Original Article
Changes of serum vitamin D levels in infertile patients with polycystic ovarian syndrome and its significance

Yuxia Niu[1], Xueling Wang[1], Dongqing Wang[2], Xiufang Jiang[1]

Departments of [1]Obstetrics, [2]MCH, Zhangqiu District People's Hospital, Jinan, Shandong 250200, China

Abstract: Objective: This study aimed to explore the changes of serum vitamin D level in PCOS infertile patients and its significance. Methods: In total, 120 PCOS patients admitted to our hospital from January 2017 to January 2019 were included and divided into the PCOS Infertility Group (PCOS IG) (n=67) and the control group (CG) (n=53) based on their fertility. The two groups were compared for serum levels of 25-(OH) D3, adropin, leptin and Galectin-3. The serum 25-(OH) D3's association with adropin, leptin, Galectin-3, obesity and insulin resistance (IR) were discussed and analyzed. The clinical data of PCOS infertile patients were analyzed by multiple logistic regression to explore the hazards related to the infertility of PCOS patients. Results: As compared with the CG, patients in the PCOS IG demonstrated sharp reduction in serum levels of 25-(OH) D3 and adropin, and a rise in levels of leptin and Galectin-3 ($P<0.05$). Serum 25-(OH) D3 is positively associated with adropin, and negatively associated with leptin and Galectin-3 ($P<0.05$). In the PCOS IG, obese patients reported significantly lower serum 25-(OH) D3 and HOMA-IS, and higher HOMA-IR and HOMA-β than those in the CG ($P<0.05$), IR patients yielded lower serum 25-(OH) D3 and higher BMI and waist-hip ratio (WHR) than those in the CG ($P<0.05$). Serum 25-(OH) D3 was found to be positively associated with HOMA-IS, and negatively associated with HOMA-IR, HOMA-β, BMI and WHR ($P<0.05$). According to the multiple logistic regression analysis, independent hazards related to PCOS infertility include BMI ≥28 kg/m^2, deficiency of Vitamin D, and HOMA-IR >3.8 ($P<0.05$). Conclusion: Compared with females of childbearing age, PCOS infertile women were lower in serum vitamin D level, which further reduced in the case of IR or being concurrently obesity. Therefore, lower-levels of Vitamin D may play an important role in the infertility mechanism of PCOS patients.

Keywords: PCOS, infertility, 25-(OH) D3, deficiency in Vitamin D, IR, obesity

Introduction

Polycystic Ovarian Syndrome (PCOS) is a common anovulatory disorder frequently found in women in adolescence and childbearing age [1]. According to studies, PCOS patients are characterized by endocrine disturbance, low endometrial receptivity, and abnormal ovulatory function, which often lead to infertility, obesity, abortion, amenorrhea, crinosity and such other poor outcomes [2]. Currently, the causes are uncertain but suggested to be associated with factors such as malfunction of hypothalamic-pituitary-ovarian axis, heredity, metabolism, and environment according to some studies [3]. In recent years, the incidence of PCOS is rising in China, accounting for about 5% to 10% of women in childbearing age, leading to most of the infertile cases, other than

those induced by the oviduct [4]. Previous studies have found that Vitamin D can maintain the stable metabolism of calcium and phosphorus in the body, which plays a key role in human reproduction. Calcium is found in the ovaries, intima and placenta, and once deficient, it will result in disorders of calcium regulation, affecting the endometrium and further leading to follicular development obstruction, menoxenia and anovulatory infertility [3-6]. In addition, Vitamin D can, through elevating local 25-(OH) D3 levels, induce the transcription of specific genes and increase the sensitivity of insulin, so as to improve the status of calcium [7]. So far, there are some domestic and foreign studies reporting a higher possibility of pregnancy when Vitamin D level rises higher in serum and liquor folliculi [8, 9], but less is known on the relationship between vitamin D

and a woman's fertility. In this study, 120 PCOS patients admitted to our hospital were studied to explore the changes of serum vitamin D level in PCOS infertile patients and its significance, and any possible role of vitamin D in the infertility of PCOS patients.

