

RESEARCH ARTICLE

Effect of Homocysteine and Vitamin D in Polycystic Ovary Syndrome with Iraqi Women

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ABSTRACT

This research has been carried out on the effect of some biochemical parameters on women with polycystic ovaries. The presented work aims to determine the levels of a few biochemical parameters in females with polycystic ovaries syndrome (PCOS) attending the Department of Biotechnology, College of Science, University of Baghdad's. The study comprised 35 women between 18 and 35 who had PCOS based upon complete Rotterdam criteria. The control group comprised 35 healthy females of one age who had a regular menstrual cycle. The results indicated that females with PCOS have been obese or overweight when their body mass index (BMI) was more than 30. Insulin, fasting blood sugar (FBS), glycated hemoglobin (HbA1c%), and homeostatic model assessment for insulin resistance (HOMA-IR) have all been higher (≤ 0.05) in females with PCOS in comparison with a control group, showing that insulin resistance (IR) has been present. Total cholesterol (TC) and low-density lipoprotein (LDL) levels have been higher (≤ 0.05). Still, very-low-density lipoprotein (VLDL) and triglycerides (TG) levels were within the normal reference range without considerable differences compared to the control group. High-density lipoprotein (HDL) has been lower than the control group. According to this work, the majority of females with PCOS had a high BMI. Those patients showed signs of IR. It was discovered that the patient had dyslipidemia. Those results vary from previous studies that found no evidence of IR. The primary goal of this work is to assess the effects of homocysteine and vitamin D in females who have PCOS, and the findings revealed a significant increase in the homocysteine levels and a considerable drop or deficiency in vitamin D in Iraqi females who have PCOS.

Keywords: HbA1c, HOMA-IR, Homocysteine, Lipid profile, Polycystic ovary syndrome, Vitamin D.

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INTRODUCTION

PCOS can be defined as a complex, chronic, and major endocrine condition that impacts both adolescents and women of reproductive age (Azziz *et al.*, 2016).¹ Without particular diagnoses, the syndrome is heterogeneous in form and has been identified through a mix of symptoms and signs of ovarian dysfunction and androgen excess (Escobar-Morreale, 2018).² Symptoms of hirsutism, oligomenorrhoea, and infertility are common in females with PCOS when they are in their early adulthood or adolescence.³

PCOS etiology is unknown. However, it is most likely multifactorial. The spectrum of anomalies in PCOS is not entirely explained by any single etiologic factor.⁴ In PCOS, the ovarian theca cell synthesizes an excessive androgens amount in the responses to enhanced luteinizing hormone stimulation. Preferentially, ovaries synthesize androgen in the case when the luteinizing hormone's concentration exceeds that of follicle-stimulating hormone (FSH).⁴ There's a reduction

in the ratio of luteinizing hormone to FSH in females with PCOS because they have a higher luteinizing hormone pulse frequency, favoring the transcriptions regarding the beta-subunit of luteinizing hormone over the beta-subunit of FSH.

IR, also known as hyperinsulinemia, stimulates the ovary's theca cells, which synergistically act with luteinizing hormone to create too much testosterone, resulting in the clinical signs of hyperandrogenism (hirsutism, acne, and alopecia).⁵ Insulin inhibits the synthesis of sex hormone-binding globulin as well, which leads to an increase in the fraction of free testosterone when total testosterone levels are normal or only slightly raised.⁴ Inflammation is known to be a major contributor to PCOS. An increase in the levels of inflammatory markers (ferritin, leukocyte TNF α , IL-18, CRP), IL6) were demonstrated to be directly linked to the PCOS development.⁶ A pathogenic association of iron overload indicators with PCOS is one of the most recent emerging difficulties. An increase in the transferrin and ferritin levels, also a high frequency of HP2/

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HP2 haptoglobin α -chain genotype were identified, resulting in a decrease in antioxidant molecules and anti-inflammatory cytokines, resulting in a chronic inflammatory response.⁷

