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Serum 25-hydroxyvitamin D is associated with homocysteine in infertile patients with polycystic ovary syndrome (PCOS)

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Serum 25-hydroxyvitamin D is associated with homocysteine in infertile patients with polycystic ovary syndrome (PCOS)

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ABSTRACT

Objectives: The aim of the study was to investigate whether there is a relationship between serum 25-hydroxyvitamin D and homocysteine in infertile-related PCOS.

Material and methods: We retrospectively reviewed 208 participants (86 PCOS and 122 non-PCOS) who met the inclusion and exclusion criteria from March 2020 to October 2021 at the Department of Obstetrics and Gynecology of the Second affiliated hospital of Xi'an Jiaotong University. Methods of Pearson correlation and linear regression were used to evaluate the associations between serum levels of 25-hydroxyvitamin D and homocysteine in infertile-related PCOS, and a smooth curve fitting were used to address potential nonlinearity.

Results: An inverse association between serum 25-hydroxyvitamin D and homocysteine was observed ($r = –0.392$, $p < 0.001$) in PCOS groups. Multiple linear regression analysis showed serum 25-hydroxyvitamin D was independently negatively associated with homocysteine levels after controlling for confounding factors ($\beta = –0.316$, $p = 0.006$). Age, BMI-stratified multivariate linear regression showed that serum 25-hydroxyvitamin D were independently associated with hyperhomocysteine especially in PCOS women aged 30 years or younger after adjusting age, BMI, and AMH.

Conclusions: Herein, the current findings suggest that 25-hydroxyvitamin D levels

was negatively associated with serum homocysteine in women with infertility-related PCOS.
Keywords: homocysteine; infertility; PCOS; vitamin D

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifaceted and heterogeneous reproductive, metabolic and psychological abnormality affecting 8–13% of reproductive-aged women [1]. Its pathogenesis is unknown, however, it is thought to be the most common cause of female infertility [2]. So yet, neither a recommended genetic screening test nor identified specific environmental substances have been linked to PCOS [3]. Polycystic ovary syndrome, a heterogeneous condition, has a multitude of symptoms including persistent anovulation, hyperandrogenism, insulin resistance, obesity, and dyslipidemia [4]. It is crucial to consider the possibility of substantial metabolic side effects, such as an increased risk of diabetes and cardiovascular disease while selecting a long-term PCOS treatment plan [5, 6]. It is well known that PCOS and metabolic diseases are closely related and mutually aggravated [7, 8]. More than half of the women with PCOS exhibit varying degrees of abnormalities in metabolic markers such as glucose, lipids, and amino acids [6]. International evidence-based guidelines for the diagnosis and treatment of PCOS have pointed out that improving the metabolic disorders of PCOS patients is essential to improve the menstrual disorders, reproductive disorders and long-term complications [9].

Several studies have shown that serum homocysteine levels or follicular fluid homocysteine levels were increased in patients with PCOS [10, 11]. Homocysteine, a sulfur-containing non-proteic amino acid, is an intermediate formed by demethylation during the metabolism of the essential amino acid methionine (Met) [12]. The metabolism of this amino acid requires vitamin B12, folic acid and vitamin B6 as cofactors for the different pathways. Any defect in enzymes involved in these pathways, deficiencies of vitamin cofactors, or drugs that cause hyperhomocysteinemia may lead to long-term cardiovascular complications or short-term adverse reproductive outcomes in women with PCOS [13]. Elevated plasma homocysteine levels are known to be associated with a variety of diseases, including cardiovascular and neurodegenerative diseases. Similarly, elevated plasma homocysteine has numerous adverse effects on PCOS patients, such as decreased

oocyte quality, aggravated insulin resistance, increased risk of cardiovascular disease and adverse pregnancy outcomes.

Vitamin D, a fat-soluble vitamin, is a steroid substance and is mainly synthesized by the skin upon exposure to sunlight [14]. 25-hydroxyvitamin D (25OH-D) is produced by hepatic 25-hydroxylase through two hydroxylation processes. Since 25(OH)D is an easily measurable molecule with a long half-life, it is considered the best indicator of vitamin D levels in the human body [15]. Vitamin D deficiency is a prevalent complication in women with and without PCOS [16, 17]. Several studies have linked vitamin D deficiency to metabolic disorders such as adverse serum lipids and glucose, and elevated homocysteine levels [18–20]. However, no study has investigated whether there was an independent association between vitamin D deficiency and elevated homocysteine in PCOS, so this was the aim of the present study.

