Association of vitamin D deficiency and endometriosis - Study of fertile women

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Abstract

Background: The pathophysiology of endometriosis remains inadequately understood, leading to ongoing debate regarding the roles of various factors, including vitamin D, in its etiology and severity. Low vitamin D levels have been implicated in the pathophysiology of endometriosis, potentially contributing to proliferative and inflammatory processes. Recent studies have reported conflicting associations between serum vitamin D levels and endometriosis; while some suggest a protective role of vitamin D in mitigating the risk and severity of the disease, others report no significant correlation.
Aim: To find the relationship between vitamin D status and endometriosis in fertile women.
Methods: An observational retrospective case-control study of reproductive age women. 360 women were included and divided into 2 groups. In Group I-138(38.3%), fertile patients with surgically and morphologically proven diagnoses of endometriosis, and in Group II-222 (61.7%), fertile, generally healthy women of reproductive age (18-50). The association between endometriosis status and blood concentration of 25-hydroxyvitamin D (25OHD/calcifediol) was studied. Confounding factors such as age, Body Mass Index (BMI), and season were considered. The connection of vitamin 25OHD levels with the phenotype and stage of endometriosis, according to the American Society for Reproductive Medicine (ASRM) classification, was analyzed. The severity of chronic pelvic pain, menstrual cycle regularity, parity status, Antral follicle count (AFC) in connection with endometriosis, and vitamin D levels were studied. Data analyses were made using IBM SPSS 27.
Results: Extreme vitamin D deficiency (<10ng/ml) was more common in women with endometriosis than in healthy controls - 31.2% vs 7.2% (p-0.001). The mean 25OHD level in Group I (n=138) - 17.1 ng/ml was significantly (p<0.001) lower than in Group II (n=222) -20.9 ng/ml. The seasonal variations did not influence this result in vitamin D levels. In cases of deep endometriosis combined with the ovarian endometrioma or bilateral ovarian endometrioma levels of 25OHD were observed to be significantly lower compared to other - milder phenotypes. Size of endometrioma significantly negatively correlated with 25OHD levels (p<0.001 r=-0.5). The lowest mean 25OHD was identified in stage IV endometriosis according to ASRM classification and the difference in vitamin D levels between the stages was significant (p<0.001). The severity of dysmenorrhea was in negative association with 25OHD concentration independently from endometriosis status (r=-0.42 p<0.001).
Conclusion: Women with endometriosis have significantly lower 25OHD levels in the bloodstream compared to the control group, and women with advanced stages or complex forms of endometriosis are even more vitamin D deficient. This study adds weight to the hypothesis that low vitamin D levels are associated with the **severity** and **phenotype** of the disease. M**ore research** is needed to establish **causality** and vitamin D's potential role in endometriosis treatment. **(TCM-GMJ June 2025; 10 (1): P22-P27)**

Keywords: vitamin D, endometriosis, 25-hydroxyvitamin D, 25OHD, ovarian endometrioma

Introduction

Endometriosis, a complex gynecological disorder characterized by the presence of endometrial-like tissue outside the uterus, affects a significant proportion of reproductive-age women and is associated with chronic pelvic pain, dysmenorrhea, and infertility(1). The pathophysiology of endometriosis remains inadequately understood, leading to ongoing debate regarding the roles of various factors, including vitamin D, in its etiology and severity. Low vitamin D levels have been implicated in the pathophysiology of endometriosis, potentially contributing to proliferative and inflammatory processes(2) (3) (4). Recent studies have reported conflicting associations between serum vitamin D levels and endometriosis; while some suggest a protective role of vitamin D in mitigating the risk and severity of the disease, others report no significant corre-

