

Vitamin D levels in patients with premature ovarian failure

Poziom witaminy D u pacjentek z przedwczesnym wygasaniem czynności jajników

Ebru Ersoy, Ali Ozgur Ersoy, Gulcin Yildirim, Umran Buyukkagnici, Aytekin Tokmak, Nafiye Yilmaz

Zekai Tahir Burak Women's Health Care, Training and Research Hospital, Ankara, Turkey

Abstract

Objective: To investigate the role of vitamin D in the pathogenesis of premature ovarian failure.

Method: Forty-eight women diagnosed with POF, and 82 women recruited as controls were included in this cross-sectional study, between January 2014 and April 2014, in a reference center of infertility in the capital of Turkey. 25(OH)D3 levels of the patients were determined with the use of a specific enzyme-linked immunosorbent assay kit.

Results: There was no difference between two groups in terms of age, body mass index, smoking status, or sunlight exposure. 25(OH)D3 levels did not differ between the POF group (7.75 [3-21.22] µg/L) and the control group (6.74 [3-25.54] µg/L) ($P = 0.477$). There was no significant correlation between vitamin D and follicle stimulating hormone levels or between vitamin D and estradiol levels.

Conclusion: Although vitamin D level was reported to play a role in ovarian physiology, it seems not to have a role in the etiology of POF. Larger nation-wide or world-wide studies should be carried out to clarify the exact mechanism of POF.

Key words: **vitamin D deficiency / etiology / ovarian reserve / premature ovarian failure /**

Autor do korespondencji:

Ebru Ersoy
Zekai Tahir Burak Women's Health Care, Training and Research Hospital, Talatpasa Bulv. Hamamonu,
06230, Altindag, Ankara, Turkey
e-mail: eebuersey09@gmail.com

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Streszczenie

Cel pracy: Ocena roli witaminy D w patogenezie przedwczesnego wygasania czynności jajników.

Metoda: Do badania przekrojowego włączono 48 kobiet ze zdiagnozowanym POF i 82 kobiety jako grupa kontrolna, w okresie pomiędzy styczniem 2014 a kwietniem 2014, w ośrodku referencyjnym leczenia niepłodności w stolicy Turcji. Poziom 25(OH)D₃ u pacjentek oznaczono przy użyciu specyficznego powiązanego z enzymem immunoabsorpcyjnego zestawu testowego.

Wyniki: nie znaleziono różnic pomiędzy grupami dotyczących wieku, BMI, palenia tytoniu i ekspozycji na promieniowanie słoneczne. Poziom 25(OH)D₃ nie różnił się pomiędzy grupą z POF (7.75 [3-21.22] µg/L) a grupą kontrolną (6.74 [3-25.54] µg/L) (P = 0.477). Nie było istotnych związków pomiędzy witaminą D a poziomem hormonów stymulujących jajczkowanie oraz pomiędzy poziomem witaminy D a poziomem estradiolu.

Wnioski: chociaż mówi się, że witamina D pełni istotną rolę w fizjologii jajnika, to wydaje się że nie ma znaczenia w powstawaniu POF. Dalsze szerzej zakrojone badania krajowe i światowe są potrzebne aby wyjaśnić dokładny mechanizm POF.

Słowa kluczowe: niedobór witaminy D / etiologia / rezerwa jajnikowa /
/ przedwczesne wygasanie czynności jajników /

Introduction

Premature ovarian failure (POF) is a clinical entity that is characterized with the cessation of the folliculogenesis before the age of 40, caused insufficiency and loss of ovarian hormonal functions. Its incidence is estimated as ~1% in women under the age of 40 [1]. Amenorrhea for the previous 4 or more months, hypergonadotropism (Follicle stimulating hormone; FSH \geq 40 IU/L), hypoestrogenism [2], and extremely low levels of Anti-Müllerian hormone (AMH) in serum (below the level of 0.02 µg/mL) are the principal features for POF diagnosis [3]. POF generally arises from premature depletion of the pool of primordial follicles in the ovaries. Recently, fear of infertility with the clinical presentation of POF has been seen more frequently because of delayed marriages (i.e., at more advanced ages). Hormonal deficiency may also cause serious neurologic, metabolic, or cardiovascular problems, as well as early onset osteoporosis. Although autoimmunity, toxic compounds, drugs and genetic defects have been implicated in the etiology of POF, an exact etiology cannot be clarified in most cases [4-6]. Therefore, POF is considered a multifactorial and heterogeneous disease.

