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The Relationship Between Vitamin D Levels and Amh and Other Ovarian Reserve Parameters in Infertile Women

Serap Mutlu ÖZÇELİK[1], İlhan ÖZDEMİR[2]

[1]MD, Department of Gynecology and Obstetrics, Diyarbakir Gazi Yasargil Training and Research Hospital, Diyarbakır, Türkiye

[2]Department of Gynecology and Obstetrics, Faculty of Medicine, Atatürk University, Erzurum, Türkiye

[1]Orcid: 0000-0001-5124-9031,[2]Orcid: 0000-0001-9957-0211

ABSTRACT: Objective: Anti-Müllerian Hormone (AMH) is one of the biochemical markers used to predict the ovarian response and determine the size of the primordial follicle pool. Obtaining data on the presence of vitamin D receptors in the central and peripheral reproductive organs in recent studies is shown as proof that this vitamin plays an important role in reproduction. There are studies conducted in recent years showing that Vitamin D plays an important role in reproduction. We aimed to determine the possible connection and function of vitamin D by analyzing the AMH and other ovarian reserve parameters of the patients who applied to the infertility clinic.

Material Methods: This study was realized retrospectively, and the files of 284 patients with infertility problems were examined. The patients were evaluated in 2 groups; Group I, those with vitamin D levels below 20 ng/mL and those with II. The group was compared as those with vitamin D levels above or equal to 20ng/ml.

Results: Vitamin D level was determined as $p<0.008$ according to age among the groups, and $p<0.001$ in the summer season when analyzed according to the seasons. Estradiol (E2) level was observed as $p<0.013$. In addition, no statistical significance was found between vitamin D and vitamin D according to AMH, follicle stimulating hormone (FSH) and antral follicle number (AFC) ($p>0.05$).

Conclusion: It was determined that there was no significant correlation between ovarian reserve parameters, especially AMH, FSH and AFC, and vitamin D levels of female patients who applied to our clinic due to infertility. Multicenter and prospective studies on Vitamin D, which is very important for both female and male infertility, need to provide new data for clinical applications.

KEYWORDS: Anti-mullerian hormone (AMH), antral follicle count (AFC), vitamin D, infertility, follicle stimulating hormone (FSH).

INTRODUCTİON

In recent years, evaluation of ovarian reserve in female infertility has become mandatory for determining treatment options. Some hormones (follicle stimulating hormone, estradiol), age of the woman and number of antral follicles are routinely used in the clinic to evaluate ovarian reserve by ultrasound examination in the early follicular phase. Evaluation of FSH and E2 low ovarian reserve has been used as a marker for years. However, sometimes when infertility occurs due to impaired ovarian function, it has been observed that the FSH level is also above the normal value[1]. Later, counting became easier by determining the number of follicles with high-resolution sonographic systems[2,3]. However, determination of AMH levels has gained importance in the evaluation of ovarian reserve. It is known as Müllerian inhibitory substance that a transforming growth factor - β family glycoprotein[4,5]. It has been reported that AMH, which is found in the serum of women with puberty to regulate early follicle development, is usually secreted by the granulosa cells of the ovarian follicles[6]. AMH inhibits the uptake of the first primordial follicle and decreases the sensitivity of pre-antral and antral follicles to FSH in ovary[7]. The AMH level remains constant throughout the menstrual cycle and can therefore be assessed on any day of the menstrual cycle[6,8].

Vitamin D exerts its effect through the vitamin D receptor (VDR), which has been identified many female genital organs, especially in granulosa cells[9]. These divergent expressions of the VDR suggest a potential role for vitamin D in female reproduction and infertility[10,11]. Reproductive physiology has little known mechanism, it has been suggested that vitamin D has a direct effect on steroidogenesis. In some experimental studies, it has been reported that estrogen levels of VDR mutant mice are lower than those of heterozygous mice, resulting in impaired folliculogenesis[12]. In addition, vitamin D stimulates steroidogenesis in ovarian cells[13].

The Relationship Between Vitamin D Levels and Amh and Other Ovarian Reserve Parameters in Infertile Women

According to the data obtained, animal models were made showing that vitamin D affects AMH signaling during follicular development[14], and the relationship between AMH and vitamin D was evaluated with studies conducted in human granulosa cells[15]. However, although the relationship between these molecules are consistent in the results obtained from basic research, although the data in clinical studies support the relationship between vitamin D and ovarian reserve, it is quite contradictory when compared to some studies[3] and in some studies, no significant relationship was found between these two important molecules[16]. Considering the unknowns of vitamin D in the female genital system, this study was conducted to evaluate the relationship between AMH and 25-OH-D vitamin, which are the most important ovarian reserve markers, especially in infertile women.