Oligo/amenorrhoea, endocrine or clinical evidence of hyperandrogenaemia, and polycystic ovaries are all symptoms of PCOS. Menstrual irregularities (oligomenorrhoea or amenorrhoea), which typically result in infertility (in 73–75% of situations), T2DM (about 10%), and abdominal obesity (30–70%), are the major abnormalities linked with PCOS (Woczyski & Zgliczyski, 2012).⁸ As it has been stated earlier, PCOS may lead to various metabolic, reproductive, anthropometric, and psychological problems in females. IR, dyslipidemia and abnormal glucose metabolism are all metabolic consequences of PCOS, which manifest as T2DM.⁹ Furthermore, females with PCOS might gain too much weight, exacerbating such symptoms. Oxidative stress, chronic inflammation, and poor fibrinolysis are all elevated cardiovascular risk factors, and there is evidence that cardiovascular disease (CVD) is more prevalent in those females.¹⁰ IR is expected to be affecting 70% of obese females with PCOS and 30% of lean females with PCOS.¹¹ When put to comparison with height- and age-matched healthy women, the ones with PCOS had greater risks of glucose intolerance and IR.¹¹

Lipid abnormalities are found in roughly 70% of PCOS women, which is far greater compared to that in healthy women (Legro *et al.*, 2001). With high serum Tg and free fatty acid concentration levels, there are increased levels of VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C), also reduced levels of HDL-cholesterol (HDL-C) because of reduced apolipoprotein A-I (apoA-I) levels (Legro *et al.*, 2001). Obesity has a significant impact on the metabolic and clinical symptoms of PCOS. In 2 systematic reviews, the obesity prevalence in females with PCOS was examined, and higher risks of obesity were identified.¹² According to a meta-analysis, women with PCOS had a 2- to 3-fold higher chance of being obese or overweight in comparison to their non-PCOS equivalents, and such prevalence has been impacted as well by ethnicity, with Caucasian females having a high prevalence compared to Asian women.¹³

Current systematic studies and meta-analyses analyzing complications and comorbidities of PCOS have been summarized in a workshop conducted by Gilbert *et al.* (2018). The researchers looked at many reviews ($n = 23$), covering 575 reviews and more than a million participants (1090072). According to the researchers, females with PCOS have more risks of surrogate markers for CVD and more prevalence of CVD.¹⁴ Females with PCOS seem to be having a greater chance of developing hypertension later in life, at least in the post-reproductive years. Pre-menopausal females with PCOS have a greater prevalence of hypertension (9–25.7%) than the general population.¹⁵ IR and obesity are two major factors, and androgens play a separate pathogenetic function by activating the rennin-angiotensin system.

The disturbance caused in the coagulation and fibrinolysis systems, such as increased levels of fibrinogen and plasminogen activator inhibitor 1 (PAI-1) and were linked to PCOS, making

it a potential prothrombotic state.¹⁶ Homocysteine levels have been found higher in people with PCOS, regardless of their BMI.¹⁷ Because of the ovulatory disorders as well as other endocrine irregularities, females with PCOS might have less fertility.¹⁸ According to recent research, infertility is ten times more likely in PCOS females than in healthy controls.¹⁹

A total of two systematic evaluations have looked at a considerable increase in risks of endometrial cancer in females who have PCOS.²⁰ As seen by oligomenorrhoea and IR, endometrial proliferation, prolonged endometrial exposures to the unopposed estrogen during anovulation, and/or associated risk factors like T2DM and obesity might all contribute to this.²¹

ACCORDING TO RECENT RESEARCH, Vitamin D Deficiency (VDD) is widespread in patients experiencing PCOS, and VDD might be linked to endocrine and metabolic disorders in PCOS patients.²² Compared to the general population, patients experiencing PCOS have a high prevalence of VDD.²³ Vitamin D is considered a steroid hormone that aids in the balance of bone mineralization and calcium phosphate.²⁴ Vitamin D receptors have been found in over 30 distinct tissues, such as the liver, pancreas, brain, immune cells, and ovaries, and affect the expression of 229 genes.²⁵

