The role of cholecalciferol deficiency in the pathogenesis of polycystic ovary syndrome

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Abstract

Objectives: to evaluate and compare clinical presentations, medical history, and laboratory data of patients with polycystic ovary syndrome, including vitamin 25(OH)D3 level.

Methods: In total, 81 patients were examined. The patient group included 51 patients with signs of polycystic ovary syndrome. The control group included 30 healthy women without signs of polycystic ovary syndrome, comparable according to gender and age to the patient group. Polycystic ovary syndrome was verified based on the diagnostic Rotterdam and international polycystic ovary syndrome guidelines' criteria. The levels of cholecalciferol were determined by mass spectrometry (ng/mL). At the second stage of the study, the patient group with polycystic ovary syndrome was divided into two subgroups depending on the waist circumference and compared with each other by the level of insulin, low-density lipoproteins, triglycerides, anti-Mullerian hormone, follicle-stimulating hormone, and luteinizing hormone. Statistical analysis was carried out using the parametric t-test for two-independent samples with equal or different variance. For nominal data—Pearson's chi-test, when the means are not calculated and a test is carried out for the presence of a relationship between the nominal variables.

Results: Patients with polycystic ovary syndrome and without polycystic ovary syndrome did not have a statistically significant difference in 25(OH)D3 level. Statistically significant differences in the level of 25(OH)D3 were found in women with polycystic ovary syndrome with the waist circumference $\geqslant 80\,cm$. In these subgroups, differences in insulin, low-density lipoprotein, and triglycerides levels were also revealed.

Conclusion: The correlation of the 25(OH)D3 level does not differ in the groups of patients with polycystic ovary syndrome and without polycystic ovary syndrome, but significantly correlates with the metabolic profile of patients.

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction with main features such as hyperandrogenism and polycystic ovary morphology.[1–3] This condition is also known as Stein–Leventhal syndrome which was named after American gynecologists Irving F Stein and Michael L Leventhal.[4] This endocrine disorder affects 5%–15% females of reproductive age.[1,5] The normal action of hormones plays an important role in the ovarian function and regulation of the menstrual cycle that maintains fertility. If there is a constant disturbance of hormonal level in females,

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then it will disturb ovary functioning which leads to the formation of a cyst inside the sac of an ovary.[4]

The clinical manifestations of PCOS may include menstrual irregularities, signs of androgen excess, and obesity.[2,4,5] Currently, to establish the diagnosis of PCOS, revised Rotterdam diagnostic criteria[3] are used: (1) oligo- or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome). Presence of two out of three criteria confirms PCOS. The symptoms increase serious complications among females: 15%–20% of females affected by PCOS are facing serious complications related to infertility and menstrual cycle irregularity.[5,6] Stress, obesity, and fluctuation in hormonal level are the major issues related to PCOS worldwide.[3,6,7]

Even though insulin resistance and hyperinsulinemia are not included as criteria, it has important etiologic factors associated with the typical clinical signs and hormonal disorders of PCOS.[4,8] The results of numerous studies have shown a connection between PCOS and peripheral insulin resistance.[4,8,9] Insulin resistance is characterized by a process in which normal insulin concentrations affect glucose homeostasis and its utilization.[4,9–11] The insulin resistance and hyperinsulinemia affect up to 50% of normal and obese PCOS patients.[4,8] While obese women with PCOS have much higher prevalence of insulin resistance—80%.[1,4,8–10] It contributes to the progression of type 2 diabetes and increases the risk of cardiovascular disease.[3,8,12]

Although the Rotterdam criteria[3] have been widely accepted, it has recently become clear that dysmetabolic features of insulin resistance are a further clinical element that needs to be taken into account.[10] One of the underestimated factors that can lead to insulin resistance and aggravate it, as well as reduce fertility due to ovarian and metabolic disorders, is a deficiency of 25(OH)D3.[13,14] The authors' opinions about the role of 25(OH)D3 is controversial. Some studies demonstrate that women with PCOS have a significantly lower serum 25(OH)D compared to fertile controls. A compromised vitamin D status in PCOS women is associated with a higher insulin resistance and an unfavorable lipid profile.[14–17] However, some other authors have not found evidence that vitamin D supplementation reduces metabolic and hormonal dysregulations in PCOS.[1]

