Journal of Women's Health, Issues & Care

Research Article

A SCITECHNOL JOURNAL

Comparison of Vitamin D Status for Hirsutism, Biochemical Hyperandrogenism, Glycemia and Dyslipidemia among Subjects with Polycystic Ovarian Syndrome (PCOS)

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Abstract

Background: Data indicates that Vitamin-D (VD) insufficiency (low level of serum VD) has a close association with many metabolic disorders. Polycystic Ovary Syndrome (PCOS) is considered to be a metabolic disorder and VD administration has been attempted to ameliorate PCOS. VD insufficiency has been reported to be present in women with PCOS. However, coexisting conditions with PCOS (hyperandrogenism, insulin resistance, obesity, glucose intolerance, and dyslipidemia) have prevented to confirm this association.

Methods: We measured and compared serum VD level in patients with PCOS (n=169) *vs.* those without PCOS (n=164), who visited PNS HAFEEZ Hospital Islamabad between Jan 2018-July 2019. We also compared VD level in relation to the following: BMI (using General Linear Model (GLM)), glucose levels and lipid parameters for patients with *vs.* without PCOS. Free Androgen Index (FAI) and modified Ferriman Gallwey (mFG) scores were compared between VD groups using one-way ANOVA. GLM was used to compare the effects of VD status and presence or absence of PCOS on insulin resistance.

Results: Patients with PCOS, compared with those without it, showed lower VD levels regardless of BMI. Similarly, Lower level of Vitamin-D was observed in hyper-androgenism (Free Androgen Index (FAI)) and hirsutism (modified FG scores). Using univariate GLM with insulin resistance as dependent (confirmed) variable and PCOS and VD defined groups as independent factors, an increase in insulin resistance was observed with lower VD and the presence of PCOS.

Conclusion: Patients with PCOS had lower VD levels than those with non-PCOS.

Keywords

Vitamin D; Clinical hyperandrogenism; Free androgen index (FAI); Modified ferriman-gallway scoring (mFG score); Rotterdam PCOS criteria; Polycystic ovarian syndrome (PCOS)

Abbreviations

Polycystic Ovarian Syndrome (PCOS); Homeostasis Model Assessment for Insulin Resistance (HOMAIR); General Linear Model (GLM); Free Androgen Index (FAI); modified Ferrimen Gellwey (mFG) Score

Introduction

Recent data has shown Vitamin D to be a pivotal factor in mediating useful effects apart from bone health to extra-skeletal benefits like immunity, anti-cancer and related effects reducing the effects of dyslipidemia and glucose indices [1,2]. Metabolic diseases including hypertension, diabetes and Coronary Artery Disease (CAD), obesity and Polycystic Ovarian Syndrome (PCOS) have been associated with one the highest mortality and morbidity. Notwithstanding the disease-related agony the patient suffers in terms of PCOS, diabetes and Coronary Artery Disease (CAD), the entity is considered a major burden on the health economy of most developed countries [3]. In this regard literature, certain studies have highlighted specific benefits of Vitamin D for human and animal subjects with diabetes, metabolic syndrome and PCOS [4,5].

However, few controversies in the association between Vitamin D status and metabolic disease processes have also emerged in the literature, which have prompted us to replicate the findings in our study. First of the issue refuting the relationship between Vitamin D and insulin resistance and metabolic disorders do exist in literature. Poomthavorn et al. have found no association between Vitamin D deficiency and metabolic derangements in glucose metabolism [6]. Similarly, Jorde et al. have not demonstrated any beneficial effect in reducing dyslipidemia after Vitamin D supplementation [7]. Associated with the above contrasting evidence linking metabolic disorders with PCOS, there is data supporting an association between Vitamin D and metabolic syndrome, PCOS and coronary artery disease. Zhao et al. have demonstrated pregnancy outcomes and insulin resistance improves with Vitamin D treatment in subjects having PCOS [8]. Similarly, another study employing Vitamin D supplementation in subjects with PCOS was able to demonstrate beneficial effects on lipid parameters and insulin resistance [9]. Apart from the pathology related aspects affecting metabolic disorders in subjects with PCOS, studies have identified regional differences related to latitude, type of clothing used, sun-exposure practices, diets, and age-wise requirements all contribute to Vitamin D level variability [10]. Moreover, PCOS phenotypes have also been observed to be slightly different from other parts of the world. Ganie et al. have observed PCOS population from Delhi and Srinagar were able to identify two phenotypes as one with hyperandrogenism and the lean type and other as obese with high insulin level category [11]. So these categories may also suggest probable regional variability thus emphasizing the need for more regional data on the subject.