MATERIAL AND METHODS

Study Design

The presented work has been carried out in the Dept. of Biotechnology, College of Science, Univ. of Baghdad. There are 35 patients who were either attending the diabetic clinic or being admitted in the department and experiencing polycystic ovary. Specific investigations include fasting blood sugar (FBS) and glycated hemoglobin (HbA1c). Serum insulin, total cholesterol (T-cho), TG, HDL, LDL, VLDL, BMI, and homeostatic model assessments for insulin resistance (HOMA-IR) has been carried out in the present study. Samples were collected, and analysis of samples was conducted in Chemical Laboratory, Univ. of Baghdad, College of Science, Dept. of Biotechnology. In the morning, the subject's Blood samples were collected after 16 hours of fasting. A needle and syringe were utilized for collecting 5 mL of blood samples from subjects.

Determining Total Serum Cholesterol (T.cho)

The T.cho concentration was evaluated with the use of the enzymatic approach²⁶ using the commercially obtainable kit (bio-Merieux). The value of the T.cho is spectrophotometrically specified at 500 nm.

Determining the Serum High-Density Lipoprotein (HDL-c)

Level of HDL-c was measured with the use of the enzymatic approach (Burstein, 1970) by using the (bio-Merieux) kit. This approach's idea is precipitating lipoproteins and chylomicrons of the LDL and VLDL by adding the phosphotungstic acid in the presence of magnesium ion. Supernatant produced after centrifuging included the HDL from which phospholipids and cholesterol may be found. HDL was specified spectrophotometrically at 500 nm.

Determining the Serum Triglycerides (TG)

The total concentration of the serum TG was assessed through the use of the enzymatic approach of Fossati and Prencipel (1982)²⁷ by using the Bio-Merieux kit. The TG total serum concentration has been identified at 500 nm.

Determining the Serum VLDL-C

The VLDL was specified based on the classical equation of (Friedewald *et al.*, 1972).²⁸ $VLDL-c \text{ (mg.dl}^{-1}\text{)} = 0.20 \times TG \text{ (mg.dL}^{-1}\text{)}$.

Determining the Serum LDL-c

The serum LDL was specified according to Friedewald's equation: $LDL-c = T.Chol. - (VLDL-c + HDL-c)$.

Determination of FBS

Fasting blood sugar is estimated enzymatically by utilizing glucose oxidase GOD PAP(Kit) (Liquid)GL2624.

Determination of HbA1c

Glycated hemoglobin (HbA1c) was determined by (Stan bio Glyco hemoglobin pre-fil-procedure NoP350) quantitative colorimetric determination of Glycohemoglobin in whole blood.

Determination of Serum Insulin

Determination of serum insulin is performed by AESKULISA ELISA kit, Germany. Serum samples diluted at 1:101 are incubated in micro-plates coated with a particular antigen—antibodies from the patient bind to the antigen in the case where they are present. In the following phase, the unbound fraction is washed away. After that, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated with the antigen-antibody complex in micro-plates and react with it. The unbound conjugate is then rinsed away in the next step. When TMB-substrate is added, an enzymatic colorimetric (blue) reaction occurs, stopped by the diluted acid (the color is changed to yellow). The amount of conjugate bound to antigen-antibody complex determines the intensity of color production from chromogen, which is proportionate to the original concentration of corresponding antibodies in the sample of the patient.

Assay Process

- Pipetting 10 mL of the standards 1 to 6 to duplicate wells was done.
- Pipetting 10 µL of every one of the diluted samples 1:101 to duplicate wells was made.
- The microplate was covered by a plate sealer, then incubated at 37°C for 30 minutes.
- Washing 3X with 300 mL washing buffer (diluted 1:50)
- Pipetting 100µl of the conjugates to every one of the wells
- Incubating for 30 minutes at 37°C
- Washing 3X with 300 mL washing buffer (diluted 1:50)
- Pipetting 100 mL TMB substrate to every one of the wells
- Incubating for 30 minutes at 37°C, protected from the intense light
- Pipetting 100 µL stop solution in every one of the wells

- Incubation for 5 minutes
- Carefully agitating the plate for 5 sec.
- Reading the level of absorbance at 450 nm.
- Calculation of concentration of insulin by standard curve

Determination of Body Mass Index (BMI)

The calculation of BMI through dividing the weight (kg) per square height in meters.