Moreover, PCOS seems to be associated with an increased risk of developing ovarian and endometrial cancer due, at least in part, to abnormal and unbalanced levels of sex hormones.[5,9,10] In this context, insulin sensitizers together with 25(OH)D3 might have a therapeutic and an anticancer effect.[5,9,10]

The above-mentioned controversies might be the result of underestimating of additional factors as body mass index (BMI), waist circumference (WC), sex hormone–binding globulins (SHBG), and other metabolites.[18] In view of the above-mentioned, we find it interesting and relevant to study the severity of vitamin D deficiency in patients with PCOS as well as the relationship between 25(OH)D3 deficiency and PCOS, taking into account factors that affect the clinical picture such as BMI and WC.

Thus, the aim of this study was to assess the relationship of 25 (OH) D3 deficiency in patients with PCOS with various metabolic parameters (BMI, WC, insulin, low-density lipoprotein (LDL), and triglycerides (TG)), which could improve the treatment approach.

Materials

Study design

The prospective case–control study was performed from November 2017 to September 2018 in "D-Doctor" private clinic, Nur-Sultan City, Kazakhstan. The study involved 81 participants: 51 patients with various clinical and laboratory manifestations of PCOS and control group of 30 healthy women without signs of PCOS. To implement the first stage of the study, all participants were divided in two groups: (I) case group (51 women)—patients with PCOS, (II) control group (30 women)—individuals without PCOS. At the second stage of the study, the patient group was divided depending on the WC into two subgroups:

I A—patients with a WC $\geqslant$ 80 cm

I B—with a WC $<$ 80 cm.

Eligible participants were recruited "D-Doctor" private clinic, Nur-Sultan city, Kazakhstan and met the following: inclusion criteria: (1) reproductive age of female (aged 18–44 years) and (2) PCOS confirmed according to Rotterdam criteria.[3] Exclusion criteria: (1) age groups of 45 years and older and younger than 18 years; (2) confirmed ovarian cancer; (3) severe comorbidity (diabetes mellitus, severe hypertension, severe cardiac diseases in decompensation, and so on); (4) use of combined oral contraceptives, hormone replacement therapy, and vitamin D during the last 3 months. This study received ethical approval from the Institutional Review Board (IRB) of the Medical Institute of the People's Friendship University of Russia, Moscow.

Data collection

At the first stage of the study, clinical and anamnestic data were collected from all patients: age, birth weight, and especially the course of pregnancy and childbirth by them in mothers, that is, the absence or presence of complications, menstrual function, a reproductive history of the patients themselves—the presence or absence of an independent pregnancy/childbirth, pregnancy loss of up to 22 weeks—spontaneous miscarriage, missed pregnancy, and so on, the presence or absence of infertility, with an

objective examination, BMI was calculated and obesity was calculated by BMI. The study conducted an ultrasound examination of the pelvic organs on various days of the menstrual cycle to confirm the presence or absence of signs of PCOS.

The levels of 25(OH)D3 (ng/mL) were investigated by the tandem chromatography-mass spectrometry method, scientific-laboratory complex "Archimedes" ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay conditions: 20 μL of the prepared sample is injected. Chromatographic separation of the components was achieved by gradient elution on a reversed-phase column (Acquity UPLC BEH C18; 1.7 μL, 2.1×50 mm^2, with 0.2 μL pre-column filter) using the Waters Acquity UPLC chromatographic system. For detection and quantification, a tandem quadripole mass spectrometer with an ion trap AB SCIEX QTRAP 4500 (AB SCIEX; Concord, ON, Canada) equipped with a source of chemical ionization at atmospheric pressure (APCI) under the control of Analyst software, version 1.6.2, was used. The monitoring of individual components corresponding to various vitamin D metabolites and internal standards was carried out in a positive mode using optimized ion transition parameters (multiple reaction monitoring (MRM)). The measurement results were processed using the MultiQuant 3.0.1 software.

