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# Comparison of Vitamin D Plasma Level in Women with or Without Endometriosis: A Case-Control Study

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## Abstract

**Background:** Endometriosis is a debilitating gynecologic disease affecting millions of women worldwide. The potential role of vitamin D is of increasing interest. Previous studies have provoked controversy over vitamin D's role and have shown a correlation between endometriosis and low vitamin D serum levels.

**Objectives:** This study assessed and compared vitamin D serum levels between women with and without endometriosis.

**Methods:** We studied 47 patients with endometriosis who visited the Infertility and Reproductive Clinic at our tertiary hospital and compared their vitamin D levels with healthy control subjects (47 women). The analysis was performed using Mann-Whitney and chi-square tests.

**Results:** The mean age of the women was 32.49, and the SD was 5.42. The Mann-Whitney test showed no significant relationship between the mean vitamin D levels and endometriosis diagnosis ($P = 0.223$). However, a significantly higher proportion of women with vitamin D deficiency (< 30 ng/mL) were diagnosed with endometriosis. This was confirmed by the chi-square test (odds ratio [OR] = 3.410; $P = 0.008$).

**Conclusions:** In conclusion, while these results indicate an association between vitamin D deficiency and endometriosis, causality cannot be established based on this case-control study. Therefore, more studies are needed to determine the role of vitamin D in endometriosis.

*Keywords:* Deficiency, Endometriosis, Vitamin D

## 1. Background

Endometriosis is a debilitating gynecologic disease affecting millions of women worldwide, with symptoms of dysmenorrhea, chronic pelvic pain, and infertility (1). Women with endometriosis are also at increased risk of developing several cancers and autoimmune disorders (2). It takes 7 years on average before a correct diagnosis is obtained from a laparoscopic procedure. Therefore, it is of great importance to patients and clinicians to have a less-invasive method that would allow an earlier diagnosis of endometriosis. Despite decades of research, there are no sufficiently sensitive and specific signs and symptoms nor blood tests for the clinical confirmation of endometriosis, which hampers prompt diagnosis and treatment (3). The potential role of vitamin D is of increasing interest. Previous studies have demonstrated the role of vitamin D as a modulator of the immune system. Some authors have suggested a correlation between endometriosis and low vitamin D serum levels (4-6), while others have reported higher vitamin D serum levels in women with endometriosis (7, 8).



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## 2. Objectives

This study aimed to assess and compare vitamin D serum levels between women with and without endometriosis.

## 3. Methods

In this observational case-control study, we studied 47 women who visited the Infertility and Reproductive Clinic at our tertiary hospital from 2020 to 2022. We had 2 groups: (1) Patients with endometriosis and (2) control subjects without endometriosis (women who do not have symptoms that might indicate undiagnosed endometriosis, such as pelvic pain or infertility) (n = 47 per group). Endometriosis was suspected by ultrasound scan and then confirmed by laparoscopy and pathology. Whole blood and serum samples were collected. Each serum sample was divided into 2 tubes and analyzed for total 25-hydroxyvitamin D. Serum total 25(OH)D concentrations were analyzed using Elecsys Vitamin D Total Kits with the Cobas e602 module (Roche Diagnostics, Mannheim, Germany). The analysis was performed using Mann-Whitney and chi-square tests. The patients' ages were recorded, and their vitamin D levels were measured. P values less than 0.05 were considered statistically significant. This study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee.

## 4. Results

We studied patients who visited the Infertility and Reproductive Clinic at our tertiary hospital. All patients underwent laparoscopy to confirm endometriosis. All patients were diagnosed based on the pathological review of surgical specimens. Participants in this study were divided into 2 groups: Patients with endometriosis and healthy controls. Healthy controls were recruited from women who were at the clinic for health screening and did not report any symptoms of endometriosis. Whole blood and serum samples were collected. The mean age of the women was 32.49, and SD was 5.42 (Table 1). There was no significant difference in age and clinical characteristics between the 2 groups (Table 1).