MATERIAL AND METHODS

Study population

This was a retrospective cross-sectional study. We extracted 86 infertile-related PCOS women who presented to the hospital for the first time, had no history of hypoglycemic medication and had complete blood data of vitamin D and homocysteine from March 2020 to October 2021 at the Department of Obstetrics and Gynecology of the Second affiliated hospital of Xi'an Jiaotong University. Polycystic ovary syndrome was diagnosed based on the Rotterdam criteria (at least two of the following three characteristics are present: oligomenorrhea or amenorrhea, a clinical or biochemical hyperandrogenemia, as well as the presence of polycystic ovaries on sonography). The control group included 122 subjects from our outpatient department who were treated for other infertility reasons (such as diminished ovarian reserve, tubal and uterine factors) other than PCOS. Exclusion criteria included: 1) Patients with thyroid dysfunction; 2) Patients in the PCOS group and control group without blood vitamin D and homocysteine data; 3) Participants who took any vitamin supplement therapy or drugs affecting liver and kidney function. The study protocol was approved by the ethics committee of the Second affiliated hospital of Xi'an Jiaotong University. Informed consent was obtained from all study participants.

Data collection

We collected baseline data including age, history of infertility duration, type of

infertility, and body mass index (BMI; calculated as weight in kilograms divided by height in meters squared). Fasting blood included thyroid-stimulating hormone, alanine transaminase, creatinine, urea nitrogen, fasting plasma glucose, triglyceride, hemoglobin, anti-Müllerian hormone, 25-hydroxyvitamin D, and plasma homocysteine were obtained from all participants upon their first hospital visit or the following day for the laboratory testing.

Laboratory assays

Venous blood was obtained from all participants in the morning after overnight fasting, and left at room temperature for 15 min, and centrifuged at 3 000 r/min for 5 min, then supernatant (serum) was isolated for laboratory analysis. Serum levels of 25(OH)D were quantitatively determined by automatic electrochemiluminescence combined assay and serum levels of homocysteine were detected by a Roche automatic biochemical analyzer at the clinical laboratory of our Hospital, Shaanxi, China.

Statistical analysis

Baseline characteristics were expressed as mean values ± standard deviation for continuous variables and categorical variables were expressed as frequency (percentage, %). The differences in clinical and laboratory characteristics between PCOS and control groups were compared using Student's *t*-test or chi-square test. Pearson's correlation analysis was used to evaluate the relationship between homocysteine level and 25-hydroxy vitamin D and to determine possible confounders for the following analysis. A multiple linear regression analysis was performed to assess the factors that could be associated with the serum 25-hydroxy vitamin D level. A generalized additive model and smooth curve fitting were used to address the potential nonlinearity. All of the analyses were conducted using SPSS version 26.0 (International Business Machines Corporation, Chicago, IL, USA) other than the generalized additive model and visualization (R version 4.0.5). A two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics and comparisons of the anthropometric and biochemical parameters of control and PCOS groups were presented in Table 1. In general, The

mean age of all participants was 29.6 ± 4.9 years, with an average age of 27.2 ± 3.5 years for women with PCOS and 31.4 ± 5.0 years for the control group. Subjects in the PCOS group were more likely to be overweight or obese (22.99 ± 3.97 vs 24.75 ± 3.57, $p < 0.001$). Compared with controls, significantly lower serum 25-hydroxyvitamin D levels ($p = 0.037$) and higher serum homocysteine levels ($p = 0.013$) were revealed in the PCOS subjects.

Then the correlation between 25-hydroxyvitamin D levels, homocysteine and other parameters in women with PCOS were analyzed and the results were shown in Table 2. Serum 25-hydroxyvitamin D levels were negatively correlated with homocysteine in the PCOS group ($r = –0.392$, $p < 0.001$). Age was also associated with homocysteine levels ($r = –0.268$, $p = 0.013$) and 25-hydroxyvitamin D levels ($r = 0.306$, $p = 0.004$).