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lation (5)(6) A large population-based study evaluating 3232 American women found a significant inverse association between serum vitamin D (25OHD) and the risk of endometriosis, after considering confounding variables, such as age, social status, demography, smoking, physical activity, and diet (7). A previous meta-analysis of 9 studies concluded that lower vitamin D levels are associated with endometriosis and even related to the severity of the disease (8). According to another study, 25OHD levels were lower in the peritoneal fluid of patients with endometriosis than in healthy controls (9). Some surveys indicate elevated serum 25OHD levels in patients with endometriosis (10). A recent study from 2024 reports a positive association between ovarian endometrioma and vitamin D levels (11). Others report no significant differences between affected and control groups and conclude that existing data cannot clarify the relationship between endometriosis and vitamin D (12). The latest meta-analysis, which included 976 patients with endometriosis and 674 controls, could not come to clear conclusions because many factors could influence study results. Such confounding factors are regional, seasonal, and individual differences in sun exposure, method of laboratory assessment, etc. 3 out of 9 studies from this meta-analysis found no difference regarding vitamin D levels in women with endometriosis compared to healthy women (2). Contradictory findings complicate our understanding of this relationship.

Methods

Study Design: An observational case-control study.

Study population: Fertile women of reproductive age with endometriosis.

The medical history of 1537 women of reproductive age (18-50 years) from 4 medical centers in Georgia, Tbilisi-between December 2022 and April 2024 was evaluated. 360 women met the inclusion criteria. We divided participants into 2 groups. In Group I were included 138(38.3%) fertile patients with surgically and morphologically proven diagnoses of endometriosis, and in Group II, 222 (61.7%) fertile, generally healthy women of the same age (18-50), who underwent routine check-ups. The association between endometriosis status and blood concentration of vitamin 25OHD was studied. We considered confounding factors such as age, BMI, and season. The associations between vitamin D levels and characteristics of endometriosis, such: phenotype, ASRM stage, dysmenorrhea, dyspareunia, menstrual cycle regularity, parity status, and AFC, were analyzed. We followed Endocrine Society recommendations, and 25OHD < 20ng/ml was considered vitamin D deficiency, 20- 30ng/ml as insufficiency, and >30ng/ml as a normal vitamin D level.(13).

Inclusion criteria: For Group I – surgically and histopathological confirmed diagnosis of endometriosis after laparoscopy, for Group II – healthy reproductive status confirmed with gynecological examination, ultrasound, and medical history.

Exclusion criteria: For both groups, infertility, any serious chronic diseases, genetic diseases, malabsorption diseases, cancer, polycystic ovary syndrome (PCOS), diabetes mellitus of any type, thyroid gland hormonal dysfunctions, any hormonal or anti-hormonal treatment past 3 months, vitamin D supplementation past 6 months, frequent sun exposure past 3 months, pregnancy or lactation past 6 months, obesity, and bariatric surgery.

The ethics committee approved this study.

Statistical analysis:

Statistical analysis was done using IBM SPSS Statistics Version 27. Correlation for continuous variables was made with Pearson correlation analysis. Categorical and continuous variables were compared using an independent Student's *t*-test, and for multiple group comparisons with a one-way ANOVA test. *P*-value < 0.05 was considered statistically significant.

Results and discussion

From a total sample of 360 patients, 138(38.3%) had a surgically and morphologically confirmed diagnosis of endometriosis after laparoscopy, and 222 (61.7%) were healthy controls. The mean BMI in Group I was 24.1kg/m^2 (STD ±3.2), and in Group II, 24.3kg/m^2 (SD ±3.3); the mean age in Group I was 31.1 (SD ±6.6), and in Group II, 33.3 (STD ±8.7). Groups were comparable and there was no significant difference in age (p-0.1) and BMI (p-0.5) between the groups. Participants with obesity were initially excluded from the study due to the possible impact of body weight on vitamin D levels. The BMI range in the total study group was 18-29.9 kg/m^2.

Vitamin D levels in healthy, reproductive-age, fertile, non-obese women (n-222) from Tbilisi, Georgia (Group II) were analyzed. Although Georgia has a humid subtropical climate, 88.3% of women had deficiency or insufficiency. Extreme low levels, such as 25OHD <10 ng/ml, were detected in 7.2% of healthy women, 10-20 ng/ml in 45.1%, 20-30 ng/ml in 36%, and normal levels > 30 ng/ml only in 11.7% of Group II. (Table 1)

The mean 25OHD level in women with endometriosis (Group I) was 17.1 ng/mL (STD ±9.6) (n=138), minimum-4ng/ml, maximum-50ng/ml, whereas the mean level of 25OHD in Group II was 20.9ng/ml (STD±8.7) (n=222), minimum-6.4ng/ml and maximum 54.8ng/ml. Women with endometriosis had significantly ($p<0.001$) lower 25OHD levels compared to the control group.