A study was therefore planned to evaluate the Vitamin D levels among PCOS and non PCOS along with measuring the effects of dyslipidemia, metabolic syndrome and diabetic biomarkers in relation to both PCOS and Vitamin D levels in our population.

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Received: September 28, 2019 **Accepted:** November 22, 2019 **Published:** November 26, 2019

Materials and Methods

The study was a cross-sectional study that was carried out among female subjects presenting at family outpatients department in liaison with the department of pathology and radiology of PNS HAFEZ hospital, Islamabad from Jan 2018 to July 2019. The study had the approval of hospital's ethical review board. The target population included female subjects in reproductive age group who presented to department at gynecology with menstrual issues or related complaints. Patient selection criteria were based upon non-probability convenience sampling. These female subjects with post-2 years menarche were requested to come in medical fasting on the second day +1 day (second day) of menstrual cycle start. Subjects who finally followed up for study were interviewed and informed about study details. Patients having treatment for hypertension or diabetes, having some autoimmune disorder, acute infectious or non-infectious condition, using any oral contraceptives or hormonal treatment, using were excluded in the study. Finally selected subjects were asked to sign on the "consent form". Following consent detailed history, anthropometric, vital signs measurements, and clinical examination were carried out. Clinical hyperandrogenism for hirsutism was measured by using modified Ferriman-Gallwey scores [12]. Oligo-anovulation was defined once menstrual cycle was longer than 35 days [13].

Lab and radiological analysis

We collected almost 10 ml of blood for various analysis including fasting plasma glucose, HbA1c, albumin, lipid parameters, total testosterone, Sex Hormone Binding Globulin (SHBG) and serum insulin. Patients were sent to radiology department for radiological examination for the diagnosis of ovaries. A diagnosis of "Polycystic Ovarian Syndrome (PCOS)" was made by the radiologist only as per the Rotterdam criteria where presence of 12 or more ovary with size ranging between 2-9 mm diameter either with or without finding ovarian volume of fewer than 10 ml [6].

Lab analysis and measurements

Fasting plasma glucose was analyzed by Selctra-ProM for measuring glucose by GPO-PAP method. HbA1c, total testosterone, SHBG Vitamin D were analyzed by Chemiluminescent Microparticle Immunoassay (CMIA) on ARCHITECT iSystem, by Abbot Diagnostics. Serum insulin was analyzed by the Chemiluminescence method on Immulite® 1000. Total cholesterol and triglycerides analysis were carried out by CHOD-PAP methodology and GPO-PAP method on Selectra-ProM (clinical chemistry analyzer). LDL and HDL-cholesterol were analyzed by direct enzymatic selective end-point methods using accelerator selective detergent method on Selectra-proM. Insulin resistance was calculated by Homeostasis Model Assessment of Insulin resistance (HOMAIR) [14]. Free Androgen Index (FAI) was calculated using following formula: FAI=(Total testosterone/SHBG) × 100. Biochemical hyperandrogenism was labeled once FAI was greater than 5% [15].

Outcome measures and criteria definitions

PCOS was evaluated by Rotterdam criteria [16]. Further clinical and biochemical characteristics included for evaluation criteria included oligo-anovulation, modified Ferriman-Gallway score (Clinical hyperandrogenism), ultrasound diagnosis of PCOS [13].

We lost a few patients who could not come for repeat testing due to various reasons including insufficient sample quantity, serum hemolysis related non-follow up the second visit.

Data analysis

All data were entered into SPSS IBM version-24. Descriptive statistics were calculated using analyze module of SPSS for age, no of PCOS as per Rotterdam criteria, and knowing their marriage status. Comparisons between age, anthropometric indices, glucose levels, and lipid parameters were done between subjects with or without PCOS using independent sample t-statistics. General Linear Model (GLM) was utilized to compare the differences between Vitamin-D levels across subjects with or without PCOS at keeping BMI as an independent variable. Comparisons of Vitamin-D based groups between Free Androgen Index (FAI) and modified Ferrimen Gallwey (mFG) was done by using one way ANOVA. The General Linear Model (GLM) was used to compare the effects of Vitamin-D status and presence or absence of PCOS on insulin resistance.