Determining the HOMA-IR

HOMA-IR has been estimated with the use of the following equation: $(\text{fasting insulin} \times \text{fasting glucose (mg/dL)})/405$ or $(\text{fasting insulin} \times \text{fasting glucose (mmol/L)})/22.50$.

Determination of Serum Vitamin D

- *Serum Vitamin D determined by chroma Kit Human Vitamin D No:INS-VD-EN(Rev.00).*

Test principle: The test applies a competitive immune detection approach, a target material in the sample binds to fluorescence (FL) labeled detection antibody in detection buffer, for creating a complex as sample mix, such complex was loaded for migrating into nitrocellulose matrix, in which covalent couple 25(OH.)D3 and the bovine serum albumin (BSA) were immobilized on a test strip, and interference with binding related to target material as well as FL Labeled antibody. When more target materials exist in the blood, fewer detection antibodies will be accumulated, causing less fluorescence signal.

Determination of Serum Homocysteine

According to the manufacturer's procedure, serum homocysteine levels have been determined with the use of ELISA kits. Cell Biolabs, Inc. provided the homocysteine ELISA kit (cat no. STA670; San Diego, CA, US).

Statistical Analyses

The data analyses were carried out by utilizing the SPSS for Windows v22. Data have been represented in the form of mean \pm standard deviation (SD). The normality test of Shapiro–Wilk has utilized the determination whether the researched parameters were following the Gaussian distribution.

Bonferroni Post Hoc test for several comparisons was applied after the analysis of variance (ANOVA) tests.

The levels of the association were analyzed with the use of Pearson's correlation analysis. A $p < 0.05$ value has been viewed as significant.²⁹

RESULTS AND DISCUSSION**Effect of BMI with Polycystic Ovary Syndrome (PCOS)**

Overall, 35 patients experiencing PCOS were recruited for the presented work. The values of BMI, weight, and age in patients who have PCOS and healthy subjects have been shown in Table 1.

The current research looked into the degree of involvement regarding some biochemical parameters in PCOS problems. The researchers looked at weight, BMI, and age. In patients with PCOS, weight and BMI are significantly higher

($p \leq 0.05$) than in control. PCOS can be defined as one of the gynecological, endocrine disorders that affect mostly females of reproductive age and have a wide range of clinical symptoms.³⁰ Our results support these findings, with around 70% of patients experiencing PCOS being obese or overweight, and PCOS is linked to environmental, genetic, and behavioral factors such as lifestyle, diet, and hormone levels.¹³ Obesity is a common risk factor for women's diseases, including breast cancer.³¹ Many researches indicated that when the weight is increased, some abnormal genes like oxidative stress, Wnt signaling, along with inflammation in adipose tissues regarding the patients experiencing PCOS will be abnormal,³² indicating that obesity participates in PCOS pathogenesis,³³ triggers reproductive and metabolic disorders, and might also result in hyperandrogenemia, glycolipid metabolism, infertility, menstrual disorders and comorbidities associated with PCOS. This study also discovered that numerous aspects and problems of PCOS might cause oxidative stress and promote IR.³¹⁻³³

Effects of Glycemic Index with Polycystic Ovary Syndrome

Some of the biochemical parameters belonging to the glycemic index in PCOS patients have been examined. In the present study, there has been a considerable increase ($p \leq 0.05$) of FBS, HbA1c, HOMA-IR, and insulin in patients with PCOS compared with healthy subjects Table 2.

