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ORIGINAL ARTICLE

25(OH)D serum concentration in women with menstrual disorders –risk factors for Vitamin D deficiency

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Abstract

INTRODUCTION: Vitamin D (VD) plays a crucial role in calcium metabolism as well as immunological and endocrine homeostasis. Previous studies revealed strong inverse correlation between VD levels and insulin resistance, parathyroid dysfunctions and autoimmune thyroid disease. Insufficient evidence concerns its dependency of ovarian hormones. Malfunctioning of the ovaries results in menstrual disorders that are one of the most common endocrine impairments in young women of reproductive age.

MATERIAL AND METHODS: The study was aimed to evaluate the correlation between 25(OH)D serum concentration and estradiol, testosterone as well as body mass index (BMI) in women with oligomenorrhea. 134 women of reproductive age with oligomenorrhea were eligible for the study. 25-hydroxyvitamin D [25(OH)D], estradiol, testosterone and sex hormone-binding globulin (SHBG) were measured using chemiluminescence immunoassay. Free androgen index (FAI) and body mass index (BMI) were calculated.

RESULTS: Critical 25(OH)D deficiency (<10 ng/ml) was found in 13.4% of women, the risk of deficiency (<30 mg/dl) was diagnosed in 69.4%, while sufficient level of VD (>30 mg/ml) in 17.2% of them. Significant negative correlation was detected between 25(OH)D and estradiol serum concentrations (r=-0.2; p=0.049), as well as BMI levels (r=-0.22; p=0.01). However, no significant correlation was found between 25(OH)D and testosterone (r=-017; p=0.055), SHBG (r=0.08; p=0.4) and FAI (r=-0.1; p=0.24).

CONCLUSIONS: Thorough assessment of vitamin D deficiency/insufficiency is required among patients with menstrual disorders, especially those overweighed and obese. Early screening and VD supplementation in women with estrogen-dependent disorders may become a part of routine management in order to optimize endocrine health.

INTRODUCTION

Vitamin D (VD) deficiency is a globally widespread problem affecting 13 to 41.6% people in developed countries and varies greatly depending on the season (Cashman *et al.* 2016).

Since 1914, when it was first extracted, most of the studies had been aimed to determine VD contribution to bone metabolism and calcium-phosphate homeostasis (Wolf, 2004). Recently, a fervent discussion about its multimodal influence on human systems is observed in the literature. The term vitamin D is used for two secosteroids: vitamin D2 (ergocalciferol) and D3 (cholecalciferol). The main source of VD derives from its synthesis in the skin during sun and ultraviolet light exposure, however, it may also be delivered from diet, especially fatty fish and eggs. Both forms of vitamin D are biologically inactive. The process of hydroxylation in liver into 25-hydroxyvitamin D (25(OH)D) and in kidney into 1,25-dihydroxyvitamin D (1,25(OH)2D) allows the metabolites to bind the nuclear receptor triggering biological auto and/or paracrine effects.

Nowadays, we know that VD is involved in neuromuscular function, cell proliferation, apoptosis and cytokine secretion (Cantorna *et al.* 2015). The dependency between VD deficiency and autoimmune disorders, cancerogenesis, or fertility impairments appears particularly challenging (Bikle, 2014; Dabrowski *et al.* 2015; Danescu *et al.* 2009; Grzechocinska *et al.* 2013). Previous investigators also suggested that vitamin D deficiency might be involved in the development of endocrine impairments, such as Hashimoto's thyroiditis, Graves' and Addison's diseases (Lawnicka *et al.* 2018). All of them are mediated via immune mechanisms (Muscogiuri *et al.* 2014). High levels of antithyroid antibodies, as well as the incidence of autoimmune thyroiditis are more frequently diagnosed among patients with VD deficiency (Kivity *et al.* 2011).

Most of the clinical trials that focused on the dependency between gonadotropins and VD concentrations involved male subjects, while data concerning female patients are insufficient (Jukic *et al.* 2015; Kozakowski *et al.* 2014). In men vitamin D deficiency seems to be associated with hypogonadism and poorer semen quality (Abbasihormozi *et al.* 2017; Lee *et al.* 2012).