The data were evaluated according to the Clinical Recommendations of the Russian Association of Endocrinologists: a pronounced vitamin D deficiency of less than 10 ng/mL (less than 25 nmol/L), vitamin D deficiency of less than 20 ng/mL (less than 50 nmol/L), vitamin D deficiency of more than 20 and less than 30 ng/mL (more than 50 and less than 75 nmol/L), and adequate levels of vitamin D more than 30 ng/mL (more than 75 nmol/L).

At the second stage, all of the above parameters were compared between PCOS groups with WC $\geq$ 80 cm and WC < 80 cm. In addition, biological material (blood) was taken from PCOS subjects for 2–3 days of the menstrual cycle to determine the level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), on any day of the menstrual cycle, blood was taken for the level of anti-Mullerian hormone (AMH), TG, LDL, and insulin.

The above analyses were performed on a Roche Cobas 6000 modular platform: TG and LDL were determined using the Cobas C 501 biochemical module using the photometric principle, and LH, FSH, AMH and insulin were determined using the Cobas E 601 immunochemical module using electro-chemiluminescence (E).

Statistical methods

The descriptive statistics of demographic and clinical characteristics and laboratory test results were presented as frequencies and percentages. The values of $p \geq 0.05$ were considered to be significant. The data were analyzed using IBM SPSS Statistics, version 23.

To test the impact in this work, several types of statistical tests were applied, depending on the type of variables:

- Comparison of averages for metric variables using the parametric t-test for two-independent samples with equal or different variance;
- Comparison of means for ordinal variables using a nonparametric criterion for two-independent Mann–Whitney's U samples;
- Pearson Z-test for assessing the difference between two shares of features related to the nominal data.

Results

There was no statistical difference in age and birth weight of patients between groups I and II. The study groups were also comparable in terms of the pregnancy complications in childbirth in their mothers. The complications occurred in 58% of patients and in 50% of control group (p = 0.44).

The menstrual function in groups had some differences. In the patient group, eight women had a regular menstrual cycle, which was 15.7%, and the remaining 43 (84.3%) women with PCOS had irregular menstrual cycle (secondary oligo-, amenorrhea). In the control group, only one participant reported irregular menstrual cycle due to hyperprolactinemia (3.3%; p = 0.000).

As shown in Table 1, the study of reproductive function revealed the following: (1) in the patient group, 13 (25.5%) women had infertility problems, (2) in the control group, two women (6.7%) had infertility (p = 0.035). The study of reproductive function showed that in the patient group, 14 (27.5%) women had spontaneous pregnancies with the successful delivery, while in the control group, 21 (70%) women had a history of successful spontaneous pregnancy and delivery (p = 0.000). In the third patient group (5.9%), women went through ovulation induction/in vitro fertilization (IVF), which is comparable with the data of the control group—pregnancy and delivery in 3.3% of participants occurred after IVF (p = 0.609). Nine (17.6%) women from the patient group had miscarriage before 22 weeks of gestation, while it happened only in three (10%) women from control group (p = 0.35).

In our study, the level of 25(OH)D3 was determined using the liquid chromatography method with tandem mass spectrometry. The level of 25(OH)D3 in the patient group ranged from 2 to 32.3 ng/mL with the average level of 16.25 ng/mL.

If analyzed according to the Russian Association of Endocrinologists classification, in the patient group, the following results were revealed: 13 (25%) women had a significant 25(OH)D3 deficiency, 22 (43%) of the subjects had vitamin D deficiency, 12 (24%) women with PCOS had 25(OH)D3 deficiency, the remaining four subjects had adequate levels—8%. The level of 25(OH)D3 in the control group ranged from 6.78 to 30.96 ng/mL with the average

Table 1. The results of the Z-test between the groups of the PCOS group and the control group.

Variables	Patient group		Control group		Test statistics: Z	p value
	51		30			
	n	%	n	%		
Irregular menstrual cycle	43	0.84314	1	0.03333	7.06547	0.000
Infertility	13	0.25490	2	0.06667	2.10606	0.035
Spontaneous pregnancy and delivery	14	0.27451	21	0.70000	−3.73306	0.000
Pregnancies after ovulation induction/IVF	3	0.05882	1	0.03333	0.51131	0.609
Pregnancy loss up to 22 weeks	9	0.17647	3	0.10000	0.93555	0.350
Complications during pregnancy and childbirth in mothers	30	0.58824	15	0.50000	0.77174	0.440

IVF: in vitro fertilization; PCOS: polycystic ovary syndrome.

