]Department of Obstetrics & Gynecology, College of Medicine- University of Basra (Iraq)
[2]AI Faiha Specialized Diabetes, Endocrine and Metabolic Centre (Iraq)
[3]Department of Clinical Pharmacy, College of Pharmacy, University of Basra (Iraq)

Summary

Background: Polycystic ovary syndrome (PCOS) is a common condition among female of reproductive age. Vitamin D may involve in the pathogenesis of PCOS. Vitamin D deficiency may exacerbate the symptom of PCOS through precipitating insulin resistance, menstrual irregularities, subfertility, hyperandrogenism, and cardiovascular dysfunction. *Aim:* The aim of the study is to determine whether normalization of vitamin D level in women with PCOS and hyperandrogenism (clinical and/or biochemical) would result in improvement in the parameters of hyperandrogenism. *Materials and Methods:* One hundred one women with PCOS and vitamin D de-ficiency were involved in the study, BMI, and Gallwey-Ferriman score were estimated, 25 (OH) vitamin D, total testosterone, sex hor-mone binding globulin (SHBG), follicular stimulating hormone (FSH), and luteinizing hormone (LH) were measured and pelvic ultrasound was performed. Forty-nine of these women received weight reduction and lifestyle modification advice and metformin only, while the remaining 52 women received vitamin D in addition, after six months all parameters were re-examined. *Results:* Adding vi-tamin D did not result in significant reduction in the Ferriman-Gallwey score or in total testosterone level, also normalization of vita-min D had insignificant effect on the level of sex hormone binding globulin and ovarian polycystic morphology, however it was associated with significant reduction in LH level. *Conclusion:* Despite the fact that correction of vitamin D deficiency is important for general health, however it is of limited beneficial effect on the biochemical and clinical parameters of hyperandrogenism in women with PCOS.

Key words: Vitamin D; Hyperandrogenism; Polycystic ovary syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is a common condition among female of reproductive age with prevalence of 6-15% depending on the criteria used [1]. It is characterized by menstrual irregularities due to anovulation, acne and hirsutism due to hyperandrogenism, subfertility, obesity and polycystic ovaries on ultrasound [2, 3]. Women with PCOS have increased risk of diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, and endometrial carcinoma [4, 5]. Insulin resistance plays an important role in the pathogenesis of PCOS [6].

Hirsutism is found in 70-80% of women with PCOS, versus 4-11% in women in the general population. Hirsutism in PCOS is due to both excess of androgen of ovarian origin and to increased sensitivity of the pilosebaceous unit to androgens [7]. Additional risks associated with increased plasma androgen besides hirsutism are coronary artery diseases, alopecia, infertility, acne, and central obesity [8].

Vitamin D deficiency may precipitate insulin resistance, menstrual irregularities, subfertility, hyperandrogenism, and cardiovascular dysfunction, therefore vitamin D deficiency may exacerbate the symptom of PCOS [9, 10]. Vitamin D expresses its action through both genetic and cellular pathways [11, 12]. Vitamin D receptor (VDR) can be found across several tissues within the female reproductive system. VDR mRNA is expressed in ovarian, decidual, placental, and endometrial cells [13]. Vitamin D may be involved in the pathogenesis of PCOS through its receptors' mediated effect on insulin resistance, LH, and sex hormone binding globulin (SHBG), and androgen levels [14, 15]. Also vitamin D may affect aromatase gene expression and thus follicular estrogen level [16].

The aim of the study is to determine whether normalization of vitamin D level in women with PCOS and hyperandrogenism (clinical and/or biochemical) would result in improvement in the parameters of hyperandrogenism.