In women strong dependency between VD levels and obesity and insulin resistance, was observed. Moreover, VD deficiency affects 67 to 85% of these women, which contributes to a much higher rate than in the general population (Joham *et al.* 2016; Thomson *et al.* 2012). Nevertheless, there is no consistent evidence for the causal relationship between the events. The decreased skin synthesis with sunlight activation is suggested (Palacios *et al.* 2012). Some studies indicate that vitamin D deficiency can be associated with polymorphism in vitamin D binding protein or vitamin D receptors' genes, as it is found in women with high BMI (Almesri *et al.* 2016). The relationship between certain VD receptor genotype combinations and the risk of pregnancy complications (e.g. preterm birth) was also revealed (Baczynska-Strzecha & Kalinka, 2016).

AIM OF THE STUDY

The aim of the study was to evaluate the correlation between of 25(OH)D serum concentration and estradiol, testosterone and body mass index (BMI) in women with oligomenorrhea.

METHODS

A single center cohort study was performed at the [blinded] between March and May 2016 in the group of 134 Caucasian women. All participants were at reproductive age (16-45 years old) and diagnosed with oligomenorrhea. Oligomenorrhea was defined as menstrual cycles occurring at intervals greater than 35 days. The study was conducted with accordance to the Declaration of Helsinki for Medical Research involving Human Subject and ethical approval was obtained from the Ethics Committee of Medical University of Warsaw (AKBE/150/15). All participants gave a written informed consent for the trial. The exclusion criteria included the onset of menopause, pregnancy, any chronic diseases, known chromosomal abnormalities and current hormonal therapy or hormonal contraception. Blood samples were collected in all women between 3rd and 5th day of the cycle. The weight and height were measured and body mass index (BMI) was calculated. Serum 25(OH)D concentrations were measured by chemiluminescence immunoassay (ECLIA, Cobas e, Roche), which is widely used in clinical practice. Estradiol, testosterone and sex hormone binding globulin (SHBG) were measured by chemiluminescence immunoassay (ECLIA, Cobas e, Roche). Finally, FAI was calculated for each subject as total testosterone level divided by the sex hormone binding globulin (SHBG) level, and then multiplying by 100.

Statistical analysis was performed with Statistical Analysis Statement program (SAS 12.0). Descriptive statistics were performed. The correlation between the variables was evaluated with the Pearson's correlation coefficient test and Spearman rank correlation test. Student's t-test and Mann Whitney U test were performed to demonstrate significant differences between groups. The null hypotheses were tested at a significance level of $p< 0.05$.

RESULTS

General characteristic of the studied population

25(OH)D deficiency was estimated below 10 ng/ml. Out of 134 studied subjects 16 women (12%) had serum 25(OH)D concentration under the estimated threshold of 10 ng/ml (deficiency group, DG). Most of the measurements (92 patients, 69%) were evaluated

Tab. 1. Descriptive statistics of the study group

	N	Mean	CI min	CI max	Median	Min	Max	SD
Age	134	27.22	26.20	28.24	26.00	17.00	44.00	5.97
BMI [kg/m^2]	134	25.13	24.03	26.24	22.65	16.10	48.00	6.49
25 (OH) D [ng/ml]	134	19.49	17.94	21.03	18.35	6.00	48.00	9.02
Estradiol [pg/ml]	134	45.00	39.38	50.62	36.46	5.00	188.50	32.89
Testosteron [nmol/l]	134	1.52	1.39	1.65	1.39	0.27	3.82	0.78
SHBG [nmol/l]	134	68.00	61.46	74.55	59.40	10.80	164.20	38.32
FAI	134	3.08	2.58	3.58	2.05	0.17	16.06	2.97

Tab. 2. Descriptive statistics among patients with 25(OH)D level >10 ng/ml (NDG) and VD deficiency (DG)

	Group	N	Mean	CI min	CI max	Median	Min	Max	SD
Age	NDG	118	26.86	25.78	27.95	26.00	17.00	44.00	5.94
	DG	16	29.81	26.78	32.84	30.00	19.00	41.00	5.68
BMI [kg/m^2]	NDG	118	24.72	23.63	25.81	22.55	16.10	41.00	5.96
	DG	16	28.20	23.27	33.13	23.50	18.40	48.00	9.26
25 (OH) D [ng/ml]	NDG	118	21.08	19.55	22.62	20.00	10.00	48.00	8.41
	DG	16	7.69	7.05	8.34	7.63	6.00	9.50	1.21
Estradiol [pg/ml]	NDG	118	41.30	36.77	45.84	35.25	5.00	120.00	24.88
	DG	16	72.22	39.09	105.35	50.43	5.00	188.50	62.17
Testosteron [nmol/l]	NDG	118	1.49	1.35	1.62	1.35	0.27	3.40	0.76
	DG	16	1.86	1.29	2.42	1.77	0.31	3.82	0.93
SHBG [nmol/l]	NDG	118	68.67	61.59	75.76	59.40	10.80	164.20	38.85
	DG	16	63.08	44.47	81.68	58.65	12.40	149.00	34.92
FAI	NDG	118	3.09	2.56	3.62	2.03	0.21	16.06	2.99
	DG	16	3.03	1.29	4.78	2.50	0.17	11.29	2.88

