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Risk of metabolic syndrome in women with reproductive dysfunction and the serum levels of vitamin D.

E. A. Gafarova*, and L. I. Maltceva.

SBEI CPE "Kazan State Medical Academy", Ministry of Health of the Russian Federation, Kazan, Russian Federation

ABSTRACT

Assessment of the blood level of 25 (OH) D3 and its influence on the development of hormonal and metabolic disorders in women with polycystic ovary syndrome (PCOS) and reproductive dysfunction. For this purpose, 45 women with PCOS and 15 healthy women were examined. We have conducted a general clinical examination, the assessment of constitutional features, degree of hirsutism, hormonal levels, and carbohydrate metabolism parameters. The content of vitamin D metabolite in serum was determined by ELISA with the use of a set of 25 (OH) D2 and D3 DIA SOURCE. We observed a decrease in blood levels of 25 (OH) D2 and D3 in women with polycystic ovary syndrome. Severe deficiency (11.49±2.1 ng/ml) was observed in patients with hyperandrogenic phenotypes of PCOS, the insufficient content (21.8±3.58 ng/ml) was observed in patients without severe hyperandrogenism. A deficiency of 25 (OH) D2 and D3 in women with PCOS was accompanied by insulin resistance, hyperinsulinemia, abdominal obesity, and increased anti-Mullerian hormone by 3 times. Vitamin D deficiency in the blood of patients with PCOS is accompanied by changes in hormonal levels, hyperandrogenism, and the development of symptoms of metabolic syndrome.

Keywords: polycystic ovary syndrome, vitamin D, 25 (OH) D3, insulin resistance, hyperinsulinemia, abdominal obesity, hyperandrogenism, anti-Mullerian hormone

Corresponding author

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INTRODUCTION

Vitamin D refers to the fat-soluble vitamins. Vitamin D and its active metabolites are the structural units of the hormonal system that regulates not only the calcium and phosphorus metabolism, but also the metabolic processes. Vitamin D plays active role in the functioning of the reproductive system. A hepatogenous metabolite 25-OH-D3 is highly active, of stable level in liver, reflects the total intake of vitamin D with food, and its formation in the skin normally ranges 10 to 100 ng/ml. The most active metabolite of vitamin D_3 - $1,25(OH)_2D_3$ is synthesized in the kidney as a result of the activity of 1 α-hydroxylase. Regulation of the synthesis of $1,25(OH)_2D_3$ is performed by parathyroid hormone. Sex steroids (estrogens, androgens) have the activating effect on the 1 α-hydroxylase [1]. In recent years it has become known that $1,25(OH)_2D_3$ inhibits proliferation and accelerates differentiation of a large number of tumor cells that induce the expression of receptors for vitamin D. In the near future one can expect the use of vitamin D derivatives as mono- and combination therapy of many tumor diseases (carcinoma, human breast, colon tumors) [2].