Results

The mean age of female subjects among our data set was 27.88 (± 7.64). 169 female subjects were diagnosed to have PCOS while 164 did not have PCOS as per Rotterdam defined criteria. 93 subjects were not married while 238 subjects were married in our data set. The comparison between age, anthropometric parameters, fasting plasma glucose, lipid indices including total cholesterol, triglycerides, LDL and HDL cholesterols are shown in Table 1. Vitamin D levels regardless of high or low BMI demonstrated a regular drop of vitamin-D levels in subjects diagnosed to have PCOS in comparison to females without PCOS (Figure 1). Similarly, we demonstrated biochemical hyperandrogenism as measured by Free Androgen Index (FAI) to increase with increasing insufficiency of vitamin-D levels (Figure 2). A similar increase for hirsutism as measured by modified FG scores was noticed in female subjects with insufficient Vitamin-D

Table 1: Differences for age, anthropometric measures, lipid parameters and glycemic status between subjects with or without PCOS as per Rotterdam defined criteria.

Parameters	PCOS Rotterdam criteria	N	Mean	Std. Deviation	Sig. (2-tailed)
Age (years)	YES	169	26.75	6.98	0.005
	NO	164	29.08	8.08	
BMI (Body Mass Index)	YES	169	29.3	5.75	0.233
	NO	164	28.48	5.66	
WHtR (Waist to height ratio)	YES	169	0.58	0.074	0.674
	NO	164	0.58	0.072	
AVI (Abdominal Volume Index)	YES	169	16.73	4.15	0.617
	NO	164	16.51	3.98	
LAP (Lipid Accumulation Products)	YES	169	45.88	34.9	0.839
	NO	164	46.66	34.76	
Fasting plasma glucose (mmol/L)	YES	169	5.62	5.48	0.471
	NO	164	5.31	1.63	
Total cholesterol (mmol/L)	YES	169	4.38	0.92	0.108
	NO	164	4.2	0.82	
Serum triglycerides (mmol/L)	YES	169	1.32	0.84	0.464
	NO	164	1.39	0.78	
Low density lipoprotein cholesterol (mmol/L)	YES	169	2.67	0.85	0.007
	NO	164	2.44	0.74	
High density lipoprotein cholesterol (mmol/L)	YES	169	1.04	0.35	0.512
	NO	164	1.08	0.73	

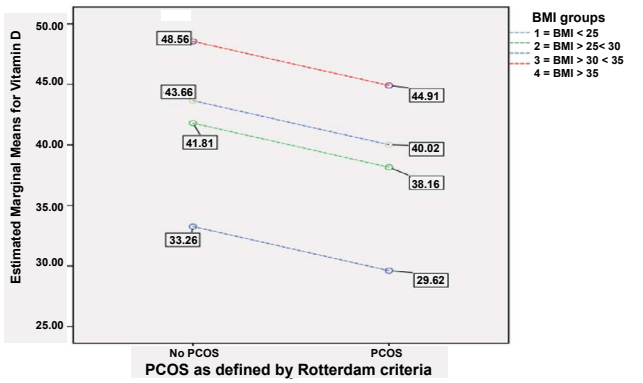

Figure 1: General Linear Model (GLM) demonstrating a gradual decline in Vitamin-D levels from subjects without PCOS to subjects diagnosed to have PCOS across various BMI (Model significance: <0.001).

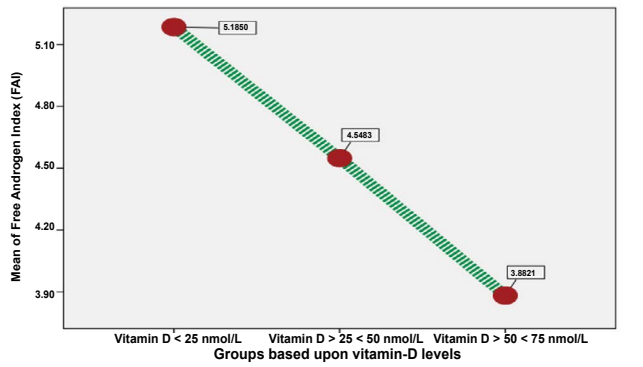

Figure 2: One way ANOVA demonstrating Free Androgen Index (FAI) between groups formulated based upon vitamin-D levels. [Group-1 (Vitamin D levels <25 nmol/L): 5.135 {95% CI: 4.28-6.12})], [Group-2 (Vitamin D levels >25<50 nmol/L): 4.58{95% CI: 3.95-5.1})], and [Group-3 (Vitamin D levels >50 nmol/L): 3.88 {95% CI: 3.20-4.56})] (p=0.059).