as border levels of 25(OH)D and ranged from 10 to 29 ng/ml. Only 26 (19%) patients had sufficient levels of 25(OH)D equal or above 30 ng/ml. The lowest serum concentration of 25(OH)D was 6 ng/ml, while the highest equaled 48 ng/ml. Mean values equaled 7.7±1.2 and 21±8.4 ng/ml in the respective groups. The measurements of equal or more than 10 ng/ml (border: 10-30 ng/ml and sufficient: >30 ng/ml) were further evaluated together (non-deficiency group, NDG). Descriptive data of the study group are presented in Table 1 and 2.

The average age of women with 25(OH)D deficiency and 25(OH)D serum concentration greater than 10 ng/ml were similar (29.8 ± 5.7 and 26.9 ± 5.9 years, respectively), Higher BMI levels were found in patients with serum 25(OH)-D below 10 ng/ml and equaled 28.2 ± 9.3 kg/m2 vs. 24.7 ± 6.0 kg/m2 in NDG. There was a significant difference in BMI between both groups depending on 25(OH)D level (t=-2.03; p<0.043). Poor negative correlation between 25(OH)D and BMI was detected (r= -0.3 with p<0.009, Figure 1).

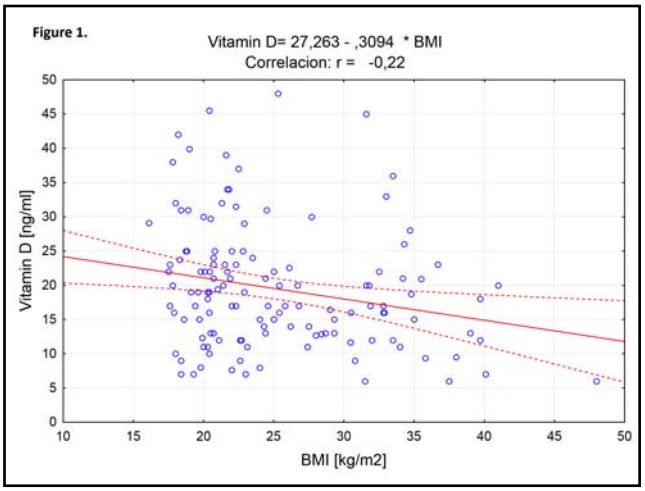

Fig. 1. Dependency between vitamin D and BMI.

Hormone concentrations

Women diagnosed with 25(OH)D deficiency had similar serum concentration of testosterone (1.86 nmol/l vs. 1.49 nmol/l) and SHBG (63.1 nmol/l vs. 68.7 nmol/l) to women with insufficient and sufficient 25(OH)Dconcentrations. FAI calculated in both groups were similar as well (3 vs. 3.1, p = 0.9). Women with 25(OH)D deficiency had significantly higher estradiol concentrations than women with 25(OH)D >10ng/ml (p<0.0003). A poor negative correlation between 25(OH)D status and estradiol concentration was found (r=-0.2; p<0.049, Figure 2). All the correlation coefficients are presented in Table 3.

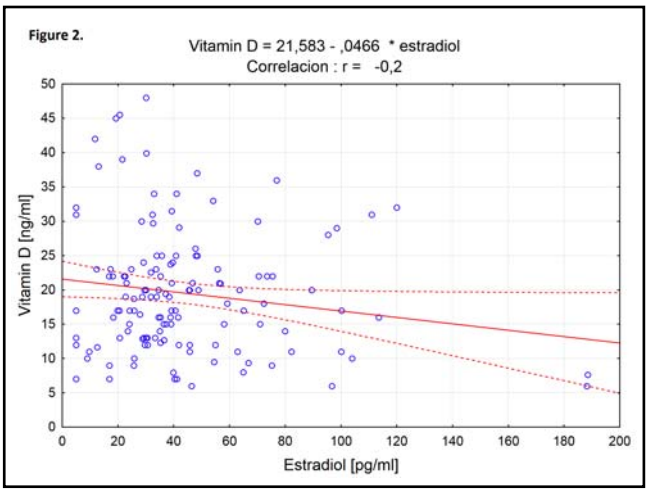

Fig. 2. Dependency between vitamin D and estradiol.