There were differences in significance in HbA1c, FBS, HOMA-IR, and Insulin in our data. There has been a considerable increase ($p \leq 0.05$) in HbA1c, FBS, Insulin, and HOMA-IR in the PCOS patients group compared with control subjects.

HOMA-IR and fasting insulin have been statistically more significant in PCOS patients compared to controls in prior research. Those findings corroborated previous research that PCOS women have high mean blood fasting insulin and HOMA-IR. According to the results, the majority of the individuals in the PCOS group have been insulin-resistant. The PCOS pathophysiology is influenced by oxidative stress and IR.

Obesity has been indicated to be prevalent in 24.8% of PCOS women, and overweight was shown to be prevalent in 21.8%, according to. According to, 54% of PCOS women have been obese or overweight according to the WHO BMI grading system.⁹⁻¹⁷ According to another research, excess

Table 1: BMI, weight, and age in patients with PCOS.

Parameters	Control subjects	PCOS Women	P-value
BMI (kg/m ²)	22.3±3.32	31.7±5.6	≤0.05
Weight (kg)	72.4±2.9	88.7±5.73	≤0.05
Age	28±2.33	28±5.3	≤0.05

Table 2: Bio-chemical parameters in PCOS and control subjects.

Parameters	Control subjects	PCOS subjects	P value
HbA1c%	4.9 ± 0.66%	6.5 ± 1.21%	≤0.05
FBS mg/dL	88.5 ± 9.32	105 ± 3.22	≤0.05
Insulin μIU/mL	11 ± 1.47	18 ± 2.23	≤0.05
HOMA-IR	2.40 ± 0.36	4.66 ± 4.76	≤0.05

triglycerides enter cells and activate the kinase of the proteins, limiting glucose uptake. Excess calorie environment, this results in compensatory hyperinsulinemia that might induce deposition of the excess fat through adipose cell hyperplasia and hypertrophy. Like a vicious cycle, this aggravates IR through increasing obesity. This aberrant fat accumulation raises IR, which leads to T2DM and glucose intolerance.¹⁰⁻¹³

Obesity is prevalent in the PCOS community, ranging between 38% and 87%. It has been shown that IR prevalence was high in obese PCOS females in comparison with the obese women with a regular menstrual cycle in the control group. Hyperinsulinemia has direct hypothalamic effects, such as increased hunger and gonadotropin secretion, which results in excessive androgen production in the ovaries in PCOS. A study conducted by Kumar *et al.* (2005) found that females with PCOS have a higher BMI than those without the condition.⁷⁻¹⁴

When there is PCOS, the obesity's pro-inflammatory state leads to the promotion of atherogenesis and IR. Although the inflammation degree corresponds strongly with adiposity and BMI, lean PCOS patients have higher inflammatory markers than their weight-matched non-PCOS controls (Carmina *et al.*, 2007).

Obesity (BMI of more than 30 kg/m²), particularly central obesity, was linked to the increase in risks of T2DM and prediabetes (Lerchbaum *et al.*, 2013). Those results are corroborated by our data, which demonstrate that obese/overweight females had a high occurrence of prediabetes than normal-weight females and that no one of normal-weight females has T2DM. Based on American Diabetes Association (ADA), diabetes screening must be done just in PCOS women who have a BMI < 25 kg/m² in asymptomatic adults.

Effect of Lipid Profile with Polycystic Ovary Syndrome

Table 3 shows differences in significance in lipid profiles in PCOS and control subjects. There has been a significant increase ($p \leq 0.05$) in TC and LDL-C in the PCOS group in comparison to control subjects. While there was a decrease significant ($p \leq 0.05$) in the high-density lipoproteins (HDL-C) in PCOS group compared to the healthy subjects.

Apart from the increased TC, LDL, and decreased HDL in the PCOS group compared to the control group, blood lipid profiles revealed no significant variations in the investigated parameters between groups.