Table 2. Comparative characteristics of the level of vitamin D in patients with PCOS and control group.

Variable	Patient group	Control group	t	p < 0.05
25(OH)D3 (ng/mL)	16.25	17.52	−0.7	0.471

PCOS: polycystic ovary syndrome.

level of 17.52 ng/mL. In the control group, severe 25(OH) D3 deficiency was observed in 4 (13%) cases, 17 (57%) had a deficiency, 7 (23%) women had 25(OH)D3 deficiency, and 2 patients had adequate 25(OH)D3 levels of 7%. The overall trend of the 25(OH)D3 levels is shown in Table 2.

The further analysis showed the presence of statistically significant differences of the following variables: WC, BMI, 25(OH)D3, insulin, TG, and LDL (Table 3).

In the study group, among the subjects with WS ⩾ 80 cm, the level of 25(OH)D3 was significantly lower compared to women with WS < 80 cm. The average level of 25(OH) D3 in women with WC of ⩾80 cm was 13.89 ng/mL, whereas in women with WC ⩽ 80 cm, the average level of 25(OH)D3 was at the level of 19.12 ng/mL (p = 0.018).

At the next stage, the level of insulin, TG, and LDL in the blood serum among women with PCOS was studied. The reference range of serum insulin is 6–27 mIU/mL. The average level of insulin in the blood serum of the subjects was 13.74 mIU/mL, the minimum level was 4.3 mIU/mL, and the maximum level was 38.8 mIU/mL. An important point was the study of insulin levels depending on the WC. Thus, in women with WC of ⩾80 cm, the average insulin level was higher—17.3 mIU/mL, while in 23 women with WC of ⩽80 cm, the average insulin level was lower—9.3 mIU/mL (p = 0.000). The average level of TG was 1.24 mmol/L, the minimum level was 0.45 mmol/L, and the maximum level was 4.81 mmol/L. Reference values of TG were from 0 to 2.25 mmol/L. The results of analyses on the level of TG are divided into several groups according to the degree of risk: the level below 1.7 mmol/L is considered acceptable, while the risk of complications is low (cardiovascular disease), the borderline level is 1.7–2.2 mmol/L,

from 2.3 to 5.6 mmol/L is considered high, and indicates a high likelihood of developing cardiovascular diseases; above 5.6 mmol/L, there is a very high risk of complications not only for the cardiovascular system but also a great danger in the development of pancreatitis. Thus, up to 1.3 mmol/L is considered the optimal level.

A study of the level of TG depending on the WS showed that for women with WS ⩾ 80 cm, the average level was 1.5 mmol/L, with WS < 80 cm, the average level of TG was lower—0.8 mmol/L (p = 0.001).

A study of LDL levels depending on the WC showed that for women with WS ⩾ 80 cm, the average level was 3.0. In subjects with WS ⩽ 80 cm, the average level of TG was lower—2.5 mmol/L (p = 0.012).

Discussion

In this study, we evaluated the relationship of 25(OH)D3 deficiency in patients with PCOS with various metabolic parameters (BMI, WC, insulin, LDL, and TG). The investigation of these variables could have an impact on the treatment choice. The results of our study show that 25(OH)D3 deficiency is associated with increased BMI, WC, insulin, TG, and LDL levels in patients with PCOS.

Results of our study have common trend with the recent study on the effects of vitamin D supplementation on insulin sensitivity and androgen levels in vitamin-D-deficient PCOS patients,[2,19–22] who found that supplementation with vitamin D reduces serum androgen levels and increases insulin sensitivity in patients with PCOS.