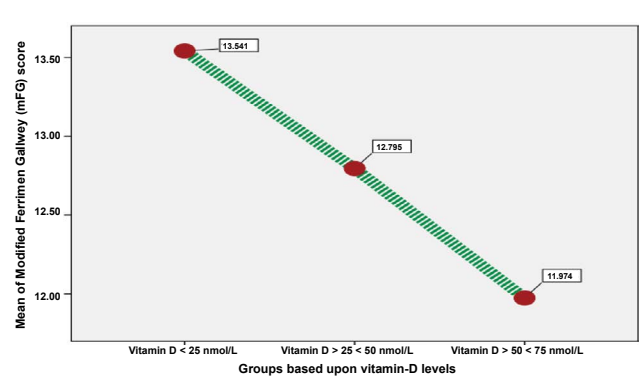

Figure 3: One way ANOVA demonstrating modified Ferrimen Gallwey (mFG) score between groups formulated based upon vitamin levels. [Group-1 (Vitamin D levels <25 nmol/L): 13.54 {95% CI: 11.88-15.20})], [Group-2 (Vitamin D levels >25<50 nmol/L): 12.80 {95% CI: 11.26-14.32})], and [Group-3 (Vitamin D levels >50 nmol/L): 11.97 {95% CI: 10.12-13.83})] (p=0.410).

levels; however the results were not statistically significant (Figure 3). Using GLM with insulin resistance as dependent variable and PCOS and Vitamin-D defined groups as independent factors, we were able

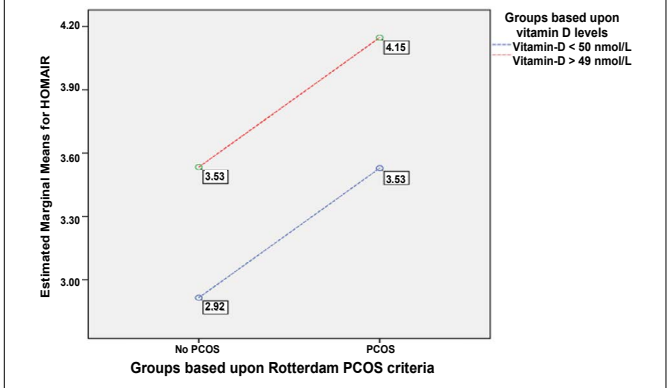

Figure 4: General Linear Model (GLM) comparing insulin resistance as measured by HOMAIR between subjects with or without PCOS along with effect of Vitamin-D status (Model significance=0.069).

to demonstrate an increase in insulin resistance with worsening Vitamin-D status and presence of PCOS (Figure 4).

Discussion

Our study highlights that Polycystic Ovarian Syndrome (PCOS) and its features including biochemical hyper-androgenism and hirsutism are associated with Vitamin-D deficiency albeit in a weaker manner and also linked with other factors like insulin resistance and body fat deposition. Our study contrasted some prevailing data where no evidence was found between PCOS and vitamin-D in some studies [6,7]. He et al in their systematic review did not find any evidence of vitamin-D supplementation to reduce biochemical abnormalities in subjects with PCOS [17]. Another study by Arslan et al did not observe 25(OH)D levels to be different in subjects diagnosed to have PCOS or otherwise [18]. However, there is plentiful data in the literature to support an association between Vitamin-D levels and polycystic disease (PCOD) [8,9]. Voulgaris et al. have identified that Vitamin-D is an important factor involved in reproductive issues and suggested therapeutic use of Vitamin-D in fertility treatment [19]. Similarly, Javed et al provided proof of concept that Vitamin D supplementation help reduces insulin resistance along with multiple other liver enzymes in subjects diagnosed to have PCOS [20]. Sharing mixed evidence and keeping our findings we can say with some confidence that there is a possibility of Vitamin-D deficiency in subjects with PCOS.