A correlation analysis was performed to compare vitamin D levels between the study groups. Although the results showed that 25(OH)D levels are significantly higher in healthy controls than in endometriosis patients, the mean level of vitamin D (in ng/mL) was not statistically correlated with endometriosis. The Mann-Whitney test showed no significant relationship between vitamin D levels and endometriosis diagnosis (P = 0.223; Table 1). To confirm the relationships between low levels of vitamin D in patients and the incidence of endometriosis in them, patients were divided into 2 groups of with and without low level of vitamin D based on their vitamin D levels. According to the 2019 Endocrine Society guidelines, abnormal levels of vitamin D were defined as serum 25(OH)D levels lower than 30 ng/mL. Further analysis of patients with vitamin D <30 ng/mL showed that most of the patients with these levels of vitamin D were diagnosed with endometriosis. This was confirmed by the chi-square test (odds ratio [OR] = 3.410; P = 0.008; Table 2). In fact, Table 2 shows that women with vitamin D deficiency (< 30 ng/mL) had a significantly higher odds of having endometriosis (OR = 3.410, P = 0.008), despite the lack of significance in mean differences.

This suggests a potential association between vitamin D deficiency and endometriosis risk, specifically in deficient individuals. Considering patients in 3 categories of normal, insufficiency, and deficiency, according to the 2019 Endocrine Society guidelines, these were defined as serum 25(OH)D levels greater than 30 ng/mL, between 10 to 30 ng/mL, and less than 30 ng/mL, respectively. Patients with normal vitamin D levels were significantly less affected by endometriosis, and those with insufficient levels were significantly affected by endometriosis (P = 0.016); however, in the deficiency group (more severe cases), this difference was not significant between subjects with and without endometriosis (P > 0.05; Table 3), which may be due to sample size limitations.

## 5. Discussion

Endometriosis is one of the most common gynecological diseases of reproductive age, with a prevalence of 5 - 10%, decreasing quality of life and fertility. Vitamin D, a classic regulator of plasma calcium concentration and skeletal mineralization, is also an effective modulator of the immune system (9). Vitamin D, as an immunomodulator, has been hypothesized to play a critical role in the pathogenesis of endometriosis (10). Although promising, several studies have not shown a cause-effect relationship between vitamin D status and endometriosis; therefore, further studies are needed to better understand the association between

**Table 1.** Characteristic of Participants [a]

| Characteristic | Control Group (n = 47) | Endometriosis Group (n = 47) | P-Value |
|---|---|---|---|
| **Age (y) (mean)** | 31.68 | 33.29 | 0.109 |
| **Marital status** | | | 0.626 |
| Unmarried | 10 (21) | 12 (26) | |
| married | 37 (79) | 35 (74) | |
| **Gravidity** | | | 0.536 |
| NG (null gravid) | 23 (49) | 26 (55) | |
| > 1 | 24 (51) | 21 (45) | |
| **Vitamin D level (ng/mL) (mean)** | 23.27 | 26.35 | 0.223 |

[a] Values are expressed as No (%) unless otherwise indicated.

**Table 2.** Cross Tabulation Shows Incidence Rate of Endometriosis and Vitamin D Level [a]

| Variables | Endometriosis Group | Healthy Group | OR | P-Value |
|---|---|---|---|---|
| **Vitamin D** | | | 3.410 | 0.008 |
| **Normal** | 9 (30) | 21 (70) | | |
| **Low (< 30 ng/mL)** | 38 (59.4) | 26 (40.6) | | |

[a] Values are expressed as No (%).