Univariate linear regression and multivariable linear regression models in Table 3 showed that 25-hydroxyvitamin D was identified as being independently negatively associated with serum homocysteine levels after adjusting different variables in PCOS patients: no covariates were adjusted in the model I (β coefficient –0.372; 95% confidence interval from –0.648 to –0.193); age and BMI were adjusted in model II (β coefficient –0.324; 95% confidence interval from –0.604 to –0.127); age, BMI and all blood variables were adjusted in model III (β coefficient –0.316; 95% confidence interval from –0.606 to –0.107). All three models were statistically significant ($p < 0.05$). Age was an important factor affecting the relationship between vitamin D and homocysteine. In Table 4, we further examined the age, and BMI-specific multivariable linear regression analysis for the association between decreasing 25-hydroxyvitamin D levels and hyperhomocysteine. The results show that serum 25-hydroxyvitamin D was independently associated with hyperhomocysteine, especially in PCOS women aged 30 years or younger. The result in a smooth curve fitting in Figure 1 showed there was a nonlinear relationship between serum 25-hydroxyvitamin D levels and homocysteine.

DISCUSSION

Our study evaluated the association between serum vitamin D concentration and homocysteine level in infertile-related PCOS patients. The results showed that serum vitamin D concentration had independent negative associations with homocysteine levels. This was the first attempt to demonstrate this negative correlation between

vitamin D levels and homocysteine in infertile patients with PCOS. This will provide guidance for future clinical management of metabolic problems such as vitamin D deficiency and hyperhomocysteinemia in PCOS patients. Our results also showed that age is an important confounding factor affecting vitamin D and homocysteine levels in PCOS patients. The results were consistent with previous studies in cardiometabolic disease populations [21]. Much indirect evidence and ideas had been obtained from similar studies of other systemic diseases.

The precise mechanism responsible for the relationship between homocysteine status and 25(OH)D levels is unclear. One important pathway of homocysteine metabolism is transsulfuration which requires the enzyme cystathionine beta-synthase (CBS) and a cofactor vitamin B6. A deficiency of the CBS enzyme is associated with hyperhomocysteinemia. Cystathionine beta-synthase is a target gene of vitamin D receptor (VDR), so the level of vitamin D may modulate homocysteine metabolism [18, 22].

In recent years, great attention has been attached to the diagnosis and treatment of PCOS. To reduce its burden on women's reproductive health and the impact of long-term complications, and simultaneously popularize disease education among the general population, great efforts have been made [1, 9]. However, the main clinical manifestations and specific needs of the PCOS individual often get high attention when the patients first came to a hospital, and some changes in metabolism-related blood parameters and vitamin deficiency have often been neglected.

It is widely known that a major role of vitamin D is to maintain calcium and phosphorus balances and promote bone mineralization. Vitamin D exerts biological effects by binding to vitamin D receptors. Evidence suggests that in addition to being involved in the typical balance of calcium and phosphorus metabolism, vitamin D also regulates reproductive processes in both women and men [14, 23, 24]. Vitamin D may play a physiological role in follicle development and luteinization, modifying FSH sensitivity, AMH signaling, and progesterone secretion [25]. However, Its role in reproductive physiology is a subject that is currently undergoing extensive research. In women, there is evidence for a role in PCOS, endometriosis, leiomyomas, in vitro fertilization, and pregnancy outcomes. However, the exact mechanism is still unclear [26]. In PCOS, although the findings are inconsistent [17], the prevailing opinion is that vitamin D has a beneficial effect on PCOS patients with metabolic disorders [16] and might be a beneficial and economic treatment for female infertility as an adjunct

to first-line treatment [27].

Homocysteine is one of the recognized cardiovascular risk markers and is often involved in oxidative stress, thrombosis, and inflammation. In PCOS, endothelial cell dysfunction may be triggered by inflammatory cytokines and homocysteine, which can lead to vascular disorders and metabolic abnormalities [28]. Several studies have demonstrated that plasma insulin levels are positively correlated with increased homocysteine levels, and PCOS women have higher levels of homocysteine when compared to noninsulin resistance PCOS [13, 29]. However, there have been inconsistent conclusions. Studies by Meng et al. [30] have concluded that high homocysteine levels in PCOS women are not associated with insulin resistance, obesity, or androgen levels and patients did not benefit from metformin treatment in reducing homocysteine levels. Polycystic ovary syndrome patients tend to have a higher AMH value than controls. There are few studies on the relationship of AMH and homocysteine, one study mentioned AMH and homocysteine, one study mentioned that betatrophin concentrations were positively correlated with AMH and the serum betatrophin level variability was explained by homocysteine, HOMA-IR and androstenedione levels [31].