Over the years there has been a growing interest in vitamin D as a factor affecting the reproductive function. A lot of data have been accumulated about the reduction of the content of vitamin D in the blood of women with various reproductive system disorders, such as polycystic ovary syndrome, infertility, uterine myoma, endometriosis, premature ovarian failure syndrome, IVF failure [3]. Al-Hendy et al., 2015 found an inverse correlation between the reduced expression of the receptors for vitamin D and increased expression of estrogen α receptors and progesterone A and B receptor. Treatment with vitamin D resulted in a significant decrease in the expression of both estrogen and progesterone receptors in the uterine fibroid tissue. Exogenous $1,25(OH)_2D_3$ induced the vitamin D receptors (VDR), which was reflected in the increased amount of associations of retinoid-X (RXR) and receptor-α. In this case, vitamin D acts as an active antagonist of sex steroid receptors (estrogen and progesterone) and a powerful anti-estrogenic agent for the treatment of uterine fibroids [4]. On the other hand, it is assumed that a high concentration of calciferol may be associated with impaired rejection of endometrial cells entering the peritoneal cavity through the fallopian tube, whereby endometriosis occurs. In case of male infertility, both low (<20 ng/ml) and high (>50 ng/ml) blood concentrations of vitamin D adversely affect the number of sperm cells per milliliter of semen, as well as their translational motion and morphology [5]. Better IVF results are observed in patients without vitamin D deficiency, which is explained by high concentration of vitamin D and its metabolites in human decidua tissue collected in the 1st trimester of pregnancy, and its proper contribution to the quality of the implant and local immunological embryo preference is assumed [6]. Vitamin D receptors are found virtually in all target tissues of the organism. The effect of vitamin D on the female reproductive organs proves the fact that the expression of mRNA receptors for vitamin D (VDR) is in the tissues of the genital organs: placenta, myometrium, endometrium, ovary, mixed ovarian cells and the cultures of treated granulosa cells, which indicates a role of sex hormones in steroidogenesis. It was found that human placenta expresses a CYP27B1 gene (encodes 1α-hydroxylase) and vitamin D receptors (VDR). Viganò et al. demonstrated that the endometrium is able to extrarenally synthesize the active form of vitamin D [7]. In addition, the authors showed that the active form of 1α-hydroxylase gene is expressed in human endometrial stromal cells regardless of cycle phase, but with a significant increase in early pregnancy in the decidua. In ovarian tissue, $1,25(OH)_2D_3$ stimulates the progesterone production by 13% and estradiol by 9%, and estrone by 21%. Studies dealing with the sufficiency of vitamin D in patients with polycystic ovary syndrome (PCOS) have been conducted in many countries around the world and discovered its decline even where there is enough sunlight.

For example, Irani M. et al., in 2014 examined the blood content of vitamin D in 57 healthy women and 20 patients with PCOS and found reduction in vitamin D levels in PCOS women below 20 ng/ml, whereas healthy patients had normal vitamin D levels averaging 34.6±2.8 ng/ml. The presence of PCOS was confirmed by a significant increase in the AMH blood content. The authors suggested that vitamin D3 improves the quality of follicles in women with PCOS, as evidenced by the normalization of abnormally elevated levels of anti-Mullerian hormone after therapy of 50,000 IU of vitamin D3 once a week for 8 weeks (from 5.3±0.6 to 3.9±0.5, p=0.003) [8].

Some studies have demonstrated a high prevalence of deficiency of 25(OH) D_3 and the relationship between low levels of vitamin D and metabolic risk factors, insulin resistance and reduced insulin sensitivity. However, the relationship between the level of vitamin D and the genetic condition of its receptor and the frequency of the metabolic syndrome or its individual symptoms remains controversial. Our study showed that in women of reproductive age the vitamin D deficiency and the presence of A-allele of ApaI polymorphism of

its receptor may pose a non-classical risk factors for metabolic disorders [9]. Blood levels of 25 (OH) D_3 of 30-45 ng/ml, optimal for the prevention of many diseases and complications of pregnancy, can be achieved with daily doses of 2,000 to 4,000 IU. However, the literature contains not enough data on the blood content of vitamin D in women with reproductive dysfunction, residing in the European part of the Russian Federation. The foregoing demonstrates the need for further study of the role of vitamin D in the functioning of the female reproductive system.

Objective of our study was the assessment of the blood level of 25 (OH) D3 and its influence on the development of hormonal and metabolic disorders in women with polycystic ovary syndrome and reproductive dysfunction.

