Ministry of higher education and scientific researches

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Serum Vitamin D Levels and Polycystic Ovarian Syndrome

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1 Introduction

Polycystic ovary syndrome (PCOS) is one of the commonest causes of female infertility. Clinical features caused by high levels of androgens, oligomenorrhea and polycystic ovarian morphology are necessary for diagnosis [1]. There is a dynamic relationship between activities of hypothalamic-pituitary-(adrenal/ and or ovarian axis) and metabolic diseases such as obesity, with involvement of compensatory hyperinsulinemia and insulin resistance [2, 3]. PCOS raises the risks of dyslipidemia, hypertension and hyperglycemia [4], thus raising the risk of developing cardiovascular diseases [5].

Vitamin D deficiency is noticed in numerous countries [6]. In vitamin D deficiency, the emergence of many diseases can be caused by defect in the metabolism of calcium and the building up of pro-inflammatory cytokines. It has been reported to lead to the development of cancer, diabetes, atherosclerosis and hypertension [7, 8]. Despite this, there is no agreement on the differences in serum vitamin D levels among women having and not having PCOS. Many studies reported that vitamin D of patients with PCOS was inversely correlated with metabolic disturbances [9-22]. Increased risk of PCOS or its associated endocrine/metabolic disturbances were linked with polymorphism of vitamin D receptor gene, which presents the effect of vitamin D in PCOS pathogenesis [12, 13, 16].

1.1 Polycystic ovarian syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women affecting approximately 4-18% of women in reproductive age, presenting with several possible combinations of signs and symptoms, which may include menstrual irregularity, anovulatory infertility, hyperandrogenism and obesity, as well as metabolic dysfunctions including insulin resistance type 2 diabetes and dyslipidemia [2]. PCOS is characterized by hypothalamic–pituitary– ovary axis dysfunction and anovulation but unlike other causes of ovulatory failure that feature insufficient ovarian follicle growth or suppressed gonadotropin secretion (or both). PCOS typically includes androgen excess and alterations in serum levels of gonadotropins and estrogens. PCOS has the potential for serious consequences, including increased risk for the development of endometrial hyperplasia and neoplasia [1]. Furthermore, extra-reproductive manifestations of PCOS include insulin resistance (IR), metabolic syndrome (MS), and cardiovascular diseases.

1.2 Pathophysiology of PCOS

Nearly all causes of PCOS are due to functional ovarian hyperandrogenism (FOH). Two-thirds of PCOS presentations have typical functional ovarian hyperandrogenism, characterized by dysregulation of androgen secretion with an over-response of 17-hydroxyprogesterone (17-OHP) to gonadotropin stimulation. The remaining PCOS with atypical FOH lack of over response of 17-OHP, but testosterone elevation can detect it after suppressing adrenal androgen production [10].