However, there is currently no research on the relationship between serum vitamin D and homocysteine levels in patients with PCOS. Results regarding the relationship between vitamin D and homocysteine levels in the populations with other different diseases were inconsistent. A large community-based cohort analysis based on data from the continuous National Health and Nutrition Examination Survey for adults without symptoms (≥ 18 years) demonstrated that 25(OH)D was negatively correlated with homocysteine when the 25(OH)D concentration was less than or equal to 21 ng/mL. However, no significant decrease in homocysteine was observed at 25(OH)D concentrations above 21 ng/mL [18]. Another larger cross-sectional study from a preventive health program in Canada analyzed serum 25(OH)D and homocysteine concentrations in 4475 participants, and additionally, monitored serum 25(OH)D and homocysteine concentrations in the vitamin D supplemented population. Their results showed that the concentration of 25(OH)D was inversely related to homocysteine, and the greater the increase of 25(OH)D concentration, the lower the risk of homocysteine elevation [32]. Ganji et al. [33] found similar results with significantly lower total homocysteine in individuals with serum 25(OH)D greater than 30 ng/mL. The latter two studies showed that a decrease in homocysteinemia was observed in patients with

higher levels of vitamin D, suggesting that increased vitamin D levels may be beneficial in correcting hyperhomocysteinemia. Vitamin D status is influenced by a variety of factors, including age, season, obesity, and chronic illness incidence [34]. A study from NHANES data showed that the incidence of vitamin D deficiency in adults aged 20–39 years old was 7.6%, which was higher than in those over 60 years and those aged 1–5 years old [35]. The study of Xu et al. [36] showed that the overall trend of plasma homocysteine concentration first decreases and then increases throughout life. Their findings show that age is one of the factors affecting homocysteine concentration. The risk of hyperhomocysteinemia was 1.8 and 3.4 times higher in women aged 18–35 years old and 55 years and older than in women aged 35–55 years old. Our findings are consistent with those of previous studies. Although the results of the current study are encouraging, they cannot yet be fully generalized due to some limitations of this study. Major limitation of the study was the small sample size and the results may be biased. In addition, due to the limitations of retrospective studies, we were unable to obtain comprehensive data about patients and did not examine relationship between vitamin D and homocysteine in different phenotypes of PCOS.

CONCLUSIONS

In conclusion, serum vitamin D was negatively associated with homocysteine concentration in women with infertility-related PCOS, and lower serum vitamin D increased the risk of hyperhomocysteine status. Future larger trials are still required to confirm these findings.

Article information and declarations

Author contributions

Jinyan Zhao and Shengyu Fu conducted analyses and wrote the draft of the article. Qing Chen conceived the study design. All authors contributed to the collection of clinical data, writing or revising of the manuscript, and approved the final version.

Conflicts of interest

All authors have declared that no competing interests exist

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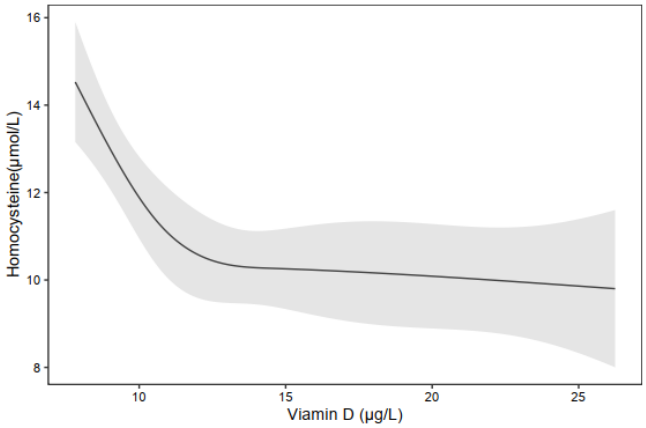

Figure 1. The associations between serum 25-hydroxyvitamin D and homocysteine in

polycystic ovary syndrome (PCOS). the solid black line represents the smooth curve fit between variables. The gray area represents the 95% confidence intervals (Cis)

Table 1. Baseline characteristics of individuals in the study (values are expressed as mean ± standard deviation except for special note)