MATERIALS AND METHODS

The study involved 45 women with impairments typical of polycystic ovary syndrome, namely oligo/amenorrhea, hyperandrogenism, ultrasound signs of PCOS, and infertility. All patients underwent general clinical and ultrasound examination, evaluation of constitutional features (height, weight, BMI, waist/hips measurement), hormonal levels (FSH, LH, the ratio of LH/FSH, prolactin, total testosterone, androstenedione, sex steroids binding globulin in the serum blood, and anti-Mullerian hormone). To exclude the thyroid disease and adrenal hyperandrogenism, which may also cause reproductive dysfunction, the TSH T3sv, T4sv, Ab to TPO, parathyroid hormone, DHEAS, 17-OH were determined by ELISA. Patients with hyperprolactinemia, thyroid dysfunction and adrenal hyperandrogenism were excluded from the study. The assessment of hirsutism severity was performed using Ferriman-Galway scale. Based on the results of a comprehensive study we determined the phenotype of PCOS. We examined all patients for the content of vitamin D metabolite in their serum using a set of 25 (OH) D2 and D3 DIA SOURCE by ELISA. The results were compared with data of 15 healthy women with normal menstrual and reproductive function, who represented the control group. Mathematical analysis of the results was performed using a standard set of statistical processing functions with Excel MS Office XP and STATISTICA 6.0 software packages.

RESULTS AND DISCUSSION

The average age of the patients did not exceed 27±3.6 years, age at menarche was 13.42±1.49 years. Somatic history of the patients was burdened with gastrointestinal diseases in 30% (chronic gastritis, cholecystitis), and chronic respiratory diseases (chronic tonsillitis, sinusitis, chronic pharyngitis) in 32% of women. Chronic cervicitis was the most common concomitant gynecologic disease (15%). The vast majority of patients complained of disturbance of the menstrual cycle and the absence of pregnancy. Menstrual cycle duration ranged 28 to 46 days and averaged 34.4±4 days. Only 7 women (17.5%) had a history of pregnancy, with later disturbances of menstrual and reproductive function.

Analysis of the blood content of vitamin D in patients of the main group showed changes in all cases. However, the degree of reduction of vitamin D was different. Twelve women were identified with severe vitamin D deficiency not exceeding the average level of 11.49±2.1 ng/ml, who formed subgroup 1. The average level of vitamin D in 28 women was significantly higher than that of the first subgroup of patients (21.8±3.58 ng/ml), but much lower than the normal range (38.2±1.3 ng/ml). These patients formed subgroup 2. We conducted a comparative assessment of two sub-groups of patients with varying degrees of reduction of vitamin D. It was found that depending on the blood content of vitamin D the women differ in stature, hormonal and metabolic parameters.

Analysis of the data presented in Table 1 showed some differences between patients of subgroups 1 and 2. Comparative evaluation of height and weight in women of both groups revealed no significant differences. Nevertheless, it turned out that the woman of subgroup 1 had significantly higher BMI (27.98±1.63) and a tendency to increase in waist to hips ratio (0.81±0.06), as compared with healthy women (BMI = 21, 76±2.3; waist to hips ratio = 0.74±0.04), whereas these parameters in two subgroups of patients tend to normal values (22.5±2.2 and 0.76±0.03, respectively). This indicates a significant manifestation of abdominal obesity in patients with severe deficiency of vitamin D in the blood. The relationship between the deficiency of vitamin D in the blood and body mass index has been demonstrated in the foreign studies [10]. Furthermore, the first group of patients had significant changes found in the parameters of carbohydrate metabolism (Table 2).

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Table 1. Constitutional parameters and morphometric indicators depending on the blood content of vitamin D in patients with PCOS.

Parameters	Main group		Control group (n=15)
	First subgroup (n=12)	Second subgroup (n=28)	
Height, m	1.64±0.03	1.67±0.05	1.68±0.03
Weight, kg	72.5±8.21	61.5±8.87	60.2±3.2
BMI	27.98±1.63*	22.5±2.2*	21.76±2.3
Waist circumference, cm	85.5±3.5	73.7±2.4	70.1±2.2
Hips circumference, cm	105±7.4	94±6.4	94±2.1
Waist to hips ratio	0.81±0.06	0.76±0.03	0.74±0.04

* - $p \leq 0.05$

As can be seen from Table 2, the oral glucose tolerance test with 75 g of dry glucose conducted according to international standards revealed no significant differences in the content of venous plasma glucose between the groups. However, 2 women with signs of severe impaired glucose tolerance (glucose level after 1 hour reached 13.2 mmol/l) were found in the first subgroup, while in the second subgroup no similar changes were detected.