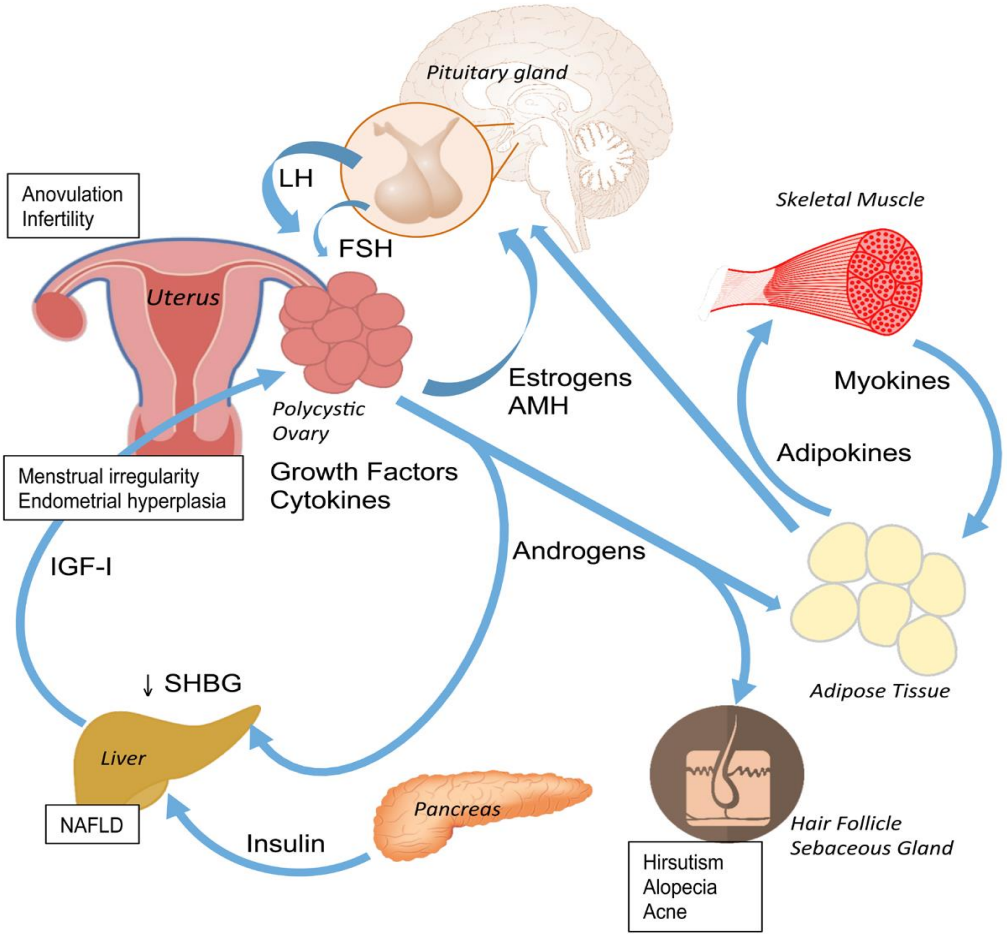


Figure 1Schematic representation of some pathophysiological mechanisms of polycystic ovary syndrome. The main clinical manifestations are shown in rectangular boxes. Androgens are responsible for dermatological symptoms, while sustained estrogen production by the ovaries and subcutaneous fat without progesterone opposition produces menstrual irregularity and increases the risk of endometria hyperplasia. Adipokines and myokines may also be involved in the metabolic alterations associated with the syndrome. Insulin resistance and the compensatory hyperinsulinemia are central mechanisms that perpetuate anovulation and lead to metabolic complications. AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; IGF-I, insulin-like growth factor I; LH, luteinizing hormone; NAFLD, non-alcoholic fatty liver disease; SHBG, sex hormone–binding globulin

Functional ovarian hyperandrogenism PCOS presents with the primary features: hyperandrogenism, oligo anovulation, and polycystic ovaries morphology. Functional ovarian hyperandrogenism is multifactorial, with a combination of hereditable and environmental factors.

Causes for this dysregulation include insulin excess, which is known to sensitize the ovary to luteinizing hormone (LH) by interfering with the process of homologous desensitization to LH in the normal ovulation cycle as well as an intrinsic imbalance among intraovarian regulatory systems.