We found Vitamin-D deficiency to be only significant once factors like BMI and insulin resistance were taken into account. Our study, in nutshell, demonstrated higher insulin resistance, LDL-cholesterol and free androgen index in polycystic females in comparison to non-polycystic females. Furthermore hirsutism and free androgen index, though being component of the broader Rotterdam criteria to define PCOS, were observed phenotypic variations between PCOS with presence or absence of certain factors to allow us to mark the syndrome "heterogeneous". Similar findings have previously been appreciated in literature in terms of so-many confounding factors from type of clothing to racial differences [10,11]. However, hirsutism, as determined by modified FG scores, was different between our study subjects with or without PCOS. Probable explanations for

this include: Firstly, mean age in our data set was 27 years and some studies like Zhao et al. have documented and hirsutism to be more significantly related to increasing age in PCOS females as depicted by average age of 35 years in their study [21]. Secondly, racial differences have been well-recognized both in terms of severity and differences and I feel this factor must be taken into account as race-related but still need to be replicated in broader epidemiological study within our population [11,22]. Moreover, Apart from various epigenetic/environmental triggers it has been recognized that genetic changes in shape of Vitamin-D receptor (VDR) play a significant role in leading to variability in hirsutism in females across races. In this regard studies by Shi et al. and Niu et al. have demonstrated specific polymorphism as VDR Apal and VDR Bsml as significant contributors towards PCOS susceptibility and hirsutism in Asian population [23,24]. In the end hirsutism measurement methods do vary and studies have provided requisite evidence in this regard that different methodologies adapted to measure hirsutism can yield variable assessment of hirsutism [25,26].

Limitations

We feel there are certain limitations to our data: Primarily our study was a non-epidemiological study and therefore it is highly recommended that a well-controlled trial covering regional data may follow this study to address the phenotypic variations among our PCOS females. Moreover, different authors have suggested multiple cut-offs for evaluation free androgen index (FAI) and hirsutism. However we have utilized an FAI cut-off of 5.0% and mFG score of >8 to define hyper-androgenism, which to our knowledge is the most prevalent cut-offs utilized in literature. Finally, we do feel a specific need to find regional PCOS phenotypes to segregate various groups within PCOS to allow personalized diagnosis and treatments.

While not being a pioneer study about PCOS in our region, the authors feel that the evidence added to link insulin resistance, Vitamin-D status and dyslipidemia and anthropometric parameters with PCOS is valuable especially with regards to our population. Recognizing the various associated factors in the final shaping up of PCOS phenotype will not only help appropriate diagnostics but will pave a "way forward" towards tailoring exact therapeutic interventions for patients.

Conclusion

PCOS subjects have lower vitamin-D levels than non-PCOS subjects. However, these effects become more prominent once associated effects related to BMI, insulin resistance are interpreted in wholesome.

Acknowledgment

The authors acknowledge the work of Miss Huma and Lab technician Iftikhar for the support, coordination, and help.

Author's Contributions

Sikandar Hayat Khan: (Corresponding author) Conception of the idea, Involved in study plan, lab testing, analysis of results, manuscript writing. Robina Manzoor: Patient history collection, initial examination, manuscript writing. Rahat Shahid: Radiological examination, and manuscript writing. Syed Aown Raza Shah Bukhari: Radiological examination, data analysis and contributed towards discussion. Roomana Anwar: Patient examination, history writing, manuscript writing. Muhammad Tariq: Study plan and statistical review, Manuscript writing. All authors approved the final manuscript.

Consent for Publication

We ensured signed written consent from all participants.

Competing Interests

There are no competing interests to declare.

Ethical Approval

The project "Comparison of Vitamin D status for hirsutism, biochemical hyperandrogenism, glycemia and dyslipidemia among subjects with Polycystic Ovarian Syndrome (PCOS)" had the approval of "hospital's ethical review committee". All participants provided written consent for the study after they were explained about the project details.

Data Funding

The study had no funding source to disclose.