Materials and Methods

This was a prospective randomized controlled study carried out at the Endocrine Centre of Al Faiha Hospital/Basra-Iraq throughout a period of 14 months from June 2017 to August 2018. The

Published: 15 April 2020

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVII, n. 2, 2020
doi: 10.31083/j.ceog.2020.02.5248

Table 1. — *General characteristic of the patients at the beginning of the study in both groups.*

	Group 1 (n=52)		Group 2 (n=49)		*p*
	Mean	± SD	Mean	± SD	
Age (years)	23.12	5.78	24.71	6.25	0.060
BMI (kg/m^2)	32.05	13.01	30.84	6.75	0.422
FGS	24.92	7.05	25.59	6.99	0.633
Serum Vit. D (ng/dl)	13.8	4.78	14.1	5.21	0.764
TT (ng/dl)	55.70	16.86	52.45	25.53	0.450
SHBG (nmol/L)	33.09	22.72	37.08	20.92	0.282
LH (IU/L)	12.07	5.52	11.80	8.14	0.846
FSH (IU/L)	5.25	2.37	4.89	2.39	0438
LH/FSH ratio	2.78	1.65	2.77	2.21	0.984

study was undertaken after achieving approval from the ethical committee of College of Medicine of Basra.

One hundred one women with PCOS and vitamin D deficiency with hirsutism as the main complaint were involved in the study. These patients were recruited randomly during their visit to the Endocrine Centre of Al Faiha Hospital. Verbal and informed consent was taken from all participants before being engaged in the study. Weight and height were recorded for each patient and BMI were calculated, and degree of hirsutism was estimated using modified Gallwey-Ferriman scoring system (score of ≤ 8 was regarded hirsutism).

Blood sample was taken from each women between the 1st and the 3rd day of menstrual cycle for 25 (OH) vitamin D, total testosterone, SHBG, follicular stimulating hormone (FSH) and luteinising hormone (LH). All participants were subjected to pelvic ultrasound to check for polycystic ovaries, PCOS diagnosed based on modified Rotterdam criteria (presence of two out of the following three criteria: clinical or biochemical hyperandrogenism, oligomenorrhea/anovulation, and polycystic ovaries on ultrasound) [17]. Vitamin D deficiency was defined as 25 (OH) vitamin D level less than 20 ng/ml.Patients with Cushing's syndrome, hypothyroidism, hyper-prolactinemia, and congenital adrenal hyperplasia were excluded from the study.

Patients were randomly subdivided into two groups: group 1 involved 52 women who received metformin 1,500 mg/day for three months, advised about weight reduction (in obese women) and vitamin D orally 300,000 IU loading dose followed by 50,000 IU (Vitamin D3) once a week for 12 weeks, after that serum level of 25(OH) vitamin D was reevaluated, those with normal level (more than 30 ng/ml) were kept on maintenance dose of 5,000 IU/day, however, in five women the level was 20-30 ng/ml (insufficient) and only in one woman the level remained less than 20 ng/ml (deficient), those six women continued on 50,000IU weekly for further six weeks followed by rechecking of serum level of 25 (OH) vitamin D to confirm normalization. The second group (group 2) involved 49 women who received only weight reduction and lifestyle modification advice and metformin 1,500 mg/day. Antiandrogens had not been used in both groups during the period of the study in order to avoid masking the effect of vitamin D. All women were re-examined six months after the initial assessment including clinical examination for degree of hirsutism, hormonal and vitamin D analysis; vitamin D was normal in all participants in the first group with average value of 52 ng/ml, and also pelvic ultrasound for the ovarian morphology was repeated.

Blood samples were collected in tubes without anticoagulants, centrifuged to obtain the serum. Serum FSH was measured using enzyme immunoassays using a FSH assay and serum LH was measured using enzyme immunoassays, and total testosterone were analyzed by elec-trochemiluminescence immunoassay. Serum level of 25(OH) vitamin D was measured by elec-trochemiluminescence immunoassay analyzers.