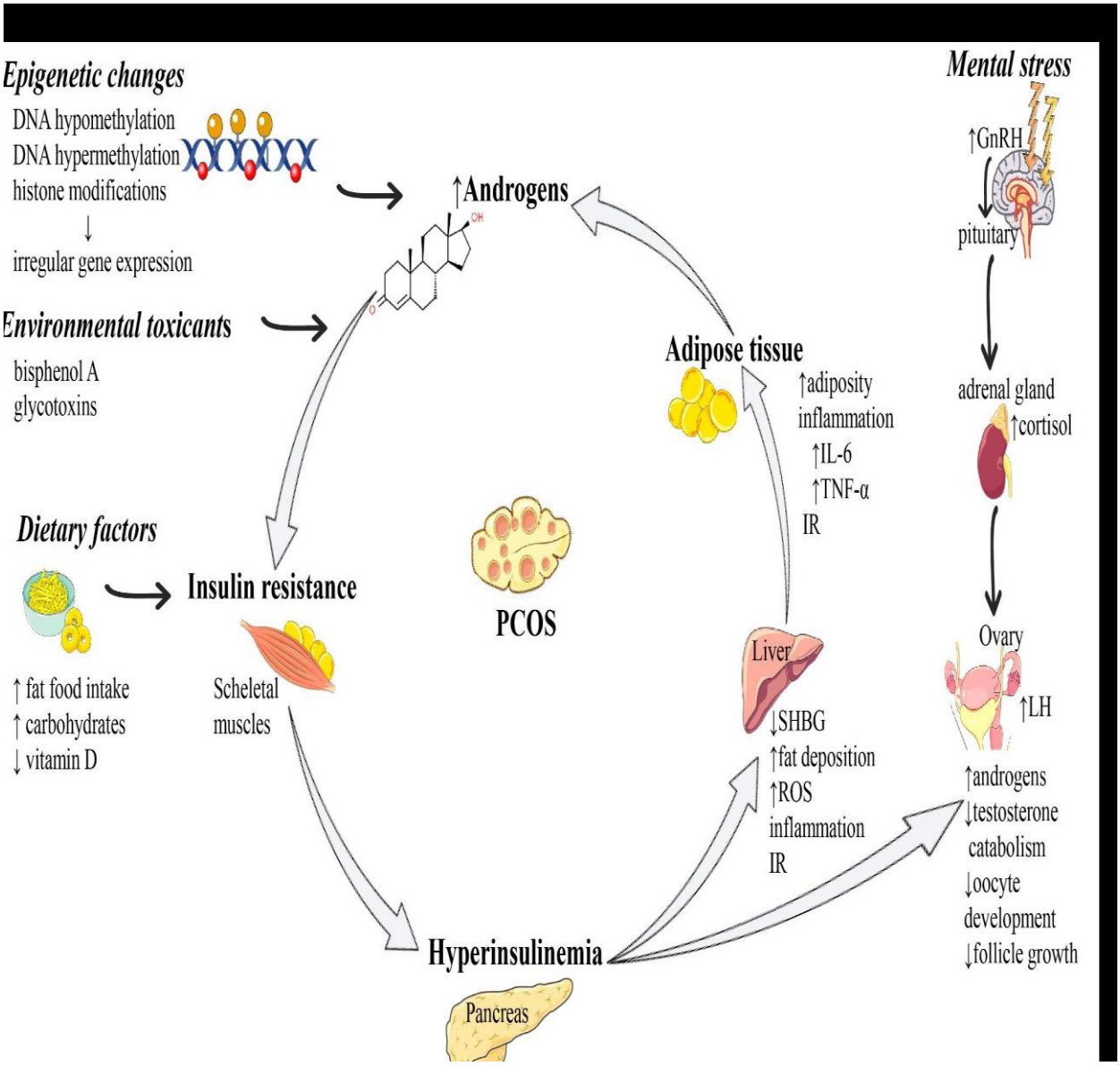
Theca cells in PCOS have overexpression of most steroidogenic enzymes and proteins involved in androgen synthesis, which suggested a prominent abnormality at the level and activity of steroidogenic enzymes, including P450c17, which has been highly identified. Granulosa cells prematurely luteinize primarily because of androgen and insulin excess. Androgen excess enhances the initial recruitment of primordial follicles into the growth pool. Simultaneously, it initiates premature luteinization, which impairs the selection of the dominant follicle. This results in classical PCOS histopathologic and gross anatomic changes that constitute PCOM [11]. PCOS is perpetuated by increased LH, but it is not caused by it. LH excess is common and is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion but is less likely to be the primary cause of ovarian androgen excess because of LH-induced desensitization of theca cells. About one-half of patients with functional ovarian hyperandrogenism have an abnormal degree of insulin-resistant hyperinsulinism, which acts on theca cell, increasing steroidogenesis and prematurely luteinizes Granulosa cells and stimulates fat accumulation. Hyperandrogenemia provokes LH excess, which then acts on both theca and luteinized granulosa sustaining cycle. Ovarian hormonal dysregulation alters the pulsatile gonadotropin-releasing hormone (GnRH) release, potentially leading to a relative increase in LH versus follicle-stimulating hormone (FSH) biosynthesis and secretion. LH stimulates ovarian androgen production, while the relative decrease of FSH prevents adequate stimulation of aromatase activity within the granulosa cells, decreasing androgen conversion to the potent estrogen estradiol. This becomes a self-perpetuating noncyclic hormonal pattern [12].