Our results agree with Swetha *et al.*, (2013) regarding higher LDL-cholesterol in the PCOS group compared to the control group. In our research, low HDL [39.81 mg/dL] was observed as a dyslipidemia variable in cases. This is similar to findings in the South Indian population, where low HDL has been seen in 93.3 percent of cases with PCOS. The cause of dyslipidemia in PCOS may be hyperandrogenemia and hyperinsulinemia. This allowed adipocytes to undergo increased lipolysis resulting from catecholamine and release free fatty acids into circulation. Increased free liver fatty acids cause VLDL secretion, leading to hypertriglyceridemia. Hypertriglyceridemia leads to reduced HDL cholesterol and elevated LDL cholesterol levels through the reverse cholesterol transfer pathway. The further

androgenic priming of adipocytes in early life predisposes PCOS-associated dyslipidemia.¹⁴⁻²²

In PCOS patients, total cholesterol and LDL were significantly higher compared to controls. Such results are in agreement with the analysis by Pagotto *et al.* (2002) where total serum cholesterol and serum triglycerides were elevated in non-obese PCOS compared to the control group whereas plasma, HDL, LDL and VLDL were not statistically significantly different in both groups. In addition, Cinar *et al.*, (2011) showed a statistically significant increase of TC and LDL in non-obese PCOS compared to the controls, while there has not been any statistically significant difference between triglycerides and serum HDL.

Have shown increased cholesterol, triglycerides, and decreased HDL in the obese PCOS females compared with the non-obese and control group. Jayasekara (2012) showed that in PCOS, there had been statistically significant increases in the serum cholesterol, LDL, and triglycerides relative to the matched control group, while serum HDL was lower in PCOS than the control group. Their study showed that obese PCOS serum cholesterol and LDL increased statistically significantly compared to obese regulation. The increase in triglycerides in obese PCOS was not statistically significant in the analysis of.²⁰⁻³⁰

Effects of Homocysteine and Vitamin D with PCOS

The goal of the present work was to see if the levels of serum homocysteine remained considerably linked to PCOS after controlling for other co-variables. The multivariate logistic regression analysis has been utilized to do this, and it has been discovered that serum homocysteine increased risks and had the strongest relation with PCOS ($p \leq 0.05$) (Table 4). Also, there is a decrease significant in vitamin D in PCOS ($p \leq 0.05$) group compared to the healthy group.

Lately, homocysteine was identified as one of the factors that are related to CVD. According to our findings, the levels of serum homocysteine in PCOS females have been substantially high compared to controls. The most striking finding was that females with PCOS have much higher levels of homocysteine. In PCOS women, metabolic inefficiency is linked to an increase in risk factors for CVD. It is unclear if the rise is due to a high cardiovascular mortality and morbidity rate among PCOS

females (Toulis *et al.*, 2011). Several researchers have found a link between high homocysteine levels and an increased risk of cardiovascular mortality and morbidity (Wald *et al.*, 2002). Given this link, it's possible that females with high levels of homocysteine, especially those with inactivating base changes in the MTHFR gene, have a high prevalence of cardiovascular-related mortality and morbidity. On the other hand, this link necessitates formal testing and is a continuous endeavor.¹⁷⁻²⁸

Ahmed *et al.* (2007) have previously published similar findings. In our research, PCOS cases had considerably higher levels of mean serum homocysteine. With a higher BMI and waist, the increase was even more obvious. When obese cases were compared to normal cases and controls, they had higher amounts. Obesity's rising global prevalence might be a major factor in PCOS development in those who are vulnerable. Furthermore, obesity aggravates pre-existing hormonal, clinical, and metabolic characteristics in the majority of PCOS female.