The previous studies confirmed lower serum vitamin D levels in PCOS women.[19–22] Moreover, the authors' findings suggest that low levels of vitamin D are correlated with a greater WC than in normal weight patients. In the study of Gallea et al.,[20] 25(OH)D3 levels were significantly lower in obese than in normal weight patients, suggesting that obesity represents a worsening condition of hypovitaminosis D. Our results are agreeing with others studies suggesting the role of central adiposity in vitamin D insufficiency.[19] The study of Seyyed Abootorabi et al.[21] showed that vitamin D supplementation for 8 weeks among

Table 3. The results of the Z-test between two groups of PCOS.

Variables	PCOS group 1A		PCOS group 1B		Test statistics: Z	p value
	28		23			
	n	%	n	%		
Irregular menstrual cycle	23	0.82143	20	0.86957	−0.47035	0.638
Infertility	5	0.17857	8	0.34783	−1.38008	0.168
Spontaneous pregnancy and delivery	10	0.35714	4	0.17391	1.45901	0.145
Pregnancies after ovulation induction/IVF	1	0.03571	2	0.08696	−0.77388	0.439
Pregnancy loss up to 22 weeks	4	0.14286	5	0.21739	−0.69476	0.487
Complications during pregnancy and childbirth in mothers	15	0.53571	15	0.65217	−0.84088	0.400
BMI deficiency	0	0.00000	4	0.17391	−2.29869	0.022
BMI normal	1	0.03571	19	0.82609	−5.75260	0.000
Overweight	9	0.32143	0	0.00000	2.99617	0.003
Obesity 1	11	0.39286	0	0.00000	3.39419	0.001
Obesity 2	5	0.17857	0	0.00000	2.13391	0.033
Obesity 3	2	0.07143	0	0.00000	1.30764	0.191

PCOS: polycystic ovary syndrome; IVF: in vitro fertilization; BMI: body mass index.

PCOS women with vitamin D deficiency led to a significant reduction in fasting glucose level and a significant increase in homeostatic model assessment of β-cell (HOMA-B), adiponectin, and serum vitamin D level. The study of Pal et al.[23] found the improvement of androgen and blood pressure profiles after 3 month intervention, suggesting therapeutic implications of vitamin D and Ca in overweight and vitamin-D-deficient women with PCOS.

Some previous studies have demonstrated that low 25(OH)D3 levels were associated with the presentations of metabolic syndrome in PCOS women.[22] It is similar with the findings of our study where in the patient group among the subjects with a WC ≥ 80 cm, the level of vitamin D was significantly lower compared to women with a WC < 80 cm (Table 3).

Another recent study of 25(OH)D3 effects on the course of PCOS has demonstrated that vitamin D supplementation for 12 weeks among vitamin-D-deficient women with PCOS significantly improved TG, LDL, and cholesterol levels,[24] which comes in line with the findings of our study.

However, there are contradictory studies regarding the effects of vitamin D treatment on insulin resistance and sensitivity. Some study results found the fasting serum insulin and the insulin sensitivity did not change significantly with vitamin D supplementation.[1] The researchers were not able to demonstrate the effect of vitamin D supplementation on insulin sensitivity and insulin resistance in women with PCOS and vitamin D deficiency.[1]

Therefore, further studies of vitamin D supplementation are necessary for better understanding of the effect of vitamin D on insulin, LDL, and WC in vitamin-D-deficient women with PCOS.

Undoubtedly, a 25(OH)D deficiency contributes to the physiology of carbohydrate and fat metabolism, but various studies have debated the relationship. Due to the urgency of the problem, an analysis of the level of 25(OH)D3 was performed in patients with PCOS and controls without PCOS, which showed no significant differences between these groups, but a statistically significant difference was revealed between the level of 25 (OH) D3 between patients with PCOS depending on the WS. The 25(OH)D3 deficiency was associated with increased BMI and WC. Among metabolic indicators, a relationship was identified with an increase in insulin and TG and an increase in LDL. The study showed that patients with PCOS with WC < 80 cm had a lower level of 25(OH)D3, which is associated with impaired anthropometric and metabolic parameters.

Conclusion

Thus, 25(OH)D3 deficiency is associated with the metabolic disorders in patients with PCOS, and this indicates that supplementation with 25(OH)D3 in addition to basic therapy can make a significant contribution to the correction of insulin resistance in PCOS patients.

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