Tab. 3. Correlation coefficient between 25(OH)D and evaluated variables

Correlated parameters	Mean	SD	r	p
25(OH)D & age	27.21642	5.97030	-0.115239	0.184868
25(OH)D & BMI	25.13470	6.49378	**-0.222810**	**0.009663**
25(OH)D & Estradiol	44.99545	32.88841	**-0.170007**	**0.049546**
25(OH)D & Testosteron	22.94149	22.83726	-0.166252	0.054876
25(OH)D & SHBG	68.00418	38.32107	0.078484	0.367376
25(OH)D & FAI	50.99568	69.25209	-0.102273	0.239641

Multiple regression analysis was conducted to isolate the factors influencing 25(OH)D serum concentration in the studied groups. It was found that among the evaluated variables (age, BMI, estradiol, testosterone, FAI and SHBG) only BMI and estradiol concentrations were important risk factors for 25(OH)D status (F = 6.90; p =0.0097 and p=0.049, respectively).

The analysis of correlation coefficients between other variables revealed a positive correlation between BMI and FAI (r=0.35; p=<0.0001). Additionally, the authors found a significant negative correlation between BMI and SHBG (r=-0.48 ; p<0.0001). Upon assessment of the whole study group , no significant correlation between BMI and estradiol levels was revealed (r=0.12 ; p=0.18). Statistically significant correlation between BMI and estradiol was detected only in the group of patients with normal (18.5-24.9) BMI (r=0.24, p<0.001). There were no significant differences in estradiol levels between the individual groups depending on BMI status.

DISCUSSION

Current opinion of National Institute of Standards and Technology (USA) recommends chromatographic resolution (HPLC) as a gold standard for assessment of vitamin D status (Phinney *et al.* 2012). In the presented study, vitamin D levels were evaluated by chemiluminescence immunassay, which is widely utilized in clinical practice. A recent study comparing available assays showed that only immunochemiluminescence characterized the results comparable to HPLC (Nikooyeh *et al.* 2017). The outcomes obtained by other methods may differ from each other up to 11.9 nmol/l (about 4.7ng/ml).

There are a lot of controversies regarding sufficient serum concentrations of 25 (OH)D. The US National Institute of Health (NIH) defines its deficiency as mean 25(OH)D levels below 12 ng/ml, < 30 ng/ml indicating the risk of deficiency, while the recommended level of 20 ng/ml (50 mmol/l) should be the goal of its daily supplementation (Ross *et al.* 2011). Severe deficiency is usually defined as the concentration below 10 ng/ml (Ginde *et al.* 2009; Looker *et al.* 2002). The threshold established by the Endocrine Society is <20 ng/ml. The Endocrine Society defines sufficient levels of 25(OH) D as 30 ng/ml or higher (Holick *et al.* 2011). However, in the general population these levels should be maintained within the target range of 40 to 60 ng/ml as optimal and recommended ones.

It is considered that 15 minutes exposure of face, arms and legs to the sunlight 2 to 3 times a week between 11 a.m. and 3 p. m. from May to October is sufficient to cover the average annual requirement for 25(OH)D (Hoel *et al.* 2016).

Parallel to the fervent discussion about multimodal functions of vitamin D and high prevalence of its deficiency in the literature, the authors proved that the incidence of 25-OH D deficiency among premenopausal women with menstrual disorders equals 12%, while its insufficiency affects 69% of such patients.

Numerous exclusion criteria for the study allowed to select a homogeneous group of patients and to minimize disruptions that could influence the results. By excluding pregnant women, postmenopausal and those receiving exogenous hormones, the authors could evaluate only patients with preserved endogenous hormonal axes.