31.2% of women with endometriosis had 25OHD <10 ng/ml, 10-20 ng/ml -37.6%, 20-30 ng/ml -20.3%, and 10.9% had normal levels above 30 ng/ml. Healthy women were more prone to have insufficiency than women with endometriosis, whereas extreme deficiency of 25-hydroxy vitamin D <10 ng/ml was more common in women with endometriosis (Table 1).

From the total sample (n360), 25OHD was measured in 29.2% (n105) of participants in spring, 26.1% (n94) in summer, 25%(n90) in autumn, and 19.7% (n71) in winter.

In nearly all seasons, Group I had a significantly lower concentration of 25OHD in blood compared to Group II's results (Table 2).

25OHD levels in Group I were significantly different in each season (p-0.01), but in the healthy control Group II, seasonal changes of 25OHD appear more serious ($p<0.001$).

We divided Group I patients based on the phenotype of endometriosis. Most of the study patients (50%, n=69) were diagnosed with deep endometriosis. The highest mean 25OHD serum levels were seen in peritoneal endometriosis (23.6 ng/ml), whereas the lowest were in deep endometriosis combined with ovarian endometrioma (14.6 ng/ml) and bilateral ovarian endometrioma (8.6 ng/ml) (Table 3).

In the case of deep endometriosis involving the ovary or bilateral ovarian endometrioma, levels of 25OHD are significantly lower (p-0.007), compared to other milder phenotypes of endometriosis. Analyzing circulating vitamin D and phenotype associations according to seasons, this tendency stays, and more complex phenotypes are characterized by extremely low vitamin D levels (9- 11ng/ml) in all seasons. Other locations did not show any order or clear association with the degree of 25OHD deficiency.

The size of ovarian endometrioma was available in 49 cases. The maximal diameter of ovarian endometrioma was 76mm, and the minimal was 17mm. The size of endometrioma significantly negatively correlated with 25OHD levels ($p<0.001$, $r=-0.5$).

In Group I, endometriosis was staged postoperatively according to the ASRM classification. Of the total 138 patients with endometriosis, stage I was diagnosed in 2.9% (n=4) of patients, stage II in 10.1% (n=14) of patients, stage III in 44.2% (n=61) of patients, and stage IV in 42.8% (n=59) of patients. The difference in 25OHD levels between the 4 endometriosis stages was statistically significant ($p<0.001$). The lowest mean 25OHD was identified in stage IV endometriosis – 12.2ng/ml, and the highest in stage II – 26.4ng/ml (Table 4).

In Groups I and II, the mean AFC was similar, -8.1 (STD ±4.1) in Group **I and** 9.7(STD ±6.5) in Group II. Results did not differ significantly. The association between AFC and 25OHD was weak and negative (p-0.07, r=-0.25).

25OHD levels were compared between women with regular and irregular menstrual cycles, independently of endometriosis status, and no difference was detected. Regularity of the menstrual cycle was related to endometriosis status. Women with endometriosis tend to have irregular menstrual cycles more frequently $p<0.001$. We could not find a relationship between menstrual cycle phases and 25OHD levels.

In this study, only fertile women were included. In Group I, 73.9% had 1, 18.1% -2, 6.5%-3, and 1.4% -4 live births. In Group II, 64.1% had -1, 23.4%-2, 9.9%-3, and 2.3%-4 live births. The number of live births between Group I and II didn't differ significantly (p-0.06). In all parity groups, 25OHD levels did not differ significantly (p-0.3).

The severity of dysmenorrhea and dyspareunia was analyzed by subjective pain feeling, using a pain scale from 0 to 10 points. The pain was in a negative association with 25OHD concentration independently from endometriosis status ($r=-0.42$, $p<0.001$).

