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Original Research

Prevalence of low vitamin D levels in infertile patients – a single center pilot study

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Summary

Purpose of investigation: The objective was to evaluate serum levels of vitamin D in patients who presented with infertility. *Materials and Methods:* For this retrospective study, the authors examined medical records of all infertile patients who visited this clinic between March and August 2017. *Results:* A total of 579 infertile women are included. The mean serum 25-OH vitamin D concentration was 16.28 ± 11.58 (range, 1-79.5) ng/mL; 220 patients were severely vitamin D deficient, 192 were vitamin D deficient, 95 were vitamin D insufficient, and only 72 patients were vitamin D sufficient. Vitamin D deficiency was more frequent in the younger patients (< 35 years) than in older patients (> 35 years) and the difference was statistically significant. *Conclusion:* This study showed that among persons presenting with infertility, more than three quarters of patients younger than 35 years and more than half of patients older than 35 years are vitamin D deficient and need treatment with vitamin D.

Key words: Infertility; 25-OH vitamin D; Body mass index; Age.

Introduction

Vitamin D plays a major role in bone and calcium metabolism and has several non-skeletal actions. Cervix, uterus, vaginal epithelia, and ovaries contain receptors and enzymes that are involved in vitamin D metabolism; therefore, it may influence women's reproductive physiology. In recent years, there have been several reports suggesting a high prevalence of low vitamin D intake and inadequate vitamin D status in many countries [1]. Its deficiency is a common problem in reproductive-aged women and low levels of vitamin D may also be linked to infertility/subfertility; however, its role in infertility remains unexplored.

It has been shown that women with endometriosis have lower vitamin 25-OH D levels than healthy women of reproductive age [2]. Beyond its role in bone and calcium metabolism, vitamin D shows antiproliferative, anti-inflammatory, and immunomodulatory effects [3], which account for this finding. In women with polycystic ovarian syndrome (PCOS), vitamin D has been found to be an independent predictor for the development of metabolic syndrome [4]. With regards to in vitro fertilization outcomes, better results [5] and increased live birth rates [6] both appear to be associated with adequate serum 25-OH D vitamin levels. These findings suggest that vitamin D has a role in pregnancy progression, or adequate serum vitamin D levels may just be related to general good health, and the latter may influence pregnancy outcomes. In any case, it seems necessary to obtain adequate serum vitamin D levels before starting assisted reproductive treatment (ART).

It is clear that vitamin D has an impact on female infertility. The purpose of this observational study was to evaluate serum levels of vitamin D in patients presenting with infertility to Istanbul University, Istanbul Faculty of Medicine, Department of Obstetrics and Gynecology, Department of Reproductive Endocrinology and Infertility. To the best of the present authors' knowledge, this is the first study regarding hypovitaminosis D among infertile Turkish women.

Materials and Methods

This study used a retrospective cross-sectional design to examine records from patients who visited the present Reproductive Endocrinology and Infertility clinic between March and August 2017. The investigators adhered to the institutional policies for protection of human participants. All participants provided informed consent and local ethics committee approval was obtained.

Infertility is defined by the failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [7]. The other inclusion criteria were voluntariness to participate in the study, giving written informed consent, and being 18-49 years of age. Patients who admitted for recurrent miscarriages or other symptoms such as hirsutism and abnormal uterine bleeding were not included in this study. Additional exclusion criteria included current pregnancy, chronic diseases, celiac disease or other causes of malabsorption: disorders that may impact calcium or vitamin D metabolism, kidney diseases, medications that could affect bone metabolism, and vitamin D or calcium supplementation.

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Demographic data (age, comorbid medical conditions, and medications) were collected from medical records. Predictors for hypovitaminosis D such as anticonvulsant use, renal and cardiovascular disease, pre-existing diabetes mellitus (type 1 or 2), malabsorption, gastrectomy, active liver disease, acute myocardial infarction, alcoholism, anorexia nervosa, and steroid dependency were investigated.

Serum 25-hydroxyvitamin D [25(OH)D] concentration is the parameter of choice for the assessment of vitamin D status because it reflects vitamin D exposure, incorporating endogenous synthesis from solar exposure, dietary intake from foods, fortified products, and/or supplements [8]. Given the concern about vitamin D deficiency, it became a common protocol to check the serum 25-hydroxyvitamin D levels of each infertile patient admitted to the present Reproductive Endocrinology and Infertility Unit.

BMI was calculated by dividing weight in kilograms by height in meters squared. Normal BMI was defined as 20-25 kg/m^2. Being overweight was defined as a BMI greater than or equal to 25 kg/m^2. Individuals with a BMI of 30 or more were considered obese.

All subjects had fasting blood taken in the morning. Maternal blood was collected by venipuncture in vacutainer EDTA-containing tubes. The serum test was performed independently of the investigators using standard medical laboratory protocols and techniques.