Table 2. — *Comparison of clinical and biochemical parameters of hyperandrogenism between both groups after treatment.*

	Group 1		Group 2		*p*
	Mean	±SD	Mean	±SD	
FGS	11.94	5.67	15.63	8.03	0.009
FGS reduction	12.98	6.17	10.69	5.40	0.083
TT	46.12	14.00	34.12	22.32	0.002
TT reduction	12.12	17.75	11.33	24.45	0.852
SHBG	56.13	33.92	52.48	39.42	0.618
SHBG elevation	23.04	20.72	14.68	35.78	0.151
LH	5.96	1.79	8.23	6.69	0.020
FSH	5.38	1.77	6.16	5.71	0.351
LH/FSH ratio	1.24	0.58	1.46	0.94	0.161
LH/FSH ratio reduction	1.54	1.53	1.32	2.24	0.555

A p value < 0.05 is considered significant.

Statistical analysis was performed using Medcalc V12. Independent *t*-test was used to discover the significance of difference between the means of demographic and hormonal parameters of the two groups of women before and after six months. Chi analysis and odd ratio were calculated to compare frequency and percentage of normalization of the studied hormones in both groups.

Results

Table 1 shows the general patient characteristics among the two main groups involved in this study. At the beginning of the study, no significant differences were found in the parameters that were studied between the two groups in terms of age, BMI, Ferriman-Gallwey score (FGS), total testosterone, SHBG, LH, FSH, and LH/FSH ratio.

Most of the involved patients were in their third decade of life; the mean age for women in group 1 was 23.12 ± 5.78 years, while for group 2 it was 24.71 ± 6.25 years. The majority of patients were obese, mean BMI for women in group 1 was 32.05 ± 13.01 *vs.* 30.84 ± 6.75 in group 2.

The mean hirsutism score for both groups was in the moderate range (24.92 ± 7.05, 26.59 ± 7 in groups 1 and 2, respectively).

Although SHBG mean levels were normal for the both groups without significant difference (33.09 ± 22.72 *vs.* 37.08 ± 20.92), the mean level of the total testosterone was elevated in both groups (55.70 ± 16.86 *vs.* 52.45 ± 25.53). The same observation can be seen regarding the LH to FSH ratio which was elevated by more than 2; 2.78 ± 1.65 *vs.* 2.77 ± 2.21.

Table 2 illustrates the effect of correction of serum level of vitamin D on the different parameters six months after the initial assessment. The mean FGS was significantly lower among patients in group 1 compared to those in

Table 3. — *Frequency and percentage of normalization of FGS, TT, LH/FSH ratio, and polycystic changes at the end of the study.*

		Group 1 n = 52		Group 2 n = 49		OR	95% CI		*p*
		n	%	n	%		Lower	Upper	
FGS	<= 8	12	23.1	9	18.4	1.33	0.51	3.51	0.369
TT	< 50	32	61.5	39	79.6	0.41	0.17	1.00	0.038
LH/FSH ratio	< 2	46	88.5	36	73.5	1.77	0.79	3.97	0.047
Polycystic changes	No	33	63.5	31	63.5	1.01	0.45	2.27	0.574

group 2, 11.94 ± 5.67 *vs.* 15.63 ± 8.03 ($p = 0.009$), nevertheless, the reduction of the scores from the baseline was not significantly different, although it was higher among patients in group 1: 12.98 ± 6.17 *vs.* 10.69 ± 5.40 ($p = 0.083$).

Although total testosterone was significantly higher in group 1 than in group 2: 46.12 ± 14.00 *vs.* 34.12 ± 22.32 ($p = 0.002$), however the reduction in TT from the baseline was not significantly different between both groups; 12.12 ± 17.75 *vs.* 11.33 ± 24.45 ($p = 0.852$).

Elevation in SHBG mean level was better in group 1, but this elevation did not reach statistical significance: 23.04 ± 20.72 *vs.* 14.68 ± 35.78 ($p = 0.151$). Likewise, there was no significant difference in SHBG levels between both groups.

