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Is there a relation between serum vitamin D and ovarian reserve markers in infertile women?: A retrospective cohort study

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Abstract

Vitamin D is an essential molecule for reproductive health. There are studies in the literature showing contradictory results regarding te relationship between serum 25(OH) vitamin D levels and ovarian reserve markers. The aim of this retrospective cohort study was to investigate the relationship between ovarian reserve markers; follicle stimulating hormone (FSH), antral follicle count (AFC), and anti-Müllerian hormone (AMH), and serum 25(OH) vitamin D levels. 195 infertil women aged between 18-45 years were included in the study. After the participants were divided into 2 groups according to their 25 (OH) D levels (those with < 20ng/ml (vitamin D deficiency) and those with ≥ 20ng/ml (vitamin D insufficiency)), the age, body mass index (BMI), AFC, AMH, FSH levels of the groups were compared. The mean age of the 25(OH) vitamin D deficient group was significantly younger (p=0.025) than the other group. There was no statistically significant difference in BMI (p=0.47) or season of blood sampling (p=0.62) between groups. The levels of the ovarian reserve markers AFC, AMH, and FSH were not significantly different between groups (p=0.95,0.18,0.86, respectively). Multiple linear regression and logistic regression analysis after adjusting potential confounders showed no significant relationship between vitamin D and AMH (p=0.628) and AFC(p=0.107). In conclusion, we found no correlation between 25(OH) vitamin D concentrations and markers of ovarian reserve. We cannot, however, ignore the critical role of vitamin D in the female reproductivity. To determine the optimal 25(OH)D3 levels during the reproductive period and the amount of vitamin D supplementation required to achieve those levels for the numerous actions of vitamin D throughout reproductivity process, high-quality, large-scale randomized clinical trials are required.

Keywords: Vitamin D, infertility, anti-Müllerian hormone, antral follicle count

1. Introduction

Vitamin D is required not only for calcium and phosphorus homeostasis regulation, but also for a variety of other functions, including female reproduction. Vitamin D receptor (VDR) and 1-hydroxylase are found in reproductive tissues such as the ovary, uterus, placenta, testis, and hypophysis (1, 2). As a result, a link between vitamin D and reproductive health appears to be almost inevitable. The importance of 25 hydroxyvitamin D (25(OH) vitamin D) in female reproduction was initially demonstrated in mice lacking in either 25(OH) vitamin D or VDR, which developed uterine hypoplasia and ovulatory dysfunction, resulting in infertility (3).

Different markers are used to assess ovarian reserve. Serum follicle stimulating hormone (FSH), which is measured during the early follicular phase, has been widely used as an ovarian reserve marker; however, it only indicates the reserve indirectly, with its blood level increasing only when ovarian follicles are severely depleted (4). In addition, ovarian reserve can also be assessed via ultrasound on the second or third day of menstruation using antral follicle count (AFC). It demonstrates the number of ovarian follicles ranging in diameter from 2 to 10 mm in both ovaries (5). On the other hand, the other ovarian reserve marker, anti-Mullerian hormone (AMH) maintains a constant level throughout the cycle (6). Nonetheless, AMH levels can fluctuate seasonally in correlation with 25(OH) vitamin D levels (7).

So far, studies on the effects of 25(OH) vitamin D on ovarian reserve markers have yielded contradictory results. While some studies show a positive relationship between serum 25(OH) vitamin D levels and ovarian reserve markers (7), others do not (8). Given the contradictory literature and the importance of 25(OH) vitamin D in reproductive health, in this study we aimed to investigate the relationship between ovarian reserve markers; FSH, AFC, and AMH, and serum 25(OH) vitamin D levels.

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2. Materials and Methods

This retrospective cohort was conducted between October 2020 and October 2021, in two specialized maternity hospital centers on North-East Coast of Turkey. Patients aged 18–45 years who were admitted due to infertility (no conception after 12 months of no contraceptive methods) were included in the study. Patients who refused to give informed consent, were taking vitamin supplements, had any surgical procedure on the pelvis, were receiving chemotherapy or radiation therapy to the pelvis, were taking menstrual cycle-affecting medications or gonadotoxic therapy, had a history of gynecological malignancy, premature menopause, or had Mullerian anomaly were excluded from the study.