This study contributes to the ongoing debate about the role of vitamin D in endometriosis. Notably, the Georgian population has no reliable data about vitamin D levels. The sample size of our study obviously cannot provide information about vitamin D deficiency prevalence. Still, only 11.7% of young healthy women with vitamin D sufficiency indicate a worldwide inappropriate interpretation of 25OHD levels or heavy deficiency despite the mild climate. A wrong understanding of vitamin D deficiency is discussed in the last Endocrine Society guideline, which highlights no clear evidence that could define the optimal target levels of 25OHD, which could benefit the prevention of diseases (14). If 25OHD reference ranges reflect the real picture, reasons for deficiency in the Georgian population may vary from limited food fortification, and indoor city lifestyle to low awareness about the importance of vitamin D. Despite high vitamin D deficiency and insufficiency in healthy women, study participants diagnosed with endometriosis have even lower vitamin 25OHD concentration in their blood. Women with endometriosis have significantly ($p<0.001$) lower 25OHD levels compared to the healthy controls. From the latest literature, similar results were obtained in a large study of American women (7) and in a meta-analysis including 9 studies (8). According to our results, the normal concentration of vitamin D (>30ng/ml) in Groups I and II is seen in practically similar frequency. 10,9% of women with endometriosis have sufficient vs 11,7% of healthy women. However, regarding severe deficiency (25OHD<10ng/ml), a huge difference is visible between the women with endometriosis and the control group. 31.2% of women with endometriosis have 25OHD levels <10 ng/ml, compared to just 7.2% of the healthy group. In our study, the smallest proportion of participants, 19.7%, were included in winter. Despite that fact, deficiency and insufficiency are prominent. The 25OHD blood concentrations of Groups I and II were compared in each season separately, which excluded the influence of seasonal variation on our results. Women with endometriosis (Group I) had lower vitamin D levels across all seasons when compared to the healthy control group. We could not find any other study that provides results according to seasons in such detail. Notably, seasonal influence affects the 25OHD concentration of healthy women more than that of women with endometriosis. It may lead to some debates that the vitamin D levels of women with endometriosis are not so dependent on natural UV rays as those of healthy women. Some other factors may play a more crucial role in vitamin D metabolism in women with endometriosis. The importance of seasons was discussed in some other studies. They mainly mention that seasons influence 25OHD levels of patients with endometriosis, and the concentration of vitamin D in winter is lower than in other seasons. (15) This is consistent with our findings, but additionally, in spring, we found a similarly low mean 25OHD concentration in both study groups, as in winter. Another study highlighted challenges posed by seasonal variations in vitamin D levels, pointing out how these fluctuations complicate the interpretation of vitamin D status in endometriosis studies (16). Exciting

evidence underscores the importance of seasonality control in studies regarding vitamin D.

Our findings suggest that vitamin D deficiency is prevalent among women with endometriosis, particularly in those with more severe forms of the disease. The study showed that women with advanced stages of endometriosis (Stage IV) had the lowest mean 25OHD levels (12.2 ng/ml), while those with Stage II had the highest. The difference between ASRM stages was statistically significant. The inverse relationship between vitamin D levels and disease severity aligns with studies suggesting that low vitamin D may exacerbate the inflammatory processes associated with endometriosis. The significant difference in vitamin D levels across ASRM stages in the current study is consistent with findings in other studies, which reported that vitamin D levels tend to be lower in patients with higher ASRM stages, especially in Stage IV endometriosis. (2,8) supporting the idea that vitamin D deficiency may be linked to disease progression. **The** phenotype of endometriosis is also in connection with vitamin D. In combined forms like deep endometriosis combined with ovarian endometrioma or bilateral ovarian endometrioma, vitamin D blood concentration is significantly lower than in mild forms. For comparison, women with unilateral ovarian endometriomas have significantly higher 25OHD levels - 18.4 ng/ml, compared to women with bilateral ovarian endometriomas - 8.6 ng/ml. The literature about the relationship between vitamin D levels and endometriosis phenotypes is scarce. Most studies indicate a trend toward lower vitamin D levels in more severe forms of the

Table 1. ***Vitamin D blood levels in women with endometriosis (Group I) and healthy women (Group II)***

25OHD level	*Group I*	*Group II*
<10ng/ml	31.2%	7.2%
10-20 ng/ml	37.6%	45.1%
20-30 ng/ml	20.3%	36%
>30 ng/ml	10.9%	11.7%

Table 2. ***Vitamin D blood concentration comparison according to seasons between women with endometriosis (Group I) and the healthy control group (Group II).***