Despite that the mean LH level was significantly lower in group 1 compared to group 2: 5.96 ± 1.79 *vs.* 8.23 ± 6.69 ($p = 0.020$), the mean LH/FSH ratio was not: 1.24 ± 0.58 *vs.* 1.46 ± 0.94 ($p = 0.161$). Also the reduction in the LH/FSH ratio from baseline was not significantly different.

Table 3 shows the rate of improvement in some clinical and biochemical features of PCOS at the end of the study. At the final clinical assessment, 23.1% of women in group 1 had normal Ferriman-Gallwey score of equal or less than 8 compared to 18.4% in group 2; however, there was no statistically significant difference between both groups.

The frequency of normalization of serum level of total testosterone among women in group 1 was significantly lower than that in group 2 (32 patients (61.5%) *vs.* 39 patients (79.6%), respectively, with a *p* value of 0.038).

LH/FSH ratio was less than 2 in 46 women (88.5%) compared to 36 patients (73.5%), respectively, with a *p* value of 0.047. There was no significant difference in the frequency of polycystic changes of the ovaries on ultrasound between both groups.

Discussion

In the present study the patients in both groups had convergent criteria as age and BMI which is important to avoid weight-related difference in results. Also patients in both groups had close TT, SHBG, FSH, LH, and FSH/LH ratio at the beginning of the study.

All women with PCOS and concomitant vitamin D deficiency recruited in this study were obese or overweight and this finding is in agreement with several studies [18, 19]. This can be explained by the fact that vitamin D is fat soluble vitamin so it can be readily sequestered in the larger pool of adipose tissue that obese individuals have; however, studies revealed that obesity did not decrease the cutaneous production of vitamin D_3, but may cause inefficient release of vitamin D_3 from the skin into the circulation; another cause for higher incidence of reduced vitamin D bioavailability in obese individuals could be due to higher rate of vitamin D sequestration in the larger pool of body fat after orally obtained vitamin D_2 had been absorbed into the lymphatic system and transferred into the bloodstream [20].

During collection of patients in the present study, the authors notice that the majority of women with PCOS had vitamin D deficiency, although they did not compare it to the level of vitamin D in women without PCOS; the possible association between vitamin D low level and PCOS could be due to the higher incidence of obesity among women with PCOS rather than true association with PCOS [9].

Ferriman-Gallwey scoring system for patients at the beginning of the study indicated moderate degree of hirsutism in both groups, after treatment the score came to the mild range (from 8-15) in both groups, with no significant difference between them, indicating that correction of vitamin D in women with PCOS did not add extra benefit in terms of correction of degree of hirsutism. Studies suggest that vitamin D may have a role in regulating the expression of aromatase enzyme gene, hence deficiency of vitamin D may contribute to the low levels of aromatase in follicles in women with PCOS females; aromatase enzyme is responsible for conversion of androgen into estrogen in granulosa cell as a result low level of vitamin D may have role in the manifestation of hyperandrogenism in PCOS, however, this thesis has been rejected by another study [21]. The present study agrees with the results of Hanif *et al.* study which revealed that hypovitaminosis D has insignificant association with clinical features of hyperandrogenism [22].

Women with PCOS usually have low levels of SHBG due to insulin resistance and obesity. This results in high level of bioavailable active free testosterone and hence a higher rate of hirsutism, therefore increased level of SHBG results in a lower level of active free testosterone and so improvement in the clinical manifestation of hyperandrogenism [23].

In the present study, adding vitamin D to the conventional treatment resulted in more elevation in the levels of

SHBG; even so this improvement did not reach statistical significance, and this can be explained by the fact that correction of vitamin D result in enhanced insulin sensitivity [24].

In the present study, correction of vitamin D deficiency resulted in a significantly higher rate of correction of LH/FSH ratio (less than 2) compared to women with uncorrected low vitamin D. Banaszewska *et al.* determined that although LH/FSH ration is of little diagnostic importance in PCOS, patients with LH/FSH > 2 and hyperinsulinemia may have increased adrenal androgenic activity and so worse symptom of hirsutism [25]. However in the present study this correction of the ratio did not improve symptoms of hirsutism, so it is of little clinical significance.