Materials and methods

General data

In total, 120 PCOS patients admitted to our hospital from January 2017 to January 2019 were included and divided into the PCOS Infertility Group (PCOS IG) (n=67) and the control group (CG) (n=53), based on if they are fertile. Sixty-seven PCOS infertile patients with mean age of (25.75±4.92) years were included into the PCOS IG, and the other (n=53) fertile patients with mean age of (25.46±4.14) years were included into the CG. All patients and their family members have been informed of the content of this study and provided written informed consent. This study has been approved by the Ethics Committee of Zhangqiu District People's Hospital.

PCOS diagnosis criteria [10].

Patients complied with the PCOS diagnosis criteria published by the International PCOS Coordination Group on the Rotterdam International Conference 2013, including (1) Clear polycystic changes of ovaria according to ultrasonic examination (12 or more follicles with a diameter between 2 and 9 mm at one or both sides, and/or the volume of ovaria exceeding 10 ml); (2) Obvious clinical manifestation and biochemical characters of hyperandrogenism; (3) Spare or no ovulation or irregular endometrorrhagia. If a patient satisfies any 2 criteria listed above being without other causes of hyperandrogenism, such as congenital adrenal hyperplasia, Cushing syndrome, and androgen secreting tumors, she is diagnosed with PCOS.

Inclusion and exclusion criteria

Inclusion criteria: (1) Aged over 18 and under 40 years; (2) Compliance with the PCOS diagnosis criteria listed above; (3) No history of drinking alcohol or smoking; (4) Complete clinical data; (5) No use of any hormonal drugs and other drugs affecting the reproductive endocrine system in the past 3 months. Exclusion criteria: (1) Abnormal heart, liver and kidney

functions; (2) Patients with mental disorders who can't cooperate with the study actively; (3) Patients who were infertile due to other reasons such as congenital deformity, problems in oviducts or abnormal female reproductive functions rather than PCOS; (4) Patients who concurrently suffered from endocrine disorders such as congenital adrenal hyperplasia, diabetes, or chronic diseases including angiocardiopathy and hypertension.

Major experimental reagents and instruments

Human 25-(OH) D3, anti-Müllerian hormone (AMH), Adropin, leptin and Galectin-3 ELISA kits and the supporting reagents were purchased from Nanjing Jiancheng Bioengineering Institute, and the ADVIA2400 automatic biochemical analyzer from Japan Sys-mex.

ELISA detection of serum 25-(OH) D_3, AMH, adropin, leptin and Galectin-3

Five ml of blood was drawn from patients in the two groups on the morning of the 2nd to 5th day during menses in a fasting status, and the blood was placed at room temperature for 2 h. After separating, the serum was placed in a centrifuge for processing at 500 r/min for 10 min to form the supernatant which was then stored in a freezer set at -80°C. ELISA kits were used to test both groups' serum 25-(OH) D3, AMH, adropin, Leptin, and Galectin-3, in strict accordance with the instructions.

Note: the normal range of 25-(OH) D3 falls between 30 and 40 µg/L. The vitamin D is insufficient if the 25-(OH) D3 is lower than 20 µg/L, or relatively deficient if the 25-(OH) D3 is between 21 and 29 µg/L, or when 25-(OH) D3 exceeds 150 µg/L, the patient has vitamin D poisoning [11].

Measurement of obesity related indexes

Body mass index (BMI) is an internationally accepted index used to measure if a woman is obese and health or not. An adult is judged as underweight if her BMI is lower than 18.5 kg/m², normal if between 18.5-23.9 kg/m², overweight if between 24-27 kg/m², obese if between 28-32 kg/m², and very obese if higher than 32 kg/m². The two groups were measured for height and weight to calculate the BMI as body weight (kg)/height (m²) and waist-hip ratio (WHR).