With its prothrombotic and atherogenic characteristics, homocysteine has a recognized role in cardiovascular mortality and morbidity. Increased inflammatory cytokine expression, apoptosis activation, oxidative stress induction, and defective methylation are some of the molecular mechanisms of the homocysteine-induced cellular dysfunctions (Forges *et al.*, 2007). Homocysteine's metabolite could be reacting with the LDL cholesterol for the formation of the foam cells and atherosclerotic plaques. The endothelial cells could be damaged directly by the free radicals that are created during the reduced homocysteine oxidation. The aggregation of the platelets might be secondary to the homocysteine's pro-aggregatory actions. The endothelial cells that have been exposed to the homocysteine over a prolonged duration produce smaller amounts of nitric oxide. Homocysteine promotes the recruitment of the leukocytes via the upregulation of production and release of the monocyte chemo-attractant protein-1 and IL-8. The homocysteine boosts collagen production and stimulates smooth muscle cell proliferation. Homocysteine's prothrombotic effects include the decreased endothelial cell tissue plasminogen activator binding sites, factor VII-a and V activation, heparin sulfate inhibition, protein C, increase in the fibrinopeptide A and prothrombin fragments 1 and 2, increase in the viscosity of the blood, and reduced endothelial anti-thrombotic activities as a result of the changes in the functions of the thrombomodulin.²³⁻³⁰

Women diagnosed with PCOS often present with insulin resistance, leading to increased inflammation mark-er levels and higher risks of type II diabetes and cardiovascular disease. These diseases were also associated with vitamin D deficiency. The reason for and nature of this association is still not fully understood. The etiopathogenesis of PCOS is a complex phenomenon arising from the interaction of genetic and environmental factors. The present study results showed that the deficiency of vitamin D caused increases in the clinical findings and in the metabolic and hormonal profiles of PCOS patients, which is consistent with other studies.²¹⁻³¹ The present study also showed that vitamin D deficiency may be a risk

Table 3: Level of lipid profile in PCOS and control subjects.

Parameters	Control subjects	PCOS subjects	P-value
TC (mg/dL)	157.33 ± 0.43	260.7 ± 3.4	≤0.05
TG (mg/dL)	91.3 ± 1.27	89.87 ± 5.2	≤0.05
HDL-C (mg/dL)	45.4 ± 0.56	39.81 ± 2.41	≤0.05
VLDL (mg/dL)	18.26 ± 0.72	17.97 ± 0.77	≤0.05
LDL-C (mg/dL)	93.67 ± 0.93	202.97 ± 4.87	≤0.05

Table 4: Homocysteine and vitamin D in PCOS and control subjects.

Parameters	Control subjects	PCOS subjects	p-value
Homocysteine (μmol/L)	5.4 ± 2.98	9.6 ± 3.7	≤0.05
S.VITD3 mg/L	29 ± 4.7	14 ± 3.22	≤0.05

factor or may play a role in PCOS pathophysiology. There is a robust association between insulin resistance and PCOS. Vita-min D is one of the factors leading to the development of insulin resistance.¹⁹⁻²¹

A correlation between PCOS and vitamin D deficiency has been reported in several studies. However, the actual pathogenesis has not yet been elucidated. Although the connection between vitamin D deficiency and the underlying causes of PCOS has not yet been clarified, previous research has revealed a positive correlation between PCOS and BMI, body fat, and insulin resistance. Previous studies have also revealed that the changes in intracellular calcium concentrations caused by vitamin D deficiency may lead to ovulation and reproductive abnormalities in PCOS.¹¹ Statistically significantly higher values for BMI, fasting glucose, and HOMA-IR were detected in the vitamin D deficient group in the present research, again in agreement with previous studies ($p < 0.05$).⁷⁻¹⁰

Results of the present and previous studies suggest that VDD is one of the risk factors for PCOS. PCOS and vitamin D deficiency have been described as risk factors for atherosclerosis and hypertensive disorders. Previous studies have shown that these increase the morbidity and mortality related to cardiovascular disease. Vitamin D replacement has also been shown to reduce systolic blood pressure and mortality associated with cardiovascular disease. However, HDL-C levels have been higher in the normal vitamin D group than in the insufficient vitamin D group with PCOS ($p < 0.05$). This finding shows that vitamin D normalization could result in the reduction of cardiovascular disease risk in PCOS patients with VDD. In the literature, as in the present study, vitamin D deficiency was related to low HDL cholesterol levels.²³⁻³³

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