The ovarian phenotype in PCOS results from arrested maturation of the FSH sensitive follicle preventing them from reaching the dominant size, and this is usually due to an increased level of insulin and insulin-related growth factors due to insulin resistance; this stimulate theca cells to produce larger amount of androgen which in turn interfere with follicular growth [26]. As vitamin D results in enhanced insulin sensitivity and so lower level of insulin, therefore the trigger for the ovarian phenotype changes in PCOS is removed which is supposed to result in reversing ovarian morphology to normal, however in the present study this was not the case; normalization of vitamin D did not result in better correction of ovarian phenotype compared to women with uncorrected low vitamin D level, and it also did not result in normalization of testosterone more efficiently than women who did not received vitamin D, probably because vitamin D supplementation did not result in significant elevation of SHBG, and this is in agreement with that revealed from the studies of Selimoglu *et al.* and of Wehr *et al.* [10, 24].

Conclusion

Despite the fact that correction of vitamin D deficiency is important for general health, however it is of limited beneficial effect on the biochemical and clinical parameters of hyperandrogenism.

Acknowledgement

The authors would like to thank all the members of Al-Fayha Specialized Diabetes, Endocrine and Metabolic Centre for their great help to complete this study.

References

[1] Fauser B.C.J.M., Tarlatzis B.C., Rebar R.W., Legro R.S., Balen A.H., Lobo R., et al.: "Consensus on women's health aspects of polycystic ovary syndrome (PCOS): The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group" .*Fertil. Steril.*, 2012, *97*, 28.

[2] Shroff R., Syrop C.H., Davis W., Voorhis B.J., Dokras A.l.: "Risk of matabolic complications in the new PCOS phenotypes based on the Rotterdam criteria". *Fertil. Steril.*, 2007, *88*, 1389.

[3] Azziz R., Carmina E., Dewailly D., Diamanti-Kandarakis E., Escobar-Morreale H.F.: "The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report". *Fertil. Steril.*, 2009, *91*, 456.

[4] Wild R.A., Carmina E., Diamanti-Kandarakis E., Dokras A., Escobar-Morreale H.F., Futterweit W., et al.: "Assessment of Cardiovascular Risk and Prevention of Cardiovascular Disease in Women with the Polycystic Ovary Syndrome: A Consensus Statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society". *J. Clin. Endocrinol. Metab.*, 2010, *95*, 2038.

[5] Moran L.J., Norman R.J,Teede H.J.: "Metabolic risk in PCOS: phenotype and adiposity impact". *Trends Endocrinol. Metab.*, 2015, *26*, 136.

[6] Barber T.M., Dimitriadis G.K., Andreou A., Franks S.: "Polycystic ovary syndrome: insight into pathogenesis and a common association with insulin resistance". *J. Royal College of Physicians*, 2015, *15*, 72.

[7] Spritzer M., Barone R., De Oliveira B.: "Hirsutism in Polycystic Ovary Syndrome: Pathophysiology and Management". *Curr. Pharm. Des.*, 2016, *22*, 5603.

[8] Harish N., Kannan L., Ganesh, Balaji R.,Jeyraveena N.M.,Harshitha, *et al.*: "A study to find out the prevalence and associated risk factors of Hirsutism in polycystic ovarian syndrome and non-polycystic ovarian syndrome individuals". *Indian Journal of Applied Research,* 2017, *7*, 65-68.

[9] Hahn S., Haselhorst U., Tan S., Quadbeck B., Schmidt M., Roesler S., *et al*.: "Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome". *Exp. Clin. Endocrinol. Diabetes*, 2006, *l14*, 577.

[10] Wehr E., Pilz S., Schweighofer N., Giuliani A., Kopera D., Pieber T.R., Obermayer-Pietsch B.,*et al*.: "Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome". *Eur. J. Endocrinol*., 2009, *161*, 575.