Elevated serum androgens are converted in the periphery to estrogens, mostly estrone. As conversion occurs primarily in the stromal cells of adipose tissue, estrogen production will be augmented in obese PCOS patients. This conversion results in chronic feedback at the hypothalamus and pituitary gland, in contrast to the normal fluctuations in feedback observed in the presence of a growing follicle and rapidly changing estradiol levels. Unopposed estrogen stimulation of the endometrium may lead to endometrial hyperplasia [13].

1.3 Clinical manifestation of PCOS

The most common symptoms of PCOS include:

- Menstrual disorders such as oligomenorrhea or amenorrhea.
- Infertility.
- High levels of masculinizing hormones manifested by acne and hirsutism.
- Metabolic syndrome, which appears as a tendency towards central obesity and other symptoms, associated with insulin resistance.
- Serum insulin, insulin resistance and homocysteine levels are higher in females with PCOS than in the normal females [2,13].



1.4 Diagnosis of PCOS

The diagnosis of PCOS must be based on the presence of at least two of the following three criteria: chronic anovulation, hyperandrogenism (clinical or biological) and polycystic ovaries (12 or more small follicles should be seen in an ovary on ultrasound examination). It is a diagnosis of exclusion and disorders that mimic clinical features of PCOS must be excluded. These include thyroid disease, hyperprolactinemia, and nonclassical congenital adrenal hyperplasia. Despite its high prevalence, PCOS is underdiagnosed and frequently takes more than one visit or different physicians to be identified, and these usually occur in more than a one-year timeframe. It is a very frustrating process for the patient. Delay in diagnosis can lead to the progression of comorbidities making it more difficult to implement lifestyle intervention, which is critical for the improvement of features of PCOS and quality of life.

1.5 Management of PCOS

Current treatment strategies aim at reducing insulin resistance in patients with PCOS and, consequently, to reach a reduction of compensatory hyperinsulinemia, improving metabolic and ovulatory features [3–5]. Insulin-sensitizer drugs are the recommended first-line therapy according to recent guidelines [6] for women with PCOS and metabolic abnormalities [7–9] with the aim at improving fertility [10–13], although physical activity and lifestyle change should be considered the first steps in overweight and obese PCOS patients to achieve weight loss [14, 15].

Indeed, the approach should be individualized for each person to meet the optimal result [8]. There is no one ideal treatment for all women diagnosed with PCOS, which leaves physicians no choice but symptomatic therapy [15].

Lifestyle Modification and Non-Pharmacological Approaches

1. Weight Loss: Elevated androgenic hormone levels lead to weight gain in women with PCOS, mainly in the abdominal area. As a result, many PCOS women have an apple shape body instead of a pear shape [7]. The first step for women diagnosed with PCOS would be weight reduction and calorie intake restriction [6].

2. Diet: An ideal diet would be rich in fibers and low in saturated fats and carbohydrates.

3. Exercise: Exercise and physical activity play a key role in weight reduction. They may be beneficial to improve insulin sensitivity [8].

4. Complementary and Alternative Medicine (CAM) Current management and accessible medications are only moderately effective in PCOS, and there are still some cases left untreated despite non-pharmacological and pharmacological treatments.

5. Supplementations: Apart from medications with USFDA approval, plenty of supplementation products has been shown to be effective in some women with PCOS. These products include vitamin D supplements, resveratrol, alpha-lipoic acid, omega-3, berberine, folic acid, myoinositol (MI), and d-chiro-inositol (DCI).

Pharmacological Treatments

Before heading to pharmacological approaches, healthy lifestyle advice must be given to all women diagnosed with PCOS regardless of their weight, complaint or anything else. This is because, in most cases and especially in mild to moderate forms, women can solely benefit from diet and exercise. However, the treatment would rely mainly on the patient's choices and condition in others.