The positive correlation between serum estradiol concentration and the incidence of vitamin D deficiency was revealed. Lower levels of 25(OH)D were observed among patients with higher estradiol concentrations at the beginning of the menstrual cycle. According to the previous investigators, overweight and obese women have higher serum estradiol levels than those who have normal BMI (Birkebaek *et al.* 2010; Karim *et al.* 2009). The correlation between VD status and estradiol is thought to be the most likely due to the dependency between BMI and 25(OH)D. Estradiol may be produced in the adipose tissue from androgens by means of aromatase, which activity is increased among obese people comparing to the age-matched women with normal or low BMI (Hubalek *et al.* 2014). The authors of the study did not find any significant dependency between BMI and estradiol, what may result from a small sample size and homogenous character of the studied population. On the other hand, previous researchers who evaluated VD levels in patients with premature ovarian insufficiency did not find significant correlation between vitamin D and estradiol or follicle stimulating hormone levels (Ersoy *et al.* 2016).

According to the aforementioned outcomes, patient's BMI positively correlates with the prevalence of vitamin D deficiency. Women suffering from this issue are significantly more prone to be overweight (mean BMI 28.2 vs. 24.7 kg/m^2).

Chae *et al.* proved no significant dependency between BMI and total serum testosterone (Chae *et al.* 2013). Similar conclusions were drawn from the presented paper. The investigators found a significant negative correlation between BMI and SHBG concentration ($r=-0.48$; $p<0.0001$) and positive correlation between BMI and FAI ($r=0.35$; $p<0.0001$). Such strict dependency was also shown in previous studies. That was the main reason why overweighed women achieved greater FAI than patients with normal BMI status. Higher values of FAI are strongly associated with clinical symptoms of hyperandrogenism. FAI and hyperandrogenism were not significantly correlated with vitamin D concentrations. On the other hand, VD supplementation seems not to improve the symptoms of hyperandrogenism (hirsutism and acne) (Tehrani *et al.* 2014). Those outcomes do not support the hypothesis that hyperadrogenic women are more likely to suffer from vitamin D deficiency as a result of sun exposure avoidance (probably due to hirsutism and other resulting in low self-esteem).

Obesity is a well-known predisposing factor to vitamin D deficiency. A meta-analysis of the studies showed increased prevalence of 24 to 35% in that group of patients (Pereira-Santos *et al.* 2015). The correlation is quite complex. Potential causes include restricted exposure to the sun resulting from distorted self-perception or greater subcutaneous fat tissue sequestering vitamin D metabolites. On the other hand, vitamin D skin production after sun exposure is decreased up to 50% in obese people in comparison with normal BMI population (Vimaleswaran *et al.* 2013). It is also another explanation of the relationship between vitamin D deficiency and obesity. The role of vitamin D receptor gene polymorphism in pathogenesis of obesity is suggested (Al-Daghri *et al.* 2014; Jedrzejuk *et al.* 2015).

The relationship between BMI, estradiol and VD has greater significance. Overweight and obesity, as well as vitamin D deficiency, are known risk factors of civilization diseases and metabolic disorders, such as metabolic syndrome and type 2 diabetes mellitus (Altintas *et al.* 2017; Smyka *et al.* 2018). Additionally, those impairments may also be enhanced by the discontinuation of physical activity due to muscle weakness among patients with lower vitamin D status (Kara *et al.* 2017; Scott *et al.* 2016).

It is well known that estrogen excess promotes the development of estrogen-dependent diseases, such as uterine fibroids, endometrial hyperplasia and endometrial cancer. On the other hand previous studies proved that sufficient level of vitamin D is associated with a reduced risk of uterine fibroids and may play an indirect role in reducing complication of endometrial hyperplasia (Baird *et al.* 2013; Tabassi *et al.* 2017). Although the results do not support a protective role of vitamin D against endometrial cancer, the dietary exposure to vitamin D seems to inhibit the carcinogenic effect of obesity on the endometrium (Yu *et al.* 2010; Zeleniuch-Jacquotte *et al.* 2010). Presented paper gives evidence of the negative correlation between 25(OH) D and estradiol serum concentration what may be the explanation of aforementioned relationship.

CONCLUSIONS

Vitamin D deficiency and insufficiency are common conditions among women with oligomenorrhea. A place for vitamin D supplementation should be established in order to optimize endocrine health. More thorough assessment of vitamin D deficiency and early initiation of its supplementation might be a part of an early prevention of metabolic and estrogen-dependent disorders.

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None to declare.

STATEMENT OF AUTHORSHIP

B Grzechocinska: project development, data collection and management, data analysis, manuscript writing, manuscript editing;
D Warzecha: project development, data collection and management, data analysis, manuscript writing, manuscript editing;
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All procedures performed in studies involving human participants were in accordance to the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

CONFLICT OF INTEREST STATEMENT AND FUNDING SOURCES

The authors declare no conflict of interest.

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