Baseline clinical characteristics including age, BMI, time of blood samples taken were obtained. AFC was determined using transvaginal ultrasonography by a gynecologist during the early follicular phase (the first five days of the cycle). AFC were defined as folicles ranging in diameter from 2 to 10 mm.

Blood samples were collected to measure AMH and FSH levels; the serum AMH level was measured by 'ECLIA' method (electrochemiluminescence immunological test), using the Cobas device (Roche Diagnostics, Risch-Rotkreuz, Switzerland) and the serum FSH level was determined using the immunochemiluminometric (ICMA) method with the ARCHITECT System kit (Abbott Laboratory Diagnostics, USA).

To measure 25(OH) vitamin D levels, the blood samples taken from the patients with EDTA tube were rotated for 5 minutes at 2500 rotation per minute (rpm) and yielded plasma was analysed by Beckman Coulter Unicel Dx1600 (Beckman Coulter, Ca, USA) immune analyser with the same branded kits. Due to the fact that none of the participants had a normal level of 25(OH) vitamin D, the patients were divided into two groups: those with < 20ng/ml (vitamin D deficiency) and those with ≥ 20ng/ml (vitamin D insufficiency) (9).

After the participants were divided into 2 groups according to their 25 (OH) D levels, the age, body mass index (BMI), AFC, AMH, FSH levels of the groups were compared. Our primary outcome was correlation of ovarian reserve markers with vitamin D levels. The study was approved by Health Sciences University Kanuni Educational and Research Hospital Ethics committee (23.12.2020/2020/80). All ethical principles of the latest version of the Declaration of Helsinki on human studies were met throughout the study.

2.1. Statistical analysis

Data were analyzed using SPSS, version 25.0 (SPSS, Chicago, IL). Categorical variables were described as percentages and compared using Pearson's chi-squared test and Fisher's exact test when necessary. For continuous variables, mean and standard deviation (SD) were given as descriptive variables. Correlation between 25(OH) vitamin D and AMH, FSH levels, and AFC was evaluated by Pearson chi-square test. We used a logistic regression model adjusting for the potential confounders (age, BMI, season of blood sampling) to estimate the adjusted odds ratios (ORs) and measure the 95 % CI for the comparison of the two groups in relation to the primary outcomes. $p<0.05$ was considered statistically significant. Sixty-five subjects in each group were required to test the 10% reduction in AFC, AMH and FSH levels at 90 percent power.

3. Results

A total of 195 participants were enrolled in the study. The mean age of patients was 32.1±5.4 years and the mean BMI was 23.8±1.7 kg/m^2. Table 1 summarizes the baseline and biochemical characteristics of patients. Of all participants, 72.3% (n=141) were in vitamin D deficieny group (<20 ng/mL) and 27.7% (n=54) were in vitamin D insufficiency group (≥20 ng/mL). The mean age of the 25(OH) vitamin D deficient group was significantly younger (p=0.025) than the other group. There was no statistically significant difference in BMI (p=0.47) or season of blood sampling (p=0.62) between groups. The levels of the ovarian reserve markers AFC, AMH, and FSH were not significantly different between groups (p=0.95,0.18,0.86, respectively) (Table 2).

Table 1. Baseline characteristics of the study population

Variables	Group 1 (25(OH) vitamin D <20 ng/mL)	Group 2 (25(OH) vitamin D ≥20 ng/mL)	*p*
Number of patients, n (%)	141(72.3)	54 (27.7)	
Age (year), mean (SD)	31.5 ±5.4	33.5± 5.4	0.025[1]
BMI (kg/m^2), mean (SD)	23.9 ±1.6	23.7±1.7	0.47[1]
Season of blood sampling, n (%)			0.62[2]
Autumn	38 (19.5)	18 (9.2)	
Spring	26 (13.3)	7 (3.6)	
Summer	35 (17.9)	11 (5.6)	
Winter	42 (21.5)	18 (9.2)	

[1]Mann-Whitney U test , [2]Pearson ×² test, Plus-minus values are mean±standard deviation SD: standart deviation