Table 1. Comparison between the 2 groups for general data

Group	PCOS IG (n=67)	CG (n=53)	t/χ^2	P
Mean age (y)	25.75±4.92	25.46±4.14	0.326	0.745
BMI (kg/m^2)	34.37±3.42	28.11±3.79	9.378	<0.001
WHR	1.51±0.08	1.15±0.12	7.713	0.001
FPG (mmol/L)	6.14±0.83	4.28±0.23	17.513	<0.001
FINS (mIU/L)	15.39±6.36	10.91±2.98	5.101	<0.001
HOMA-IR	4.37±0.42	2.11±0.79	18.828	<0.001
HOMA-IS	0.51±0.11	0.59±0.03	5.691	<0.001
HOMA-β	258.87±39.66	168.82±30.71	13.957	<0.001
AMH (ng/ml)	14.37±2.42	11.11±2.79	6.735	<0.001
deficiency in Vitamin D	34	14	7.299	0.007
adropin (ng/ml)	2.37±0.42	9.02±4.79	10.076	<0.001
leptin (ng/ml)	19.71±4.11	13.73±5.39	6.678	<0.001
Galectin-3 (ng/ml)	3.87±0.66	2.82±0.71	8.298	<0.001
25-(OH) D3 (µg/L)	19.75±4.92	25.46±4.14	6.901	<0.001

Table 2. Analysis of correlation between serum 25-(OH) D3 and adropin, leptin, and galectin-3

Related indexes	25-(OH) D3	
	r	P
Adropin	0.241	0.015
Leptin	-0.334	0.001
Galectin-3	-0.437	0.004

Detection of IR-related indexes [12, 13]

All patients were prohibited from food for 12 h and water for 8 h. On the next day morning, 5 ml of blood was drawn in a fasting status, and analyzed for the fasting plasma glucose (FPG, mmol/L) and fasting insulin (FINS, mIU/L) by an ADVIA2400 automatic biochemical analyzer in strict accordance with the instructions. HOMA-IR is an index used to evaluate an individual's IR level according to the expression of HOMA-IR = FPG×FINS/22.5. When HOMA-IR >3.8, IR is confirmed. HOMA-IS is an index used to evaluate an individual's insulin sensitivity according to the expression of HOMA-IS=1/HOMA-IR, and rises as the insulin sensitivity rises. HOMA-β is an index used to evaluate an individual's β islet cells according to the expression of HOMA-β=20×FINS/(FPG-3.5) (%). In a normal individual, the HOMA-β is 100%, and rises with the β islet cells.

Observation indexes

The two groups were compared for serum levels of 25-(OH) D3, adropin, leptin and Galectin-3, and analyzed for the association between serum 25-(OH) D3 and adropin, leptin, Galectin-3, obesity, and IR. The clinical data of PCOS infertile patients are analyzed by multiple logistic regression to explore the hazards related to the infertility of PCOS patients.

Statistical analysis

Statistical analysis was performed with SPSS 22.0. In case of numerical data it was expressed as Mean ± Standard Deviation, comparison studies were carried out through T test for data which were normally distributed; in case of nominal data, comparison studies were carried out through X^2 test. Association was analyzed by the Pearson method. For all statistical comparisons, significance was defined as $P<0.05$.

Results

General data

As compared with the CG, the PCOS IG was significantly lower in HOMA-IS, serum levels of 25-(OH) D3 and adropin, and higher in BMI, WHR, FPG, FINS, HOMA-IR, HOMA-β, AMH, deficiency in Vitamin D, and leptin, Galectin-3 ($P<0.05$, **Table 1**).