Season	*25OHD in Group I Endometriosis*	*25OHD in Group II Healthy*	*Significance of the difference in 25OHD levels between Group I and II*
Spring	14.4 ng/ml	17.4 ng/ml	p-0.06
Summer	19.5 ng/ml	25.3 ng/ml	p-0.007
Autumn	19.3 ng/ml	23.9 ng/ml	p-0.02
Winter	13.2 ng/ml	17.9 ng/ml	p-0.004

Table 3. ***Vitamin D blood concentration in women with endometriosis with different phenotypes***

PHENOTYPE OF ENDOMETRIOSIS	*Frequency*	*Mean 25OHD*
Deep endometriosis	50% (n=69)	16.7 ng/ml (STD ±9.1)
Ovarian endometrioma	32.6% (n=45)	18.4 ng/ml (STD ±10.5)
Deep endometriosis involving the ovary	10.9% (n=15)	14.6 ng/ml (STD ±7.9)
Peritoneal endometriosis	3.6 % (n=5)	23.6 ng/ml (STD ±13)
Bilateral ovarian endometriosis	2.2% (n=3)	8.6 ng/ml (STD ±2)
Tubal endometriosis	0.7% (n=1)	17 ng/ml

Table 4. *Vitamin D blood concentration in women with different stages of endometriosis*

ASRM stages	*Frequency*	*Mean 25OHD*
Stage I	(n=4) 2.9%	18.2 ng/ml (STD ±6.1)
Stage II	(n=14) 10.1%	26.4ng/ml (STD ±9.4)
Stage III	(n=61)44.2%	19.6 ng/ml (STD ±9.6)
Stage IV	(n=59)42.8%	12.2ng/ml (STD ±7)

disease, considering only ASRM stages**,** not concentrating on phenotypes. Some studies did not receive clear results. (12)(2) It's noteworthy that one study reported a higher 25OHD concentration in the blood of women with ovarian endometriomas than in healthy women (11), which is contrary to our results, even though the size of endometrioma significantly negatively correlated with 25OHD levels. The regularity of the menstrual cycle in our study was connected with endometriosis, which is an unusual finding. It seems that women with endometriosis tend to have irregular menstrual cycles more frequently. Our research could not find any relationship between menstrual cycle phases and 25OHD status. The severity of dysmenorrhea was in negative association with 25OHD concentration independently from endometriosis status, which is in agreement with the latest evidence that vitamin D supplementation may reduce the severity of dysmenorrhea, offering a potential adjunctive treatment for individuals experiencing menstrual pain. Vitamin D exhibits anti-inflammatory properties by modulating inflammatory cytokines, potentially reducing inflammation associated with menstrual cramps. Vitamin D metabolites can decrease the synthesis of prostaglandins in the endometrium, which are compounds that induce uterine muscle contractions, leading to menstrual pain. (17,18) The exclusion criteria in our study were strict. The study focused exclusively on fertile women to eliminate the confounding factor of infertility, which is, by itself, connected to vitamin D deficiency. Somigliana et al. (2007) found that vitamin D levels in infertile women with endometriosis were lower compared to fertile women with endometriosis. Ursache et al. (2024) also acknowledged that infertility could confound results (2,10). This distinction is important because the relationship between vitamin D and endometriosis may be influenced by fertility status, as vitamin D deficiency can impact reproductive health independent of endometriosis status. Many studies are highlighting the importance of vitamin D levels for fertility (19–21).. It is also important that obesity was excluded as a known associated factor with vitamin D deficiency. Obesity may interfere with vitamin D metabolism, as vitamin D is fat-soluble and may be sequestered in adipose tissue (22–24). Xie considered confounding variables, such as age, social status, demography, smoking, physical activity, and diet, but not fertility status, body mass index, and season. Many other studies also do not provide detailed information about cofactors(7,11).

Conclusion

Vitamin D deficiency is common in women with endometriosis and may be associated with disease severity and phenotype. The observational nature of the study cannot make any causal conclusions. More controlled, longitudinal studies are needed to understand the mechanism beyond the relationship between vitamin D and endometriosis.

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Conflict of Interest:

Authors declare no conflict of interest

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