MATERİAL AND METHODS

Study Design

The files of female patients aged 18-42 years who applied to the Gazi Yaşargil Training and Research Hospital infertility polyclinic between November 2019 and March 2021 were retrospectively analyzed. The relationship between 25-OH vitamin D, which is routinely checked in patients with ovulation induction, and AMH and ovarian reserve parameters was evaluated. Blood samples were taken for AMH, prolactin, FSH, E2 and LH, tiroid stimulation hormone (TSH), and 25 (OH) D vitamin. Morever, detailed information about their age, body mass index (BMI), blood draw season, causes of infertility, any factors that could affect ovarian reserves and smoking status were recorded. This retrospective study was obtained from the data of patients who came to the Gazi Yaşargil Training and Research Hospital Infertile outpatient clinic.

Inclusion criteria for the study; All of the women included in the study between the ages of 18 and 42 with an infertility period longer than 12 months and a BMI <30 were included in the study.

Exclusion criteria: 1- Patients who had previously received vitamin D therapy, 2- patients who had undergone ovarian surgery and gonadotoxic treatment, 3- women who were taking medication due to systemic disease were excluded from the study.

Serum 25-OH vitamin D measurement

The best indicator to determine the vitamin D level is the 25(OH) vitamin D level in the serum. Although there is no consensus on the value of vitamin D, if the 25(OH) vitamin D level is less than 20 ng/mL, vitamin D lack, between 21 and 29 ng/mL 21 and 29 ng/mL, vitamin D deficiency, and if it is higher than 30 ng/mL it is higher than 30 ng/mL, it is considered an adequate level. Patients included in the study with serum 25 (OH) vitamin D levels <20 ng/mL were classified as first group, and those with ≥20 ng/mL were classified as second group.

AMH levels

To determine the level of AMH, 25-OH measurement was performed after collation regardless of the day of the menstrual cycle. The blood collected from the women participating in the study was taken into serum tubes; It was quickly centrifuged for 1 hour and the serum was separated and stored at -80°C. The AMH test was found to have a functional sensitivity of 0.16 ng/mL, with variable coefficients within and between tests of <6%.

Antral follicles

While blood samples were taken and serum AMH and vitamin D levels were determined, AFC was calculated on day 1-3 of the first cycle. The calculated AFC represented the total number of antral follicles in the 2 to 10 mm area in the right and left ovaries.

Statistics

All statistics were analysed using SPSS 20. 0 (IBM Corporation, Somers, USA). Descriptive properties of data in analysis; were presented as number (n) and percentage (%) for categorical data, mean ± standard deviation, median (1st and 3rd quartiles) for numerical variables. First of all, the conformity of the data to the normal distribution was determined. For this, histogram and Kolmogorov-Smirnoff Test were applied. Then Mann Whitney U test was applied for comparison of two independent groups ($p<0.05$).

RESULTS

In the study were included a total of 284 patients, of which 264 (94.01%) patients had a vitamin D level <20 ng/mL (group I) and 17 (5.99%) had vitamin D ≥20 ng/mL (group II). detected on. All of the patients were selected among those who applied to the hospital for infertility treatment for the first time. The patients were 31.37±6.46 age of mean. The mean age of patients with a vitamin D value above 20 was found to be significantly higher ($p<0.008$).The mean body mass index was 25.82±3.18, according to smoking status, the number of group I patients was 114 smokers, 153 non-smokers, and group II patients were 7 smokers and 10 non-smokers. No significance could be determined according to BMI and smoking status. In addition, patients with low ovarian reserve, endometrioma, male factor, pcos and unexplained infertility applied to the clinic in the analysis made considering the causes of infertility. Among these, unexplained infertility leads with the highest average of 147 patients. It was determined that the relationship with vitamin D was not significant. The value of vitamin D is important according to the seasons. Vitamin D deficiency was less common in the summer period ($p<0.001$). Both demographic and other characteristics of the patients in the groups are presented in Table 1. Age, cause of infertility, antral follicle count (AFC), body mass index (BMI), serum FSH, prolactin, E2, AMH levels and 25(OH) vitamin D levels were summarized. It was determined that AMH level and AFC number were not significant with vitamin D ($p>0.05$). The mean E2 hormone level of patients with vitamin D value above ≥20 ng/mL (group II) was found to be significantly higher (Figure 1, Figure 2).