Analysis of association between serum 25-(OH) D3 and adropin, leptin, Galectin-3

According to the Pearson association detection results, serum 25-(OH) D3 is positively associated with adropin, and negatively associated with leptin and Galectin-3 ($P<0.05$, **Table 2**).
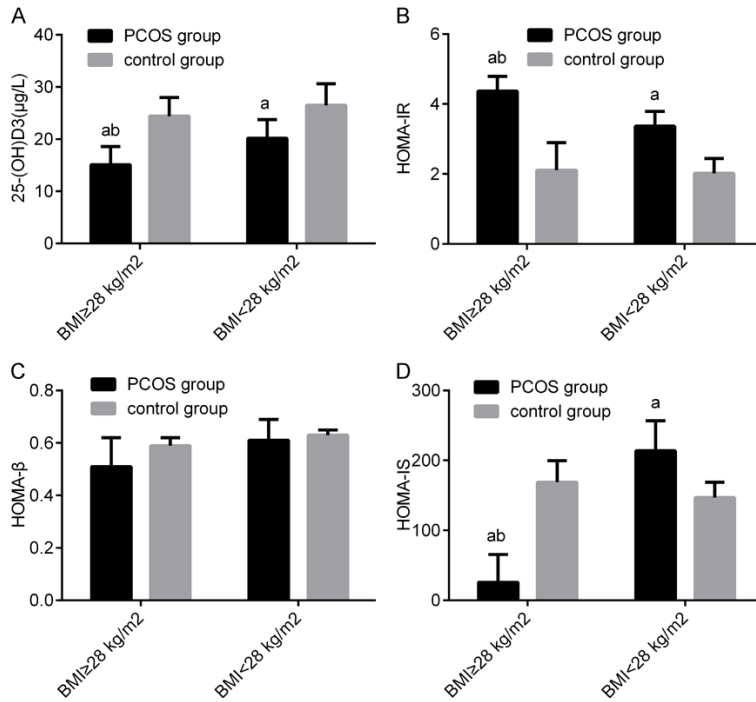
Figure 1. Comparison between obese and non-obese patients for 25-(OH) D3 and IR-related indexes. Note: [a]P<0.05 as compared with the CG, and [b]P<0.05 as compared with patients whose BMI<28 kg/m².
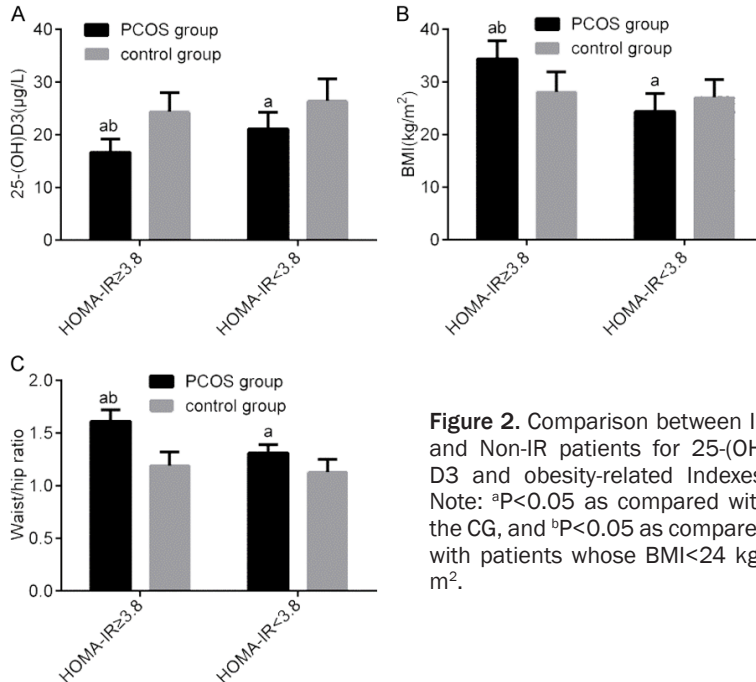

Figure 2. Comparison between IR and Non-IR patients for 25-(OH) D3 and obesity-related Indexes. Note: [a]P<0.05 as compared with the CG, and [b]P<0.05 as compared with patients whose BMI<24 kg/m².