[11] Mahmoudi T.: "Genetic variation in the vitamin D receptor and polycystic ovary syndrome risk". *Fertil. Steril.,*2009, *92*, 1381.

[12] Ramagopalan S.V., Heger A., Berlanga A.J., Maugeri N.J., Lincoln M.R., Burrell A., *et al.*: "Chip-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res.*, 2010, *20*, 1352.

[13] Lerchbaum E., Obermayer-Pietsch B.: "Vitamin D and fertility-a systematic review". *Eur. J. Endocrinol*., 2012, *166*, 765.

[14] Patra S.K., Nasrat H., Goswami B., Jain A.: "Vitamin D as a predictor of insulin resistance in polycystic overian syndrome". *Diabetes Metab Syndr.*, 2012, *6*, 146.

[15] Thomson R.L., Spedding S., Buckley J.D.: "Vitamin D in the aetiology and management of polycystic ovary syndrome". *Clin. Endocrinol.*, 2012, *77*, 343.

[16] Kinuta K., Tanaka H., Moriwake T., Aya K., Kato S., Seino Y.: "Vitamin D is an important factor in estrogen biosynthesis in both female and male gonads". *Endocrinol.*, 2000, *141*, 1317.

[17] Fr D.D., Tarlatzis R.: "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome". *Fertil. Steril.*, 2004, *81*, 19.

[18] Forrest K.Y.Z., Stuhldreher W.L.: "Prevalence and correlates of vitamin D deficiency in US adults". *Nutr. Res.*, 2011, *31*, 48.

[19] Pereira-Santos M., Costa P.R., Assis A.M., Santos C.A., Santos D.B.: "Obesity and vitamin D deficiency: systematic review and meta-analysis". *Obes. Rev.*, 2015, *16*, 341.

[20] Wortsman J., Matsuoka L.Y., Chen T.C., Lu Z., Holick M.F.: "Decreased bioavailability of vitamin D in obesity". *Am. J. Clin. Nutr.*, 2000, *72*, 690.

[21] Merhi Z., Doswell A., Krebs K., Cipolla M.: "Vitamin D Alters Genes Involved in Follicular Development and Steroidogenesis in Human Cumulus Granulosa Cells". *J. Clin. Endocrinol. Metabolism*, 2014, *99*, 1137.

[22] Hanif F., Ilyas N., Qamar T.: "Association of vitamin d deficiency with hyperandrogenism in females with polycystic ovarian syn-

drome". *Int. J. Clin. Biomed. Res.*, 2018, *4*, 31.
[23] Nestler J.E., Powers L.P., Matt D.W., Steingold K.A., Plymate S.R., Rittmaster R.S., et al.: "A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome". *J. Clin. Endocrinol. Metab.,* 1991*, 72*, 83.
[24] Selimoglu H., Duran C., Kiyici S., Ersoy C., Guclu M., Ozkaya G.,et al.: "The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome". *J. Endocrinol. Invest.*, 2010, *33*, 234.
[25] Banaszewska B., Spaczyński R.Z., Pelesz M., Pawelczyk L.: "Incidence of elevated LH/FSH ratio in polycystic ovary syndrome women with normo- and hyperinsulinemia". *Rocz. Akad. Med. Bialymst.*, 2003, *48*, 131.
[26] Broekmans F.J., Knauff E.A.H., Valkenburg O., Laven J.S., Eijkemans M.J., Fauser B.C.J.M.: "PCOS according to the Rotterdam consensus criteria: change in prevalence among WHO-II anovulation and association with metabolic factors". *BJOG*, 2006, *113*, 1210.

Corresponding Author:
M.M. ABDULRIDHA, CABOG, FICMS, MRCOG
Department of Obstetrics & Gynecology
College of Medicine, University of Basra (Iraq)
Iraq-Basra Province- Basra City -
College of Medicine, University of Basra
e-mail: Maysaram1979@gmail.com