Characteristics	**Control group (n = 122)**	**PCOS group (n = 86)**	**p value***
Age [y]	28.0 (3.5)	27.2 (3.5)	0.103
Duration of infertility [y]	1.7 (1.1)	1.5 (0.7)	0.193
Type of infertility, n (%)			< 0.001
Primary	43 (35.2)	53 (61.6)	
Secondary	79 (64.8)	33 (38.4)	
BMI [kg/m²], n(%)			< 0.001
Underweight	21 (17.2)	1 (1.2)	
Normal weight	64 (52.5)	40 (46.5)	
Overweight	32 (26.2)	36 (41.9)	
Obese	5 (4.1)	9 (10.5)	
Blood biochemical parameters			
TSH [μIU/mL]	2.9 (1.8)	2.9 (1.4)	0.824
ALT [IU/L]	20.5 (12.8)	25.0 (15.0)	0.025
FPG [mg/dL]	93.7 (12.3)	107.0 (65.0)	0.029
TG [mmol/L]	1.3 (1.0)	1.7 (1.4)	0.022
Hb [g/L]	129.1 (15.1)	137.6 (11.2)	< 0.001
AMH [ng/mL]	1.8 (1.3)	7.5 (4.5)	< 0.001
25(OH]D [ng/mL]	14.5 (5.8)	12.9 (4.7)	0.037
Hcy [μmol/L]	10.6 (4.1)	12.3 (5.3)	0.013

*Significant difference between groups determined by Student's t-test (continuous variables) or Chi-square test (categorical variables); PCOS — polycystic ovary syndrome; BMI — body mass index (calculated as weight in kilograms divided by height in meters squared); TSH — thyroid stimulating sormone; ALT — alanine transaminase; FPG — fasting plasma glucose; TG — triglyceride; Hb — haemoglobin; AMH — anti-Müllerian hormone; 25(OH)D — 25-hydroxyvitamin D; Hcy — homocysteine

Table 2. Relationship between serum homocysteine concentration and other selected interesting parameters in the polycystic ovary syndrome (PCOS) groups

Variables	Hcy	25(OH)D
25(OH)D	r = –0.392, p < 0.001****	–

AMH	r = 0.379, p < 0.001*****	r = –0.181, p = 0.096
Age	r = –0.268, p = 0.013*****	r = 0.306, p = 0.004*****
BMI	r = –0.019, p = 0.863	r = 0.051, p = 0.639
TSH	r = 0.088, p = 0.419	r = –0.089, p = 0.417
ALT	r = 0.077, p = 0.483	r = –0.033, p = 0.764
FPG	r = –0.069, p = 0.527	r = –0.065, p = 0.551
TG	r = –0.103, p = 0.346	r = –0.024, p = 0.824
Hb	r = –0.014, p = 0.899	r = 0.079, p = 0.468

25(OH)D — 25-hydroxyvitamin D; AMH — anti-Müllerian hormone; BMI — body mass index (calculated as weight in kilograms divided by height in meters squared); TSH — thyroid stimulating sormone; ALT — alanine transaminase; FPG — fasting plasma glucose; TG — triglyceride; Hb — haemoglobin

Table 3. Multiple linear regression analysis for determinants of serum homocysteine level in polycystic ovary syndrome (PCOS) patients

	Model I		Model II		Model III	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
25(OH)D	–0.372 (–0.648, –0.193)	< 0.001	–0.324 (–0.604, –0.127)	0.003	–0.316 (–0.600, –0.112)	0.005
AdjustedR^2	0.128		0.135		0.176	

Model I: no covariates were adjusted.;Model II: age and body mass index (BMI) were adjusted; Model III: model II + thyroid stimulating sormone (TSH), alanine transaminase (ALT), fasting plasma glucose (FPG), triglyceride (TG), haemoglobin (Hb) and anti-Müllerian hormone (AMH); β — standardized regression coefficient; CI — confidence interval; 25(OH)D — 25-hydroxyvitamin D

Table 4. Association between serum homocysteine and 25-hydroxyvitamin D [25(OH)D] level by multivariate linear regression, stratified analyses by selected characteristic

Variables	Total n (%)	Multivariable linear regression analysis[a]	
		β (95% CI)	p value
Age [years]			
≤30	72 (83.7)	–0.359 (–0.733, –0.174)	0.002
>30	14 (16.3)	0.400 (–0.075, 0.389)	0.120
Obesity[b]			

Yes	43 (50.0)	–0.279 (–0.784, 0.042)	0.076
No	43 (50.0)	–0.300 (–0.514, 0.023)	0.072

[a]Adjusted for age, body mass index (BMI) and anti-Müllerian hormone (AMH); [b]BMI ≥ 25 kg/m^2 was defined as obesity; β — standardized regression coefficient; CI — confidence interval