Comparison between the obese and non-obese patients for 25-(OH) D3 and IR-related indexes

For obese and non-obese patients, the PCOS IG yielded significantly lower serum 25-(OH) D3

and HOMA-IS, and higher HOMA-IR and HOMA-β (P< 0.05). In the PCOS IG, patients with BMI ≥28 kg/m² reported serum 25-(OH) D3 and HOMA-IS levels far lower and HOMA-IR, HOMA-β far higher than that of the PCOS infertile patients with BMI<28 kg/m² (P<0.05, **Figure 1**).

Comparison between the IR and non-IR patients for 25-(OH) D3 and obesity-related indexes

For IR and non-IR patients, the PCOS IG yielded significantly lower serum 25-(OH) D3 but higher BMI and WHR (P<0.05). In the PCOS IG, patients with HOMA-IR >3.8 reported serum 25-(OH) D3 far lower and BMI and WHR far higher than that of the PCOS infertile patients with HOMA-IR ≤3.8 (P<0.05, **Figure 2**).

Analysis of correlation between serum 25-(OH) D3 and indexes related to obesity and IR

According to the Pearson association detection results, HOMA-IS is positively associated with serum 25-(OH) D3 (r=0.253; P<0.05), while OMA-IR, HOMA-β, BMI and WHR were negatively associated with serum 25-(OH) D3 (r=-0. 324, -0.411, -0.361, -0.504 respectively; P<0.05, **Table 3**).

Simple analysis results of PCOS infertility

Referring to the simple analysis results, PCOS infertility is related to BMI, deficiency in Vitamin D, HOMA-IR, and family history of PCOS (P< 0.05), and independent of age, crinosity and acne (P>0.05, **Table 4**).

Table 3. Analysis of correlation between serum 25-(OH) D3, obesity and IR-related indexes

Related Indexes	25-(OH) D3	
	r	P
HOMA-IR	-0.324	0.001
HOMA-IS	0.253	0.005
HOMA-β	-0.411	0.004
BMI (kg/m^2)	-0.361	0.035
WHR	-0.504	0.027

Table 4. Analysis results of PCOS infertility

Related Factors		PCOS IG (n=67)	CG (n=53)	X^2	P
Age (y)	>30	42	29	5.684	0.061
	≤30	25	24		
BMI (kg/m^2)	<28	18	41	10.039	0.003
	≥28	49	12		
Deficiency in Vitamin D	No	33	29	11.095	0.014
	Yes	34	24		
HOMA-IR	≤3.8	20	21	12.345	0.022
	>3.8	47	32		
Family history of PCOS	No	24	26	5.341	0.004
	Yes	43	27		
Crinosity	No	32	25	0.154	0.341
	Yes	35	28		
Acne	No	29	31	0.020	0.876
	Yes	38	22		

Multiple logistic analysis results of PCOS infertility

The 4 factors with statistical difference, listed below and in **Table 5**, were taken as the independent variables, valued based on if the PCOS patients is fertile, and multiple analysis by logistic regression method. Hazards related to PCOS infertility include BMI ≥28 kg/m^2, deficiency in Vitamin D, and HOMA-IR >3.8 (*P*<0.05, **Table 5**).

Discussion

PCOS is a common but complicated disease due to the disorder of the endocrine system and metabolism in women at childbearing age; and as an overall general female endocrine disease [14] it not only affects endocrine functions, leads to infertility, but also induces various diseases such as depression, diabetes, endometrial cancer as well as crinosity, obesity, lipsotrichia, etc., all of which can influence the patients' body shape and appearance [15]. So far, there are studies focusing on the important role of vitamin D in reproductive function; deficiency of which is related to abnormal calcium regulation, and may impair the follicular development in PCOS patients, resulting in menstrual disorders and infertility [16, 17].