- If the patient does not want to get pregnant and complains mostly about her menstruation irregularity, combined oral contraceptives (COCs) or progestins are the drugs of choice. The physician can choose the best oral contraceptive with a look on other symptoms rather than menstruation irregularity; for example, Yasmin®, Yaz®, or some other agents can show antiandrogenic effects and can, on the other hand, result in the reduction of androgen production. As a result, they might be helpful in those with hirsutism and/or acne complications.
- Metformin, from the biguanides category, is usually prescribed along with the first choice drugs (COCs) to restore the ovulation cycle in PCOS women because of its insulin sensitivity-increasing properties. Metformin has an antihyperandrogenic effect in the short term too. In other patients who just want relief from dermatological manifestations due to hyperandrogenism, agents such as aldosterone receptor antagonists (e.g., spironolactone) and 5-alpha reductases (e.g., finasteride) would be more beneficial.
- Therapy options change for those with infertility who should take agents for ovulation induction like clomiphene citrate and/or aromatase inhibitors [4]. Of course, there are lots of limitations and precautions, and not everyone can benefit from the agents mentioned above owing to their adverse effects or contraindications.

Many COC agents cause nausea and vomiting as they try to stimulate the pregnancy situation for the body. In addition, depression, headaches, and migraine are commonly seen in those taking them. Metformin also causes nausea and vomiting in the first days of consumption which may not be tolerated in all patients and leads to abandonment of the therapy. Spironolactone, a widely used and prescribed agent for androgen-related complications, can cause hyperkalemia. Therefore, it is suggested to look up the adverse reactions or contraindications in reliable drug literature or ask the patient's history of any possible reaction before the prescription.

Drug Repurposing in PCOS

Drug repurposing, or in other terms drug repositioning or drug re-tasking, actually means finding new indications in other diseases or conditions for a medication that has previously been in the market and has USFDA approval for a specific therapeutic goal [9]. Using this method has shortened the duration of the research and development process, given the thought that the medicines have passed pre-clinical and clinical, safety and immunological tests. As mentioned before in this review, PCOS still does not have a single ideal pharmacological treatment, and doctors typically tend to cure patients' symptoms with other agents.

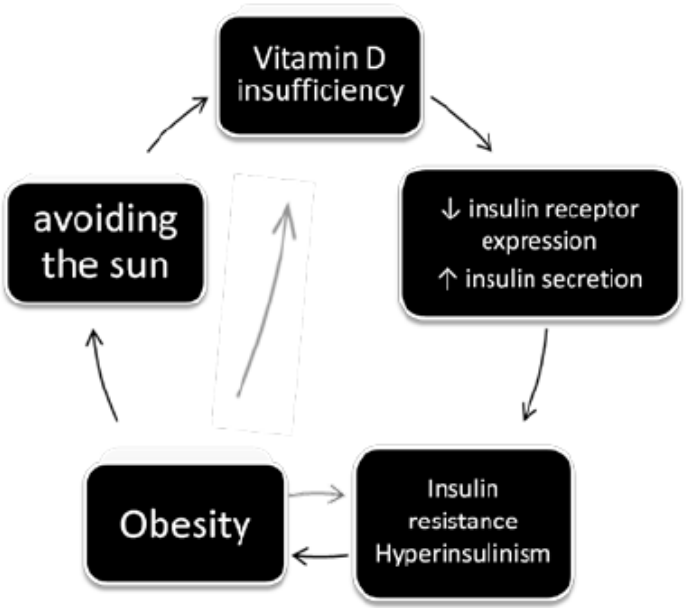
Laparoscopic ovarian surgery

Laparoscopic ovarian diathermy

Assisted reproductive technology (ART)

In vitro fertilization, intrauterine insemination, intracytoplasmin sperm injection.