DISCUSSION

In this study, we investigated whether there is any relationship between serum AMH and vitamin D and other ovarian reserve indicators in women who applied to IVF clinic for the first time due to infertility. According to the results of the study, there was no statistically significant difference in serum vitamin D levels between AMH and AFC. Most of the patients included in the study were in the region of vitamin D deficiency (<20 ng/ml) and we found that serum vitamin D level had no effect on ovarian reserve parameters. Vitamin D has an important role in reproductive system[17]. Vitamin D receptor (VDR) is found in ovary, endometrium, tuba uterina, placental and decidual cells and its expression increases during pregnancy[18]. It has been reported that there is a relationship between vitamin D and female infertility. Vitamin D supports follicular development and endometrial proliferation. As a result, vitamin D level was found to be associated with reproductive functions[19]. Although there are many studies examining the relationship between infertility and vitamin D, which is one of the indicators of ovarian reserve. In the few clinical studies that show vitamin D plays an important role in reproduction, there continues to be a correlation between adequate vitamin D levels in women with infertility and successful fertility treatments[20]. Studies show that there is a correlation between the in vitro fertilization method and these successful fertility treatments[21,22]. Since fluctuations in vitamin D levels are associated with exposure to ultraviolet light, they vary seasonally[23,24]. It is therefore reasonable to assume that vitamin D may affect ovarian function and AMH production[16]. Some researchers have observed a positive linear relationship between AMH and vitamin D[3]. In our study, we found that vitamin D levels differed significantly seasonally. However, no such difference was found between the level of AMH and vitamin D. It is also thought that high doses of vitamin D intake can rapidly increase the level of AMH in women of reproductive age. However, dietary vitamin D levels are claimed to be lower[25].

The results were not replicated when compared with previous clinical studies that supported the link between vitamin D and AMH in previous studies. When we examine another study, especially Drakopoulos et al. in a cross-sectional, nested study, it was revealed that the relationship between serum AMH and 25(OH) vitamin D in women during the late reproductive period was minimal and a significant result was reached, and showed that there was no relationship in younger patients. When we consider another study, especially if we make an explanation for the data obtained in line with the results obtained by Drakopoulos, it would be correct to think that the data are retrospective and originate in order to provide the correlation in the comparisons between them[26].

If we consider another study, in this study, especially women diagnosed and infected with HIV were included, and only patients with vitamin D data were analyzed. For this reason, it is reported that there is a high probability of selection error in line with this analysis. Also, Dennis et al.[3] in another study he carried out, he analyzed 33 women who did not go through menopause and who had pre-menopausal age, and dealt with the possibility of a seasonal change in AMH and showed in his study that it was related to the magnitude of the change in AMH level and vitamin D level. On the contrary of this study, our study will keep serum AMH levels at normal levels in pre-pmenopausal women with vitamin D deficiency by providing 1,25-dihydroxyvitamin D3 supplementation, and in order to test this thought, 67 patients who could not find any beneficial effect of vitamin D were included in the supplementation of serum AMH levels. We can say that it is compatible with a retrospective study[20]. In a study similar to the previous study, 340 women were included in this recent retrospective study, and neither in this case, which we call polycystic ovary syndrome (PCOS), nor in patients with ovulatory diagnosis, no correlation was found in serum AMH and vitamin D levels[16].

Although a study is planned to address the relationship between the most important ovarian reserve markers (AMH and AFC) and serum vitamin D in line with the information obtained from the studies, cross-sectional findings are shown due to the nature of the study. We must accept. From this point of view, we cannot form or exclude any causal effect hypothesis. Despite these, the data we obtained in our study show that the relationship between vitamin D and ovarian reserve markers is very low.

Some limitations of our study should be taken into account. First, as this is a retrospective observational study, the influence of factors that have the potential to influence the outcome cannot be excluded. However, when the demographic and cycle characteristics of the two groups in our study were examined, the potential confounding factors were not statistically different between the two groups. The second limitation is that potential seasonal changes in women undergoing IUI were not evaluated in our study; Quantitative evaluations were made only on serum vitamin D levels. One of the strengths of our study is that it was conducted with a relatively large number of patients over a wide period of time. Another strength is that the study was conducted in a single center with a standard treatment approach that did not change between cycles.

CONCLUSION

The data presented in this study show that vitamin D levels do not affect pregnancy outcomes in the treatment of intrauterine insemination following ovulation induction. Although vitamin D supplementation is necessary for general health in those with serum vitamin D levels <20 ng/mL, there is insufficient evidence whether this supplementation will increase pregnancy rates in infertile couples. Therefore, large-scale studies are needed to explain the relationship between vitamin D and pregnancy after IUI, especially since no clear results have been found in IVF and vitamin D studies published so far.

ETHICS STATMENT

Ethical protocol was approved by XXX Ethics Committee, approval number (2021-758).

Conflict of Interest: The authors declared no conflicts of interest.

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Table 1. Demographic characteristics of the harvests included in the study.