vitamin D and endometriosis (11). However, Qiu et al. (11) recently published a meta-analysis and concluded that women with endometriosis had lower vitamin D status compared with controls, and a negative relationship between vitamin D levels and severity of endometriosis was observed (4). Baek et al. indicated no association between vitamin D and the severity of endometriosis (12). In an observational study of 49 women, Ciavattini found a relatively high rate of women with ovarian endometriosis and hypovitaminosis D (13). Recently, Delbandi et al. studied the serum levels of 25(OH)D in 56 healthy women and 54 patients with endometriosis and showed that subjects with vitamin D deficiency were at higher risk of endometriosis (9). However, in a case-control study, Buggio et al. assessed 434 women and concluded no association between vitamin D levels and endometriosis (9, 14). Regarding vitamin D levels in subjects with and without endometriosis, some previous studies have reported inconsistent findings. Our study shows free 25(OH)D concentrations between women with endometriosis and healthy controls. Women with endometriosis (n = 47) and healthy controls (n = 47) were enrolled in this observational case-control study. Our analysis showed no statistically significant difference in the mean vitamin D levels between the two groups, which is consistent with some previous studies (9, 14). However, when stratifying participants based on vitamin D status (< 30 ng/mL), we found that a greater proportion of individuals with vitamin D deficiency were diagnosed with endometriosis. Stratification into insufficiency and deficiency categories showed that the insufficiency subgroup was significantly associated with endometriosis, while the deficiency subgroup was not. These results suggest that while vitamin D status may be relevant in endometriosis, the relationship is nuanced and may depend on the degree of deficiency. While this shows a possible trend, the association did not reach statistical significance, indicating that future investigations with larger sample sizes are needed to clarify whether a true relationship exists. A limitation of our study is that we did not determine Body Mass Index (BMI), parathyroid hormone (PTH), and smoking. Our study also did not account for sunlight exposure, all of which are known to significantly influence serum vitamin D levels. These variables may have contributed to variability in vitamin D status independent of endometriosis diagnosis. Given the heterogeneity and diversity of the different studies, more research with careful control of confounding factors is required to elucidate the association between vitamin D and endometriosis. In fact, mean-level comparisons may not

**Table 3.** Cross Tabulation Show's Incidence Rate of Endometriosis and Insufficiency/Deficiency Vitamin D Level [a]

| Variables | Endometriosis Group | Healthy Group | P-Value |
|---|---|---|---|
| **Vitamin D** | | | |
| **Normal** | 9 (30) | 21 (70) | 0.008 |
| **Insufficiency** | 17 (68) | 8 (32) | 0.016 |
| **Deficiency** | 21 (53.8) | 18 (46.2) | 0.530 |

[a] Values are expressed as No (%).

capture distributional differences or subgroup trends. Our subgroup analysis suggested a potential pattern, but due to the limited sample size, the results did not achieve statistical significance. Therefore, additional research with large sample sizes is warranted, particularly studies designed to evaluate the hormonal mechanisms and the immunomodulatory role of vitamin D in endometriosis pathogenesis, which may help clarify these preliminary findings.

### 5.1. Conclusions

In conclusion, while these results indicate an association between vitamin D deficiency and endometriosis and there may be a relationship between vitamin D and endometriosis, causality cannot be established based on this case-control study. Therefore, more studies are needed to determine the role of vitamin D in endometriosis and also to explore whether vitamin D assessment or supplementation could contribute to the prevention, diagnosis, or management of endometriosis.

## Footnotes

**AI Use Disclosure:** The authors declare that no generative AI tools were used in the creation of this article.

**Authors' Contribution:** Study concept and design: N. Z.; Acquisition of data: D. N. and Z. P.; Analysis and interpretation of data: F. Kh.; Drafting of the manuscript: Sh. A.; Critical revision of the manuscript for important intellectual content: N. Z.; Statistical analysis: F. Kh.; Administrative, technical, and material support: D. N.; Study supervision: F. K.

**Conflict of Interests Statement:** The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

**Data Availability:** All data generated or analyzed during this study are available for review by the Editor-in-Chief of this journal on request.

**Ethical Approval:** This study was conducted in accordance with the Helsinki Declaration and was approved by the Tehran University of Medical Sciences ethics committee (IR.TUMS.IKHC.REC.1397.34 ).

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