Table 2. Parameters of carbohydrate metabolism in patients with PCOS depending on the blood level of vitamin D.

Parameters	Main group		Control group (n=15)
	First subgroup (n=12)	Second subgroup (n=28)	
Vit D, ng/ml	11.49±2.1	21.8±3.58	38.2±1.3
Fasting insulin, µIU/ml	15.135±5.46*	6.2±1.7*	5.7±1.1
Fasting blood glucose, mmol/l	4.99±0.38	4.6±0.2	4.3±0.2
Blood glucose after 1 hour, mmol/l	8.58±2.7	6.3±1.1	6.28±0.8
Blood glucose after 2 hours, mmol/l	6.38±1.25	5.5±0.6	5.4±0.3
HOMA index	3.35±1.2	1.26±0.07	1.08±0.02

* - $p \leq 0.05$

This suggests the possible development of insulin resistance depending on the blood content of vitamin D in patients with PCOS. It is the first subgroup where more significant hyperinsulinemia (GI) was established. Insulin levels were 2.44 times higher in women with vitamin D deficiency than in patients of the second subgroup. It is known that increased levels of fasting insulin may be an indicative measure of insulin resistance [11]. In addition, there is a significant difference in the HOMA index between the groups, which in the first subgroup was 2.6 times higher than in the second. The described constitutional and laboratory abnormalities in patients with severe deficiency of vitamin D in the blood indicate a relationship between the risk of metabolic syndrome in PCOS patients with vitamin D deficiency, and the lower the level of 25 (OH) D3 is, the more pronounced changes are observed. This dependence of carbohydrate metabolism parameters on the blood levels of vitamin D is described in the literature [12].

In addition to metabolic changes, the examined women had hormonal disorders characteristic of polycystic ovarian syndrome. Despite the similar clinical manifestations and the lack of significant differences in the duration of the menstrual cycle, there has been a number of significant differences in hormonal levels in patients with vitamin D deficiency (Table 3).

Table 3. Hormonal levels in PCOS women depending on the blood level of vitamin D.

Parameters	Main group		Control group (n=15)
	First subgroup (n=12)	Second subgroup (n=28)	
Vit D, ng/ml	12.49±3.45	21.8±3.58	38.2±1.3
FSH, mIU/l	5.02±1.45	5.84±1.9	4.8±1.2
LH, mIU/l	9.21±3.6	5.97±2.65	3.9±0.8

LH/FSH	1.83±0.86	1.03±0.27	0.81±0.12
Total testosterone, nmol/ml	2.96±1.8	1.5±0.9	1.4±0.02
Androstendione, ng/ml	8.88±1.48	5.28±2.37	5.27±0.51
SSBG, nmol/l	30.5±9.6	60.01±10.3	64.5±7.5
AMH, ng/ml	24.8±10.4	10.34±4.27	7.8±2.4
Parathyroid hormone	58.33±11.37	39.24±12.86	33.8±9.5

* - $p \leq 0.05$

The patients of subgroup 1, as compared to subgroup 2, had an increased LH/FSH index - 1.83±0.86 against 1.03±0.27, whereas in healthy women this index did not exceed 0.81±0.12 and was accompanied by a regular menstrual cycle. A significant difference was revealed in androgen indices. Total testosterone in subgroup 1 was almost 2 times higher (2.96±1.8 nmol/l) than in the subgroup 2 (1.5±0.9 nmol/l), androstenedione was 1.7 times higher in the first subgroup as compared with the second (8.88±1.48 ng/ml and 5.28±2.37 ng/ml, respectively). Double reduction of SSBG level in the first subgroup (30.5±9.6 nmol/l) as compared with the second (60.01±10.3 nmol/l) and a group of healthy women (64.5±7.5) may be a logical reason for the increase of androgens in the blood and may depend on the blood content of vitamin D in women.