Vitamin D is mostly synthesized in the skin with the presence of ultraviolet (UV) light (especially UV-B). It is converted to its active form of 1, 25 dihydroxy-vitamin D₃ by the enzymes 25-hydroxylase and 1 α -hydroxylase, located in the liver and kidneys respectively. 1 α -hydroxylase is also present in the ovary, brain, and prostate; 1, 25 dihydroxy-vitamin D₃ can also be synthesized locally in these organs. Vitamin D affects not only the skeletal system, but also cell production and demolition, and suppression of immunity. Vitamin D exerts its effects via its receptor (Vitamin D receptor [VDR]) in the cell. VDR is found in the ovaries, uterus, hypophysis, and hypothalamus [7,8]. Its localization to these regions prompted us to consider a crucial role for vitamin D in the female reproductive system [9]. Vitamin D is also reported to have an effect on the secretion of the Anti Müllerian Hormone (AMH) from the granulosa cells in the ovary [10]. As is widely known, AMH decreases the levels of FSH by a negative feed-back mechanism. In this context, we aimed to investigate the potential role of vitamin D in the etiology of POF.

Materials and Method

Our study was conducted as a cross-sectional study in the Zekai Tahir Burak Women's Health Care Training and Research Hospital, Ankara, which is a referral medical center, between the dates of January 2014 and April 2014. The institutional review board approved the study, and the universal principles of the Helsinki Declaration (as revised in 2000) were applied. Two groups were established; one of them included Caucasian women diagnosed with POF (n = 48), and the other group included Caucasian women recruited as controls (n = 82). All of the patients underwent physical examination regarding determinants of secondary sex characters and certain syndromes associated with ovarian failure (e.g., syndromes with karyotype abnormalities, Fragile X syndrome). The diagnosis of POF was made in case of FSH \geq 40 U/L (confirmed with sequential analysis) and amenorrhea for at least six months in patients below the age of 40. A control patient was defined as below the age of 40 with regular menstruation cycles, having had at least two children and with no history of infertility. Women were excluded for the following reasons: detection of fetal anomalies, history of systemic and chronic diseases (e.g., liver disease, renal disease, thyroid disease, skin disease, and, malignant and immunologic diseases), history of ovarian surgery, chemotherapy, radiotherapy, use of various drugs/substances likely to affect the levels of vitamin D, and ovarian reserve determinants (e.g., hormonal drugs, alcohol, or cocaine). The concentration of 25(OH)D₃ is accepted as the best indicator for vitamin D levels, because it reflects vitamin D levels from both dietary intake and in-skin synthesis. 25(OH)D₃ levels do not change throughout the menstrual cycle [11]. As such, 25(OH)D₃ was used to assess serum vitamin D levels. The study period was entirely within the winter season, during which sun-light levels were relatively low, in the region of Ankara (Longitude: 40° 40' N, Latitude: 32° 34' E, Altitude: 891m, the average temperature is 0.4–11.3 °C) [12]. Due to its altitude and inland location, Ankara has a continental climate, with cold, snowy winters and hot, dry summers. Rainfall occurs mostly during the spring and autumn. The weather was rainy for ~5 days, and snowy for ~5 day of each month during the study period. It was sunny for ~4 h per day during the study period. All of the patients have been exposed to direct sun-light for a mean time

Table I. Comparison of demographic characteristics of the patients.

Characteristic	POF (n = 48)	Control group (n = 82)	P value
Age (years)	33.3±5.2	32.4±3.9	0.293
Age > 30 years [n(%)]	34 (70.8%)	50 (61%)	0.342
Gravidity	0 (0 – 4)	2 (0 – 6)	<0.001*
Parity	0 (0 – 3)	2 (0 – 5)	<0.001*
Abortus	0 (0 – 1)	0 (0 – 2)	<0.001*
Live Born	0 (0 – 3)	2 (0 – 5)	<0.001*
BMI (kg/m ²)	25.7±3.9	24.7±3.9	0.155
Smoking	14 (29.2%)	21 (25.6%)	0.813

Values were given as mean±standard deviation or median (range) or number (percentage). *Statistically significant; POF: Premature ovarian failure; BMI: Body mass index.

Table II. Comparison of basal hormone and 25(OH)D₃ levels between two groups.

Characteristic	POF (n = 48)	Control group (n = 82)	P value
FSH (mIU/mL)	60.5 (40 – 149)	6 (2.42 – 10.0)	<0.001*
LH (mIU/mL)	32 (12 – 61)	4 (1 – 10)	<0.001*
Estradiol (pg/mL)	14 (5 – 29)	43 (19 – 93.63)	<0.001*
Prolactin (µg/L)	9.62±4.42	11.11±4.62	0.074
TSH (mIU/L)	1.87±0.80	2.00±0.91	0.406
25(OH)D ₃ (ng/mL)	7.75 (3 – 21.22)	6.74 (3 – 25.54)	0.477

Values were given as mean±standard deviation or median (range). *Statistically significant; POF: Premature ovarian failure; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; TSH: Thyroid stimulating hormone.

of 30 min per day. Veiling habits were similar among patients as a result of cold weather in winter. Two groups were matched for daily intake of calcium, phosphorus, and vitamin D by a dietitian.