Table 2. Relation of ovarian reserve markers and vitamin D level

	Group 1 (25(OH) vitamin D <20 ng/mL)	Group 2 (25(OH) vitamin D ≥20 ng/mL)	*p*
AFC, n (%)			0.95[1]
≥6	96 (49.2)	37 (19)	
<6	45 (23.1)	17 (8.7)	
AMH (ng/mL), mean (SD)	3.5 ±3.3	35 ± 4.4	0.18[2]
FSH, mean (SD)	8.6 ±5.2	9.01 ± 6.2	0.86[2]

Plus-minus values are mean±standard deviation SD: standart deviation. SD: standart deviation.[1]Pearson ×² test, [2]Mann-Whitney U test

Multiple linear regression analysis was conducted in order to evaluate the relationship between serum 25(OH) vitamin D levels and AMH levels, after adjusting for potential confounders (age and BMI) According to the adjusted

analysis no correlation was found between 25(OH) vitamin D and AMH levels (standardized coefficient=0.486, p=0.628) (Table 3).

Logistic regression analysis was conducted in order to evaluate the relationship between serum 25(OH) vitamin D levels and AFC, after adjusting for potential confounders (age, BMI, AMH and season of blood sampling) According to the adjusted analysis no relation was found between 25(OH) vitamin D levels and AFC (p=0.107) (Table 4). We found no correlation between serum 25(OH) vitamin D and AMH (r=-0.78, p=0.280), AFC (r=0.005, p=0.949) and FSH levels (r=0.049, p=0.497) (Fig. 1 and Fig. 2).

Table 3. Linear regression coefficients between all covariates and serum anti-Müllerian hormone

	Unstandardized Coefficients		Standardized Coefficients	t	*p* value	95,0% Confidence Interval for Beta	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	9.965	3.450		2.889	0.004	3.161	16.770
Age (year)	-0.329	0.043	-0.496	-7.666	0.000	-0.414	-0.244
25(OH) vitamin D (ng/mL)	0.017	0.034	0.031	0.486	0.628	-0.051	0.084
BMI (kg/m^2)	0.162	0.135	0.076	1.199	0.232	-0.104	0.428

a. Dependent Variable: AMH, BMI: Body mass index

Table 4. Logistic Regression coefficients between covariates and AFC

Covariates	*p* value	OR	95% CI for OR	
			Lower	Upper
Age (year)	0.008	1.122	1.031	1.223
25(OH) vitamin D(ng/mL)	0.107	0.952	0.897	1.011
AMH (ng/mL)	0.000	0.493	0.358	0.679
Constant	0.137	0.097		

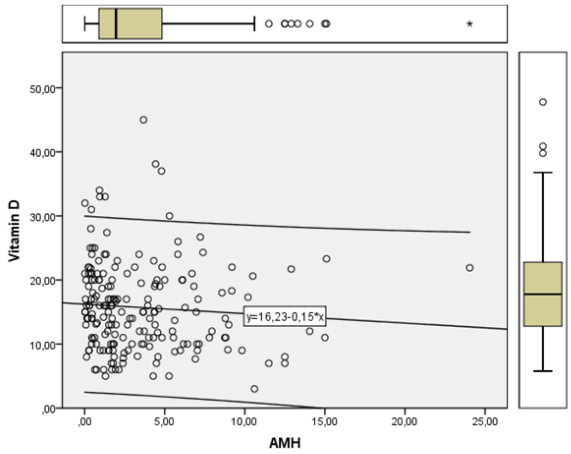

Fig. 1. Correlation plots of 25(OH) vitamin D and anti-Mullerian hormone (AMH)

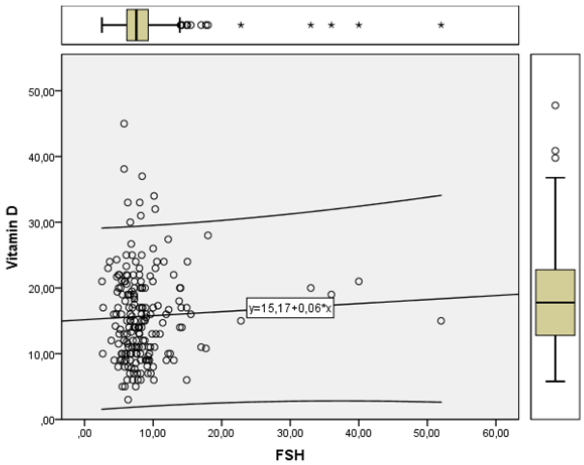

Fig. 2. Correlation plots of 25(OH) vitamin D and follicle stimulating hormone (FSH)