Deficiency in vitamin D is a hazard related to the infertility of PCOS patients. In this report, patients' vitamin D level was measured by serum 25-(OH) D3, which, if at an excessively low levels, indicates that the patient is deficient in vitamin D, and may worsen the endocrine disturbance of PCOS patients, leading to abnormal ovulation and infertility. Consistent with previous studies, the present study also found that the PCOS IG as compared with the CG, had a lower serum 25-(OH) D3 level [18] and higher BMI and WHR [19] through measurement of indexes related to obesity. Patients with BMI ≥28 kg/m^2 were considered obese, which is a hazard related to infertility of PCOS patients; interestingly whose ovulation may recover to a certain degree after losing weight. When HOMA-IR >3.8, IR is established as another factor leading to the infertility of PCOS patients. In this report, HOMA-IR, HOMA-IS, and HOMA-β were used to measure the IR level. So far, while HOMA-IR is extensively applied, HOMA-IS is used to evaluate an individual's insulin sensitivity and is negatively associated with the IR; HOMA-β is an index representing the function of an individual's β islet cells, between which, a positive relation is established.

Studies on the relationship between 25-(OH) D3 and obesity and IR found that, in the serum of obese PCOS infertile patients, 25-(OH) D3 was at a level far lower than that of a non-obese patient, while HOMA-IR was far higher. In PCOS infertile patients with IR, serum 25-(OH) D3 level was clearly lower than and the BMI was far higher than those patients who were not

Table 5. Multiple logistic analysis results of PCOS

Item	Regression Coefficient β	SE	Wald	P	OR (95% CI)
BMI ≥28 kg/m²	4.516	1.258	19.132	0.004	2.967 (1.642~5.259)
deficiency in Vitamin D	0.925	0.285	10.841	0.019	2.231 (1.349~4.625)
HOMA-IR >3.8	0.748	0.234	6.877	0.047	4.005 (2.041~6.793)
With family history of PCOS	2.918	0.842	0.421	0.079	2.377 (0.249~3.641)

resistant to insulin. Through Pearson correlation detection, serum 25-(OH) D3 was shown to be positively associated with HOMA-IS and negatively associated with BMI. The reasons may include endocrine dyscrasia and compromised insulin function in PCOS infertile patients due to obesity, which lead to IR [20]. In addition, obesity resulted in deficiency in Vitamin D, a liposoluble substance, restricted vitamin D in fat tissue and reduced circulating level, leading to lower bioavailability [21]. Furthermore, with less willingness to exposure themselves to the sun, obese patients may contain less vitamin D in their skin.

Studies in recent years have found that adropin, leptin, and Galectin-3 were related with obesity, insulin level and high androgen level; which, if suppressed, could contribute to the improvement of IR [22, 23] and play an important role in the occurrence and development of PCOS. According to the discoveries in this study, the serum levels of 25-(OH) D3 and adropin were clearly lower and the leptin, Galectin-3 were clearly higher in the PCOG IG. According to Pearson correlation detection, serum 25-(OH) D3 was positively associated with adropin, and negatively with leptin and Galectin-3. An excessively low 25-(OH) D3 level indicates a deficiency in Vitamin D, which may lead to anovulatory infertility and IR. Adropin protein is one of the important indexes used to evaluate IR and is positively associated with IR [24].

In conclusion, compared with females of childbearing age, PCOS infertile women were lower in serum vitamin D level, which was further reduced in the case of IR or concurrently obesity. Therefore, lower-levels of Vitamin D may play an important role in the infertility mechanism of PCOS patients.

Disclosure of conflict of interest

None.

Address correspondence to: Xiufang Jiang, Department of Obstetrics, Zhangqiu District People's Hospital, No. 1920, Huiquan Road, Zhangqiu District, Jinan 250200, Shandong, China. Tel: +86-531-83250519; E-mail: xiufangjiangjiang@163.com

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