All women in the study gave written informed consent to participate. Following 10 h of fasting, blood samples were obtained from the antecubital vein early in the morning, and transferred to the laboratory in a light-proof case (so as not to be exposed to light). Serum samples were separated by centrifugation at 5,000 rpm (2,236 g) for 10 min within 15–20 min of blood sampling and studied immediately after centrifugation.

The serum 25(OH)VitD₃ levels were measured using an ELISA kit (Immunodiagnostic AG, Leverkusen, Germany). All blood samples were analyzed at the biochemistry laboratory of our hospital. The intra-assay and inter-assay coefficients of variation were 8.9 and 10.6% for serum 25(OH)VitD₃. Basal levels of FSH (with the use of an Immulite 2000 Analyzer, EURO/DPC Ltd, Gwynedd, UK; intra-assay variation of 4.0%, inter-assay variation of 6.7%) and LH (Immulite 2000 Analyzer; intra-assay variation of 4.8%, inter-assay variation of 10.7%), estradiol (with the use of an Immulite 2000 Analyzer; intra-assay variation of 6.7%, inter-assay variation of 9.7%), prolactin, and thyroid stimulating hormone (TSH) were all determined using the same device.

Anthropometric data were collected by a dietitian on the same day of blood sampling. Height and weight of the patients were measured using a professionally calibrated device. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (i.e., kg/m²).

Data were recorded and analyzed with the use of the IBM-SPSS for Windows Version 21.0 (IBM-SPSS, Armonk, NY, USA) program. The distributions of the variables were assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were presented as the mean and standard deviation (SD) or median (range), and categorical variables are presented as the number and percentage of subjects. Continuous variables were compared using independent-samples *t*-test if they were normally distributed or with the Mann-Whitney *U* test if they were non-normally distributed. Categorical variables were analyzed using the χ^2 test or Fisher's exact test. Spearman's rank correlation coefficients were calculated for non-normally distributed continuous variables. In all analyses, two-tailed *P*-values of < 0.05 were considered statistically significant.

Results

One-hundred-thirty patients between the ages of 22 and 39 were recruited for this study. Of these, 48 patients (36.9%) were in the POF group, and 82 (63.1%) were in the control group. The mean age of the patients in the POF group was 33.3±5.2 years, and 32.4±3.9 years in the control group (Table I). There was no difference between the two groups in terms of ages (*P* = 0.293). The number of patients above the age of 30 was 34 (70.8%) in the POF group, and 50 (61%) in the control group; the two groups were similar for this variable (*P* = 0.342). Obstetric history characteristics were significantly different between groups (Table I). There was no significant difference between the two groups in terms of BMI and smoking status (*P* = 0.155 and *P* = 0.813, respectively) (Table I).

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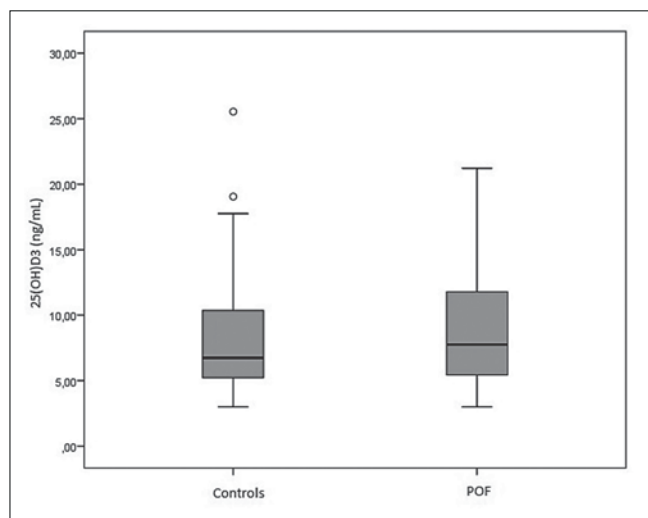


Figure 1. The concentration of 25(OH) D_3 and its changing levels in sera of the patients diagnosed with POF and controls. The 95% confidence interval was used.