	Overall	**Vitamin D <20 ng/mL**	**Vitamin D ≥20 ng/mL**	***P* value**
Number of patients, n (%)	284	267 (94.01)	17 (5.99)	
Age, mean (SD)	31,37 (6,46)	31,09 (6,46)	35,18 (5,24)	0,008
BMI (kg/m^2), mean (SD)	25,82 (3,18)	25,92 (3,22)	26,12 (2,75)	0,779
Smoking (%)				0,902
Smoker	121 (42.61)	114 (94.21)	7 (5.79)	
Non- smoker	163 (57.39)	153 (93.87)	10 (6.13)	
Infertility cause, n (%)				0,727
Ovulatory	65 (22.89)	60 (92.30)	5 (7.70)	
Endometriosis	13 (4.57)	12 (92.30)	1 (7.70)	
PCOS	49 (17.25)	45 (91.84)	4 (8.16)	
Male factor	10 (3.52)	9 (90)	1 (10)	
Unexplained	147 (51.77)	141 (95.92)	6 (4.08)	
Season of blood sample, n (%)				<0,001
Spring	63 (22.19)	63 (100)	0 (0)	
Summer	26 (9.16)	9 (34,62)	17 (65.38)	
Autumn	74 (26.05)	74 (100)	0 (0)	
Winter	121 (42.60)	121 (100)	0 (0)	
AFC, median (IQR)	7,99 (5,08)	8,01 (5,092)	7,76 (4,99)	0,838
AMH (ng/ml), mean (SD)	3,31 (2,97)	3,32(2,97)	3,44 (3,11)	0,938
TSH, (mIU / L), mean (SD)	2,04 (1,2)	2,04 (1,2)	2,28 (1,29)	0,439
E2, (Pg/mL), mean (SD)	45,9 (26,6)	45,37 (26,99)	54,29 (21,38)	0,013
FSH, (mlU/ml), mean (SD)	7,47 (3,43)	7,49 (3,47)	7,32 (3,06)	0,895
LH, (IU/L), mean (SD)	7,84 (4,75)	7,77 (4,77)	9,25 (4,80)	0,057
Prolactin, (µg/L), mean (SD)	18,4 (10,35)	18,49 (10,25)	18,34 (12,43)	0,563

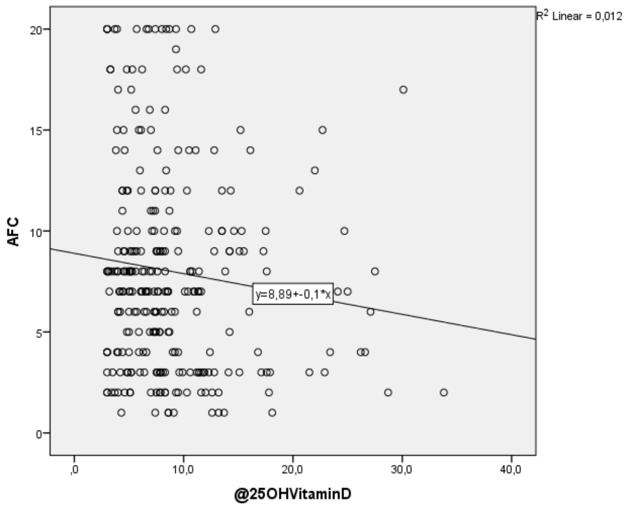

Figure 1: No correlation was found between AFC and Vitamin D (p:0,072, r:-0,107)

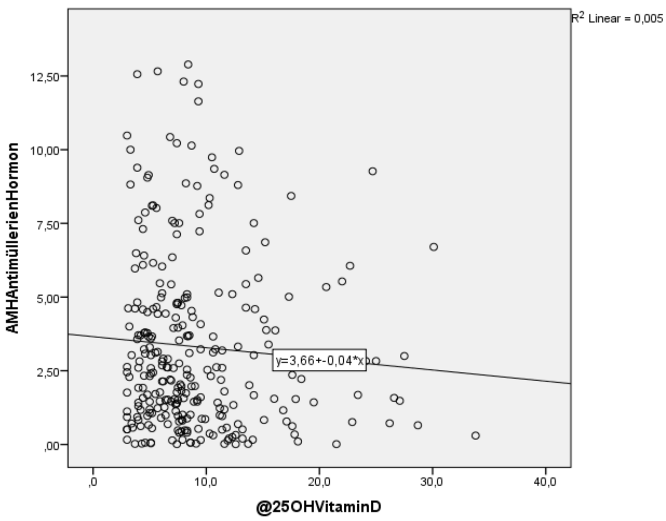

Figure 2. No correlation was found between AMH and Vitamin D (p:0,243, r:-0.088)