Biochemical hyperandrogenism is reflected in the phenotype of PCOS - the women with very low levels of vitamin D had predominant phenotypes A, B, C as compared with higher values of 25 (OH) D3, with predominant phenotype D. It is known that the first three phenotypes are characterized by the presence of clinical and laboratory signs of hyperandrogenism and a higher Ferriman-Galway score. According to the population-based study conducted by academician Dedov I.I., 75% of women with hirsutism and/or hyperandrogenism with or without oligo/anovulation have polycystic ovary syndrome [13]. Data from modern literature indicate an increased AMH level as an important sign of polycystic ovary syndrome [14, 15]. The AMH level was high in all patients, but significant increase in anti-Mullerian hormone values was observed in women with very low levels of 25 (OH) D3 - 24.8±10.4 ng/ml, which is about 3 times higher than in healthy women without PCOS (7.8±2.4 ng/ml). The second group had AMH level only 1.3 times higher (10.34±3.6 ng/ml). This suggests a possible effect of vitamin D on ovarian function.

An interesting fact is that the prolonged hyperandrogynism in general and the increased testosterone concentration in the blood in particular lead to hypercalcemia caused by calcium release from bone depot. This process is regulated by calcitonin - a hormone of thyroid C-cells, which increases in response to the administration of exogenous testosterone, as well as PTH, which promotes an increase in the blood level of calcium. Calcium enters the cells at a concentration gradient that against hypercalcemia can lead to excessive accumulation of calcium ions within the cell and facilitate cell damage [16]. As is known, the concentration of calcium in blood serum is regulated with high precision and varies within a very narrow range [17]. It is natural that in our study the patients with very low levels of vitamin D and marked clinical laboratory hyperandrogenism had PTH level 1.5 times higher than in women of the second group and the control group, which can be explained by a compensatory response of the body to reduced absorption of calcium on the background of lack of vitamin D and the triggering of the regulation mechanism of the constant blood level of calcium.

Considering the obtained results (reduced levels of vitamin D in all patients with PCOS, hormonal changes, metabolic disorders) and literature data about the possibility of the use of high doses of vitamin D to restore the reproductive function, we have included vitamin D in the treatment scheme for PCOS. All patients received Aquadetrim, 12 drops (6000 IU vitamin D) once a day for 3 months, after which the second study was conducted. It turned out that the constant administration of high doses of vitamin D for three months led to the restoration of normal blood levels of 25 (OH) D3 only in the patients of the second group with a moderate decline in vitamin D. While patients with severe vitamin D deficiency only showed a tendency toward the normalization of parameters and required longer drug intake. However, the change in the blood content of vitamin D led to reduced levels of androstenedione, AMH, and increased SSBG already by the end of the third month of therapy. There was a trend toward normalization of the ratio of gonadotropins (LH/FSH) to indicators of glucose-tolerance test, with simultaneous reduction in the blood level of insulin, which is reflected in changes of anthropometric indicators - reduction in waist circumference and body mass. Women reported

shortening of intermenstrual intervals and renovation of the menstrual cycle. One patient turned to have a spontaneous pregnancy.

SUMMARY

- The blood content of vitamin D was low in all patients with PCOS, with a pronounced deficit observed in women with hyperandrogenous phenotypes of PCOS, and the lack of vitamin D - in women without hyperandrogenemia.
- Vitamin D deficiency in patients with PCOS is accompanied by changes in hormonal levels: increased ratio of gonadotropins (LH/FSH>1.0), androstenedione, AMH, and decreased SSBG, which, in turn, is reflected in the clinical manifestations of the disease.
- An expressed reduction in vitamin D levels leads to the development of symptoms of metabolic syndrome (visceral obesity, hyperinsulinemia, insulin resistance), which exacerbates reproductive disorders in patients with PCOS.

CONCLUSION

The blood content of vitamin D in patients with PCOS plays a certain role in the development of symptoms of menstrual dysfunction, manifestations of hyperandrogenism and a phenotype of the disease. The possibility of using vitamin D in the treatment of PCOS, the selection of the optimal dose and duration of therapy also require further investigation.

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