1.6 The role of vit D in PCOS:



Vitamin D, a steroid hormone, is essential in skeletal growth and development, calcium/ phosphorus metabolism, and bone mineralization[8], [9] Vitamin D receptors regulate the expression of 229 genes in ~ 30 different tissues, including the pancreas, liver, immune cells, brain and ovaries. Studies indicate that there might be an involvement of vitamin D in the pathophysiology of PCOS [2]. Vitamin D plays a physiologic role in reproduction including ovarian follicular development and luteinization via altering anti-müllerian hormone (AMH) signaling, follicle-stimulating hormone sensitivity and progesterone production in human granulosa cells[1]. It also affects glucose homeostasis through manifold roles. The potential influences of vitamin D on glucose homeostasis include the presence of specific vitamin D receptor (VDR) in pancreatic β-cells and skeletal muscle, the expression of 1-α-hydroxylase enzyme which can catalyze the conversion of 25-hydroxy vitamin D [25(OH) D] to 1,25-dihydroxyvitamin D and the presence of a vitamin D response element in the human insulin gene promoter2. Low 25(OH) D levels are found to be significantly correlated with insulin resistance in women with PCOS [2]. Thus, genes involved in vitamin D metabolism have been suggested as candidate genes for the susceptibility to PCOS. A few polymorphisms in the *VDR* gene, such as Cdx2, Taq1, Bsm1, Apa1, and Fok1, were reported to play an influential role on insulin secretion and sensitivity in PCOS women [8]. The *VDR* Fok1 polymorphism was found to have a protective effect on the risk of type 2 diabetes mellitus, while the Bsm1 had a precipitating effect on the risk of type 2 diabetes. Besides, the Apa1 polymorphism was reported to confer a reduced risk of vitamin D deficiency [8]. The present study was designed to compare the serum level of vitamin D in PCOS women and healthy controls and explore the role of vitamin D in pathogenesis of PCOS.

Study design

49 women, diagnosed with PCOS were recruited as cases and another 49 women without PCOS served as controls. Demographic data like age, BMI, menstrual pattern, clinical features like hirsutism, acne were recorded for both cases and control. The serum 25(OH) D and metabolic markers were measured. The primary outcome was the difference in vitamin D status between the cases and controls, the secondary outcomes were correlations between serum 25(OH) D concentration and metabolic risk factors in women with PCOS.

Results

Vitamin D level was significantly lower in cases than in controls ($P < 0.05$). The prevalence of IR was significantly greater in the Vitamin D deficiency group among cases ($P < 0.05$), but not so for BMI and fasting insulin. In addition, prevalence of obesity and deranged lipid profile was not significantly increased in the vitamin D deficiency group of PCOS patients.

Table 1Comparing baseline characteristics of case and control groups

Characteristic	Case n=49	Control n=49	p-value
Age	23.78±5.67	27.45±5.45	0.001
BMI	25.35±5.44	23.11±2.81	0.013
SBP (systolic BP)	113.67±10.74	127.35±6.38	0.000
DBP (diastolic BP)	73.59±8.30	69.84±8.61	0.030
LH/FSH	2.61±4.91	2.80±4.89	0.846
Testosterone	22.98±22.37	28.31±21.92	0.236
PRL	15.24±5.18	15.05±5.04	0.854
Vit D Level	19.40±11.49	28.81±10.73	0.0001
Fast Insulin	11.63±16.01	13.47±19.30	0.609

The differences in age, BMI, SBP, DBP, and PRL were found to be statistically significant (p value <.05). No statistically significant differences could be found in the other parameters between the two groups.

Vitamin D level status between PCOS women and controls

The serum 25(OH) D concentration was significantly lower in PCOS women than in controls (19.3967± 11.4876.vs. 28.8120± 10. 7247. p<0.0001) [Table 1]. In addition, the prevalence rates of 25(OH) D deficiency and insufficiency were significantly higher in women with PCOS than in controls (65.3% vs. 18.4%, P <0.01; 26.5%) % vs. 22.4%, P < 0.0001). Furthermore, the prevalence of normal 25(OH) D status in women with PCOS was significantly lower than that in controls (8.2% vs. 59.2%, P < 0.01).

Table 2 Comparing metabolic factors with vitamin D deficiency, insuffciency and normal sub-groups.