FSH levels in the POF group (60.5 U/L, ranging from 40 to 149 U/L) were significantly higher than in the control group (6 U/L, ranging from 2.42 to 10.0) ($P < 0.001$). The LH levels in the POF group (32 U/L, ranging from 12 to 61 U/L) were significantly higher than the control group (6 U/L, ranging from 2.42 to 10.0 U/L) ($P < 0.001$). Estradiol levels in the POF group (14 pg/mL, ranging from 5 to 29 pg/mL) were significantly lower than the control group (43 pg/mL, ranging from 19 to 93.63 pg/mL) ($P < 0.001$). Prolactin and TSH levels were similar between the two groups ($P = 0.074$; $P = 0.406$, respectively) (Table II).

The 5(OH) D_3 levels did not differ between the POF (7.75 μ g/L, ranging from 3 to 21.22 μ g/L) and control (6.74 μ g/L, ranging from 3 to 25.54 μ g/L) groups ($P = 0.477$) (Table 2 and Figure 1).

There was no significant correlation between the 25(OH) D_3 and FSH levels of all 130 patients ($r = 0.070$; $P = 0.42$). Also, there was no significant correlation between the 25(OH) D_3 and estradiol levels of all 130 patients ($r = -0.086$; $P = 0.331$).

Discussion

POF occurs as a result of apoptosis and atresia of ovarian follicles and is thought to have an idiopathic etiology [13]. Various genetic and molecular studies have addressed its pathogenesis. Mutation of the FMR1 gene [14] and polymorphisms of the SF1 [15] and inhibin- α gene (INHA) are examples of substantial findings [16]. Furthermore, occupational hazardous substances such as ethylene glycol methyl ether; 2,2-bis(bromomethyl)-1,3-propanediol; benzo[a]pyrene; dimethylbenzanthracene [17], and smoking [18] were shown to lead to POF.

According to the guidelines of North American Endocrine Society, vitamin D deficiency is defined as < 20 ng/mL, and its insufficiency as between 20 and 30 ng/mL [19]. In a study conducted in west Turkey, where sun exposure is high, these high levels of vitamin D deficiency (74.9%) were accentuated [20]. In a study by Jukic et al., conducted in North Carolina (USA), reported a similar rate of vitamin D deficiency (75%) [21].

When planning this study, we postulated that vitamin D, which has also endocrinologic roles, may play a role in the

pathogenesis of POF. Nevertheless, we found no difference between our POF and controls groups for vitamin D levels. In a study by Irani and Merhi, it was found that vitamin D has substantial roles in ovarian physiology [22]. Moreover, various researches have recently shown that vitamin D deficiency causes changes in steroidogenesis and in levels of AMH [21, 23].

Vitamin D was found to be associated with ovarian reserve in an *in vitro* study and exerted its effects on granulosa cells via the expression of the receptor genes for AMH-2 (AMHR-2) and FSH (FSHR) [21], while a cohort study reported a positive correlation between serum AMH and vitamin D levels [10]. However, in these two studies, this association was only seen when the granulosa cells were present in ovarian preantral and early antral follicles. Vitamin D increases the synthesis of AMH in granulosa cells and AMH decreases FSH levels by negative feed-back inhibition of its FSH synthesis. In this context, we hypothesized that vitamin D deficiency might decrease AMH levels, resulting in elevated FSH levels and subsequently POF. Nevertheless, we found no difference in the vitamin D levels between of the POF and control groups in our study, and no significant correlation between the vitamin D and FSH levels. These findings do not support a role for vitamin D in the pathogenesis of POF. However, Jukic et al. found a highly significant inverse association between 25(OH) D_3 and FSH levels, with a 10 ng/mL decrease in 25(OH) D_3 being associated with a nearly 10% increase in FSH [24]. Likewise, another Turkish study found that Serum 25(OH) D_3 levels were negatively correlated with FSH levels [25].

The sample population being limited to women from Ankara was a limitation of our study. Therefore, there is a need for randomized studies with larger samples recruited from different geographic regions who have different sun-light exposures. Another limitation was the absence of AMH level assessments. If the AMH levels were available, its associations with FSH and vitamin D levels might be clarified.

Conclusion

Although vitamin D level were reported to play a role in ovarian physiology, it seems not to have a role in the etiology of POF. There is a need for the determination of the prevalence, etiology, and strategies for therapy of POF, which could be met by larger nation-wide or world-wide studies.

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Authors' contribution:

1. Ebru Ersoy – study design interpretation of data, article draft corresponding author.
2. Ali Ozgur Ersoy – acquisition of data, analysis, revised article critically.
3. Gulcin Yildirim – study design, acquisition of data, article draft.
4. Umrhan Buyukkagıncı – concept, interpretation of data, analysis, revised article critically.
5. Aytekin Tokmak – interpretation of data, analysis, revised article critically.
6. Nafiye Yılmaz – study design, interpretation of data, revised article critically.

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Persons in Charge: Anna Botto - Martina Di Paolo