The normal value for vitamin D (25-OH vitamin D concentration in plasma) is considered as ≥ 30 ng/mL [75 nmol/L] according to the Institute of Medicine [9]. Severe deficiency is considered < 10 ng/mL [25 nmol/L], deficiency < 20 ng/mL [50 nmol/L], and insufficiency 21-29 ng/mL [51-74 nmol/L].

Continuous variables are presented as mean, standard deviation (SD), and minimum-maximum. Patients were analyzed according to age groups and BMI indexes. The Chi-square test (χ^2) was used to compare differences in the various indices. Significance was accepted at < 0.05. Excel 2010 and SPSS 15.0 were used for statistical analyses.

Results

Between March 2017 and August 2017 (spring and summer in Turkey), 711 consecutive outpatients, all Caucasians, aged between 18 and 49 (mean ± SD, 30.6 ± 5.49) years were enrolled for this study. Sixty-one patients did not give blood for vitamin D measurement (economic issues, forgetting to give blood, cessation of treatment in the present hospital or other unknown reasons). Eleven patients were actively taking vitamins including vitamin D and were therefore excluded from the study. Related medical conditions found that could interfere with blood vitamin D levels: endometrioma (n=28), polycystic ovarian syndrome (n=39), hypo- or hyperthyroidism (n=32), systemic lupus erythematosus (n=4), rheumatoid arthritis (n=2), diabetes (n=13), and other diseases (Hodgkin's lymphoma, familial Mediterranean fever (FMF), epilepsy, psoriasis, tuberculosis, sarcoidosis, chronic ulcerative colitis, scoliosis, and adenoma of the parathyroid gland (n=9) were excluded.

The prevalence of endometriosis and polycystic ovarian syndrome are high in the apparently healthy normal population, affecting 5-20% of reproductive-age women [10, 11]. These patients were included in the study but the other 60 patients were not. In total, 579 infertile women met the inclusion criteria and were included in the final analysis.

Table 1. — *Vitamin D status in all patients.*

Prevalence of vitamin D deficiency and insufficiency.		
	n	%
Severe deficiency (< 10 ng/mL)	220	37.99
Deficiency (< 20 ng/mL)	192	33.16
Insufficiency (21-29 ng/mL)	95	16.41
Sufficiency (≥ 30 ng/mL)	72	12.44
Total	579	100

The mean serum 25-OH vitamin D concentration for the present study population was 16.28 ± 11.58 (range, 1-79.5) ng/mL. Two hundred twenty patients were severely vitamin D deficient (serum concentrations (< 10 ng/mL or 25 nmol/L), 192 patients were vitamin D deficient with a serum concentration less than 20 ng/mL (50 nmol/L), 95 patients were vitamin D insufficient (serum concentrations between 20-29 ng/mL or 50-74 nmol/L), and 72 patients were vitamin D sufficient with serum concentrations above 30 ng/mL (75 nmol/L) (Table 1); only 12.44% of the present patients (72 persons) had sufficient levels of vitamin D (above 30 ng/mL).

According to age groups, vitamin D deficiency was more prevalent in younger patients (< 35 years) than in older patients (> 35 years) (Table 2) and the difference was statistically significant ($p < 0.05$). Vitamin D sufficiency in patients in the 18-35 year age group was 9.75% and 16.67% in patients of the 35-40 and > 40 year age groups (Table 2). This difference in vitamin D sufficiency was statistically significant between the < 35 year and > 35 year groups ($p < 0.05$) (Table 2). There was also a statistically significant difference between the mean vitamin D levels of younger and older patients ($p < 0.05$) (Table 2).

The mean BMI of all participants was 26.14 ± 5.11 (range, 16-49.6) kg/m^2. Two hundred sixty-four patients (45.60%) were of normal weight with a BMI < 25 kg/m^2; 197 patients (34%) were overweight with a BMI between 25-30 kg/m^2, and 118 patients (20.40%) were obese with a BMI > 30 kg/m^2 (Table 3). When the patients were divided into three groups according to their BMI, no difference was seen between the groups regarding mean vitamin D levels (Table 3).

Discussion

The present authors conducted this study to determine the prevalence of vitamin D deficiency and insufficiency in a large population of patients with infertility. The present study indicates an alarming prevalence of deficient and insufficient vitamin D levels in this patient population. Severe deficiency and deficiency was found in 71.15% of this study group. Vitamin D insufficiency, which means a serum value of 21-29 (51-74) ng/mL, was found in 16.41% of the present patients. Only 12.44% of the present patients had

Table 2. — *Distribution of vitamin D deficiency and mean vitamin D values according to age groups.*

Age group (years)	Deficient and severely deficient	Insufficient	Sufficient	Total
(% within the age group)	< 20 ng/ml	> 20 ng/mL	> 30 ng/mL	
18-35	337 (76.42%)	61 (13.83%)	43 (9.75%)	441 (100%)
35-40	59 (54.63%)	31 (28.7%)	18 (16.67%)	108 (100%)
>40	16 (53.33%)	9 (30%)	5 (16.67%)	30 (100%)
Total	412 (71.16%)	101 (17.44%)	66 (11.40%)	579 (100%)