Metabolic Factor	Deficiency (n=32)	Insufficiency (n=13)	Normal (n=4)	p-value
BMI	25.72±5.07	24.91±6.10	23.77±7.37	0.760
Fast Insulin	13.70±19.50	7.93±2.90	7.06±2.25	0.469
Insulin Resistance (IR)	4.09±3.80	1.42±0.42	1.52±0.49	0.026

[Table 2] illustrates the difference in the metabolic factors (BMI, Fasting Insulin, and IR) between the three subgroups of deficiency, insufficiency and normal Vit D level among the PCOS patients. Only the difference in IR was found to be statistically significant. (P value <0.05).

Table 3Comparing prevalence of obesity, lipid derangement and Insulin resistance between

Metabolic parameters	Vit D Deficiency (n=32)	Vit D Insufficiency (n=13)	Normal Vit D (n=4)	p-value
Obese (BMI>28kg/M^2)	18 (66.67%)	7 (25.93%)	2 (0.54%)	0.967
Non-obese (BMI <28kg/M^2)	14 (63.6%)	6 (27.27%)	2 (9.1%)	
Lipid profile deranged	21 (75%)	6 (21.43%)	1 (4.55%)	0.195
Lipid profile normal	11(52.38%)	7 (33.33%)	3(14.28%)	
Insulin resistance present	26 (100%)	0	0	0.000
insulin resistance absent	6 (26.1%)	13 (56.5%)	4 (17.4%)	

[Table 3] shows the prevalence of obesity, abnormal lipid profile and HOMA – IR among different vitamin D categories in women with PCOS. There was statistically significant difference in HOMA-IR, among the three groups ($P < 0.05$). No significant difference was found when comparing prevalence of obesity and deranged lipid profile among different groups ($P > 0.05$).

Table 4 Shows the percentage of cases with a specific lipid derangement within different categories

Lipid derangement type n=22	Deficiency (n=33)	Insufficiency (n=13)	Normal (n=4)	p-value
Cholesterol n=8	5 (62.5%)	3 (37.5%)	0 (0%)	0.191
HDL n=2	1 (50%)	0 (0%)	1 (50%)	0.028
LDL n=7	6 (85.7%)	1 (14.28%)	0 (0%)	0.160
VLDL n=1	1 (100%)	0 (0%)	0 (0%)	0.221
Triglyceride n=4	3 (75%)	1 (25%)	0 (0%)	0.241

[Table 4] shows that 62.5% of those PCOS women with hypercholesterolemia, 50% with increased HDL cholesterol, 85.7% with increased LDL, 100% with increased VLDL and 75% with increased triglycerides had Vit D deficiency. In addition, excepting for one person with increased HDL, none of those PCOS women with normal Vit D level had any lipid derangements.

1.7 Vit D supplementation

Vitamin D supplementation can lower the abnormally elevated serum AMH levels and increase serum anti-inflammatory soluble receptor for advanced glycation end products in vitamin D-deficient women with PCOS [1]. In particular, vitamin D and calcium supplementation in addition to metformin therapy in women with PCOS could result in the beneficial effects on menstrual regularity and ovulation [6]. Consequently, intervention with vitamin D at doses as high as (≥4000 IU/d) administered for a period of at least 12 weeks have provided improvements in the hyperinsulinemia and androgenic and fertility factors in PCOS women. Moreover, vitamin D replacement significantly decreased serum VEGF levels correlating with a decrease in serum TG [28] and also led to significant reductions in serum high-sensitivity C-reactive protein (hs-CRP), plasma total antioxidant capacity (TAC) levels [24,25] and plasma malondialdehyde (MDA) levels [25], showing that VDD women with PCOS phenotype have beneficial effects on chronic inflammation and oxidative stress after vitamin D supplementation. Overall, high dose vitamin D supplementation has shown promising results in improving the treatment of the PCOS patients.

2 Conclusion

Meta-analysis of cross-sectional/case-control studies supports the existence of positive associations between Vit D deficiency and metabolic and endocrine disorders in PCOS. PCOS women are more prone to vitamin D deficiency than normal subjects. Increased Insulin resistance and increased HDL- C are significantly more common in the Vitamin D deficiency group among PCOS women.

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