4. Discussion

In this present retrospective cohort study, we tried to investigate the correlation of ovarian reserve markers; AFC, AMH and FSH with vitamin D level, to study whether vitamin D has a role in increasing female reproductivity. Our results demonstrated no significant relationship between ovarian reserve markers, FSH, AFC and AMH, and serum 25(OH) vitamin D levels even after adjusting confounders such as age and season of blood sampling.

Previous studies on this subject yielded inconsistent results so far. Similarly to our findings, Drakopoulos et al. (10) reported that there was no significant association between serum 25(OH) vitamin D levels and AFC and AMH levels after adjusting for possible confounding variables. Furthermore, an Iranian cross-sectional study of 287 infertile women found no correlation between serum 25(OH) vitamin D concentrations and both AFC and AMH levels. Additionally, the majority of participants in this study had vitamin D levels in the deficiency zone (20ng/mL), possibly as a result of their clothing and religious practices (11). In another study, which was also conducted retrospectively and included a larger sample size of 340 women, the authors concluded that AMH levels did not exhibit seasonal variation, as 25(OH) vitamin D concentrations do, and that there is no significant correlation between AMH and vitamin D levels. Interestingly, a prospective study involving 22 women with polycystic ovary syndrome (PCOS) and 45 women without PCOS found that supplementing deficient participants with vitamin D reduced AMH levels in PCOS patients but had no effect on AMH levels in the control group (12).

However, there is some evidence in the literature that vitamin D 25(OH) is associated with ovarian reserve markers. Merhi et al. (13) reported that while AMH and 25(OH) vitamin D levels were positively correlated in women in their late reproductive years, no correlation could be founded in young women. Another study involving 33 women revealed a seasonal correlation between serum AMH levels and 25(OH) vitamin D, indicating that seasonal changes can be avoided by supplementing with vitamin D, particularly during the winter

(7). The study's findings, however, should be interpreted in light of the study's sample size. Naderi et al. (14) examined 30 infertile women with 25(OH) vitamin D concentrations less than 30 ng/mL and AMH concentrations less than 0.07ng/mL. They reported that after three months of weekly vitamin D replacement at a dose of 50.000 IU, participants' 25(OH) vitamin D and AMH levels increased concurrently. Furthermore, the authors concluded that vitamin D deficiency can impair AMH production, resulting in infertility, and that vitamin D supplementation increases fertility through AMH. Additionally, an Iranian study discovered a significant positive correlation between 25(OH) vitamin D levels and AFC in 189 infertile women with an average 25(OH) vitamin D concentration of 15.46 ng/mL (vitamin D deficient) (15). These contradictory findings can be explained by a variety of factors, not just methodological differences. Disparities in several reproductive health outcomes may be explained by genetic, ethnic, and racial differences, as well as religious and dressing habits and season. Another factor that could influence the results is the use of different AMH measurement methods and blood storage times (16). Additionally, because AFC is evaluated using ultrasound, which is influenced by individual experience, it is possible for different results to be obtained from the same patient.

One of our study's limitations is that it was conducted retrospectively, which meant that we could not access all of the participants' data. For instance, we could present AFC in two categories, as ≥6 and <6, without specifying the follicle count. Additionally, we did not evaluate the causes of infertility. Another limitation is that, in addition to BMI, age, and season of blood sampling, several factors can influence vitamin D levels. Beyond these limitations, one of this study's strengths is its relatively large sample size, which is sufficient to create objective results. Another factor is that the study population was drawn from two different tertiary care hospitals in the Black Sea region.

In conclusion, we found no correlation between 25(OH) vitamin D concentrations and markers of ovarian reserve. We cannot, however, ignore the critical role of vitamin D in the female reproductiviy. To determine the optimal 25(OH)D3 levels during the reproductive period and the amount of vitamin D supplementation required to achieve those levels for the numerous actions of vitamin D throughout reproductivity process, high-quality, large-scale randomized clinical trials are required.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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