References

1. Wimalawansa SJ (2018) Non-musculoskeletal benefits of vitamin D. J Steroid Biochem Mol Biol 175: 60-81.
2. Mellanby RJ (2016) Beyond the skeleton: the role of vitamin D in companion animal health. J Small Anim Pract 57: 175-180.
3. Yin X, Sun Q, Zhang X, Lu Y, Sun C, et al. (2012) Serum 25(OH)D is inversely associated with metabolic syndrome risk profile among urban middle-aged Chinese population. Nutr J 11: 68.
4. Buyukinan M, Ozen S, Kokkun S, Saz EU (2012) The relation of vitamin D deficiency with puberty and insulin resistance in obese children and adolescents. J Pediatr Endocrinol Metab 25: 83-87.
5. Muldowney S, Lucey AJ, Paschos G, Martinez JA, Bandarra N, et al. (2011) Relationships between vitamin D status and cardio-metabolic risk factors in young European adults. Ann Nutr Metab 58: 85-93.
6. Poomthavorn P, Saowan S, Mahachoklertwattana P, Chailurkit L, Khlairit P (2012) Vitamin D status and glucose homeostasis in obese children and adolescents living in the tropics. Int J Obes (Lond) 36: 491-495.
7. Jorde R, Grimnes G (2011) Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. Prog Lipid Res 50: 303-312.
8. Zhao J, Liu S, Wang Y, Wang P, Qu D, et al. (2019) Vitamin D improves *in-vitro* fertilization outcomes in infertile women with polycystic ovary syndrome and insulin resistance. Minerva Med 110: 199-208.
9. Dastorani M, Aghadavod E, Mirhosseini N, Foroozanfard F, Zadeh Modarres S, et al. (2018) The effects of vitamin D supplementation on metabolic profiles and gene expression of insulin and lipid metabolism in infertile polycystic ovary syndrome candidates for *in vitro* fertilization. Reprod Biol Endocrinol 16: 94.
10. Fuleihan Gel-H, Bouillon R, Clarke B, Chakhtoura M, Cooper C, et al. (2015) Serum 25-Hydroxyvitamin D levels: Variability, knowledge gaps, and the concept of a desirable range. J Bone Miner Res 30: 1119-1133.
11. Ganie MA, Marwaha RK, Dhingra A, Nisar S, Mani K, et al. (2016) Observation of phenotypic variation among Indian women with polycystic ovary syndrome (PCOS) from Delhi and Srinagar. Gynecol Endocrinol 32: 566-570.
12. Goodman N, Bledsoe M, Cobin R, Futterweit W, Goldzieher J, et al. (2001) American Association of Clinical Endocrinologists Hyperandrogenism Guidelines. Endocrine Practice 7: 113-120.
13. Kollmann M, Klaritsch P, Martins WP, Guenther F, Schneider V, et al. (2015) Maternal and neonatal outcomes in pregnant women with PCOS: comparison of different diagnostic definitions. Hum Reprod 30: 2396-2403.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419.
15. Al Kindi MK, Al Essry FS, Mula-Abed WA (2012) Validity of serum testosterone, free androgen index, and calculated free testosterone in women with suspected hyperandrogenism. Oman Med J 27: 471-474.
16. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81: 19-25.

17. He C, Lin Z, Robb SW, Ezeamama AE (2015) Serum vitamin D levels and polycystic ovary syndrome: A systematic review and meta-analysis. Nutrients 7: 4555-4577.

18. Arslan E, Gorkem U, Togrul C (2019) Is there a relationship between vitamin D deficiency status and PCOS in infertile women? Geburtshilfe Frauenheilkd 79: 723-730.

19. Voulgaris N, Papanastasiou L, Piaditis G, Angelousi A, Kaltsas G, et al. (2017) Vitamin D and aspects of female fertility. Hormones (Athens) 16: 5-21.

20. Javed Z, Papageorgiou M, Deshmukh H, Kilpatrick ES, Mann V, et al. (2019) A randomized, controlled trial of vitamin D supplementation on cardiovascular risk factors, hormones, and liver markers in women with polycystic ovary syndrome. Nutrients 11: E188.

21. Zhao JL, Chen ZJ, Zhao LX, Geng L, Wang S, et al. (2006) Epidemiological study of clinical characteristics of Chinese Han ethnic women with polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi 41: 375-379.

22. Javorsky E, Perkins AC, Hillebrand G, Miyamoto K, Boer Kimball A (2014) Race, rather than skin pigmentation, predicts facial hair growth in women. J Clin Aesthet Dermatol 7: 24-26.

23. Shi XY, Huang AP, Xie DW, Yu XL (2019) Association of vitamin D receptor gene variants with polycystic ovary syndrome: A meta-analysis. BMC Med Genet 20: 32.

24. Niu YM, Wang YD, Jiang GB, Bai G, Chai HB, et al. (2019) Association between Vitamin D receptor gene polymorphisms and polycystic ovary syndrome risk: A meta-analysis. Front Physiol 9: 1902.

25. Tehrani FR, Rashidi H, Azizi F (2011) The prevalence of idiopathic hirsutism and polycystic ovary syndrome in the Tehran Lipid and Glucose Study. Reprod Biol Endocrinol 9: 144.

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