Age group (years)	n (%)	Mean 25 - OH D vitamin value (ng/mL)
18-35	441 (76.17%)	14.7
35-40	108 (18.65%)	20.9
>40	30 (5.18%)	20.7

Table 3. — *Prevalence of vitamin D deficiency and insufficiency/sufficiency according to BMI.*

Body mass index (kg/m^2)	n (% within all patients)	Severely deficiency and deficiency (< 20 ng/mL) (% within BMI group)	Vitamin D insufficiency and sufficiency (> 20 ng/mL) (% within BMI group)
Normal (BMI 20-25)	264 (45.59%)	189 (71.59%)	75 (28.41%)
Overweight (BMI ≥ 25)	197 (34.02%)	146 (74.11%)	51 (25.89%)
Obese (BMI ≥ 30)	118 (20.38%)	77 (65.25%)	41 (34.75%)
No. of participants (total)	579	412	167

adequate levels of vitamin D. However, this does not provide a cause-and-effect link between low serum 25-hydroxyvitamin D levels and infertility.

Vitamin D deficiency constitutes an epidemic in many populations across the world and has been reported in the healthy population across all age groups and both sexes [12]. It has been estimated that one billion people worldwide have inadequate levels of vitamin D in their blood [13]. Tangpricha *et al.* [14] reported a 32% prevalence of vitamin D deficiency in otherwise fit healthcare providers sampled at a Boston hospital. Hypovitaminosis D has also been noted in medical inpatients with and without risks for this deficiency [15]. The prevalence of vitamin D deficiency in reproductive-age women appears to be increasing worldwide over the past two decades [16-18]. To the best of the present authors' knowledge, this is the first such study with regard to the prevalence of vitamin D deficiency in patients with infertility in Turkey.

Vitamin D insufficiency/deficiency is linked to many chronic diseases such as cancer, autoimmune, and infectious diseases, as well as cardiovascular diseases, and diabetes mellitus type 2 [12, 19]. However, as vitamin D deficiency is so widespread, determining whether vitamin D deficiency is directly associated with infertility/subfertility is challenging. Another obscure point is the threshold for vitamin D for optimal fertility and response to ART. The Endocrine Society Clinical Practice Guideline defines vitamin D deficiency as 25(OH) D below 20 ng/mL (50 nmol/L), and vitamin D insufficiency as 25(OH)D 21-29 (52.5-72.5) ng/mL [20]. A sufficient level of vitamin D (> 30 ng/mL [75 nmol/L]) is associated with maximal suppression of the parathyroid hormone (PTH) and optimal calcium absorption [20]. A cut-off point of 30 (75 nmol/L) ng/mL is required for optimal maintenance of the normal structure and function of the skeletal system. We do not know which cut-off point should be obtained in infertile patients for best ART results.

Risk factors for vitamin D deficiency include inadequate sunlight exposure, genes, dark skin pigmentation, lifestyle, obesity, pregnancy, certain medical problems, and low vitamin D intake [17, 21]. There are also seasonal differences between serum 25-OH vitamin D levels. Bee *et al.* [22] showed that the difference between summer and winter levels was minuscule (around 3 ng/mL) and it did not change the prevalence of hypovitaminosis D (the mean level of vitamin D was 26.4 ng/mL in winter *vs*. 29.8 ng/mL in summer in their cohort). The authors emphasize that this study was conducted during spring and summer. If they had conducted this study in autumn and winter, they would probably have obtained lower levels of vitamin D.

In the present study, there was a correlation between vitamin D and age; vitamin D levels were higher in older patients (> 35 years). Classically, increasing age is associated with a decrease of vitamin D concentrations [23]. This is due to decreased synthesis of vitamin D by the skin, decreased dietary intake, decreased renal function, and reduced time spent outdoors [23]. This is valid for the elderly, but not for women aged 35-49 years, the constituents of the present older group.

Obese individuals, as a group, have low plasma concentrations of 25-hydroxyvitamin D [25(OH)D] [24, 25]. The present study revealed no statistically significant difference between 25 OH D vitamin levels of normal, overweight, and obese patients. The prevalence of vitamin D deficiency

and insufficiency was very high in the present study group; this may explain why the authors found no difference between normal, overweight, and obese groups.

In conclusion, from a practical standpoint, this study showed that the majority of the infertile patients required supplementation of vitamin D. More than three quarters of patients aged younger than 35 years and more than half of patients older than 35 years are vitamin D deficient and need treatment with vitamin D.

Further investigations are needed to enlighten any possible data that directly link hypovitaminosis D with infertility. More importantly, prospective studies should investigate whether correction of low vitamin D levels improve spontaneous pregnancy rates or success of infertility treatments.

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