

# JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

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Original Research Article

## Vitamin D Levels in Patients with Endometriosis Cysts

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### Article Info

#### History

Received: 27 Aug 2025

Accepted: 20 Nov 2025

Available: 24 Dec 2025

### Abstract

**Background:** Endometriosis is associated with lipid peroxidation by reactive oxygen species (ROS) which causes endometrial cell DNA damage. Vitamin D is known to have good antioxidant content, so it is thought to have the ability to inhibit proliferation, invasion and production of pro-inflammatory cytokines in endometriosis.

**Objective:** To analyze the differences in vitamin D levels between endometriosis cyst patients and normal women and the relationship between vitamin D levels and the size of endometriosis cysts.

**Methods:** Analytical observational study with cross sectional design with 60 samples consisting of 30 endometriosis cyst patients and 30 normal women. Evaluation was carried out on vitamin 25 (OH) D serum levels using the ELISA method and endometriosis cyst size. Cyst size is evaluated by assessing the largest diameter of a single endometriosis cyst assessed through an ultrasound examination by a specialist doctor.

**Results:** Vitamin D levels in the endometriosis group were significantly lower than normal women ( $13.23 \pm 5.39$  ng/mL vs  $15.83 \pm 5.30$  ng/mL;  $p = 0.048$ ). Subjects with vitamin D levels  $<13.45$  had a 4.03x (OR 4.03; CI95% 1.37-11.83) higher risk of experiencing endometriosis compared to subjects with vitamin D levels  $> 13.45$  ng/mL. There was no correlation between vitamin D levels and the size of endometriosis cysts ( $p=0.720$ ).

**Conclusion:** Endometriosis patients have significantly lower serum vitamin D levels than normal women (OR 4.03; CI95% 1.37-11.83). Serum vitamin D levels do not significantly correlate with endometriosis cyst size.

**Keywords:** Endometriosis; Endometriosis cyst size; Vitamin D.

**Permalink/ DOI:** <https://doi.org/10.14710/jbtr.v11i3.29234>

### INTRODUCTION

Endometriosis is a condition that affects women of reproductive age, including adults and teenagers. It happens when tissue similar to the lining of the uterus grows outside the womb. About 10 to 15% of women in this age group have endometriosis, and around 70% of women who experience long-term pelvic pain are affected.<sup>1</sup> The development of endometriosis is linked to damage to the DNA of endometrial cells, which is caused by lipid peroxidation due to reactive oxygen species (ROS). These ROS lead to the release of inflammatory substances and create oxidative stress, which triggers an inflammatory response. This inflammation leads to the buildup of lymphocytes and macrophages, which are cells that produce a lot of

cytokines.<sup>2</sup> These cytokines then promote enzyme activity and encourage the growth of new blood vessel cells. Vitamin D is known for its antioxidant properties. The active form of vitamin D can work as a membrane antioxidant, helping to protect cell membranes from damage caused by lipid peroxidation.<sup>3</sup> It is also known to slow down the growth, spread, and production of pro-inflammatory cytokines in endometriosis. Additionally, vitamin D helps lower the production of interleukin 6 and other inflammatory cytokines that contribute to the development of endometriosis.<sup>4</sup>

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25(OH)D, or 25-hydroxyvitamin D, is the main form of vitamin D found in the bloodstream and serves as the most reliable marker of an individual's vitamin D status derived from sunlight exposure and dietary sources. It is synthesized in the liver following vitamin D absorption, and its blood levels are measured to assess whether a person has a deficiency, insufficiency, or adequate amount of vitamin D.<sup>5</sup>

This study was conducted to analyze the differences in vitamin D levels between endometriosis cyst patients and normal women and the relationship between vitamin D levels and the size of endometriosis cysts.

## MATERIALS AND METHODS

Analytical observational study with *case control* design with 60 samples consisting of 30 endometriosis cyst patients and 30 normal women. Evaluation was carried out on vitamin 25 (OH) D serum levels using the ELISA method and endometriosis cyst size. Cyst size is evaluated by assessing the largest diameter of a single endometriosis cyst assessed through an ultrasound examination by a specialist doctor. Sampling was carried out using consecutive sampling, namely selecting research subjects based on research criteria and subjects signing an agreement to participate in the research. The study was conducted from July to October 2023 in RSUP dr. Kariadi Semarang.

Inclusion criteria for endometriosis group include 1) Women aged 18 – 45 years; 2) Single endometriosis cyst based on ultrasound results; 3) Anatomical pathology examination with endometriosis histopathology results. Inclusion criteria for normal women group include 1) Women aged 18 – 45 years; 2) No clinical symptoms of endometriosis; 3) There is no cyst appearance on ultrasound examination; 4) Ca-125 levels < 35 U/mL. Exclusion criteria include 1) Women with malignant disease, 2) Women with hormonal therapy in the last 6 months, 3) Women with vitamin D supplementation 6 months before surgery, 4) Women with systemic diseases (diabetes mellitus, cerebrovascular disease, chronic hypertension, kidney disease and heart).

The Mann Whitney U test was employed to analyze differences in vitamin D levels among the research groups. The ROC curve was utilized to analyze the cutoff level of vitamin 25 (OH) D that could indicate the status of endometriosis cysts. The association between vitamin 25 (OH) D levels and the risk of endometriosis was evaluated using Chi-Square. The Spearman Test was used to analyze the relationship between vitamin D levels and the dimensions of endometriosis cysts. Analysis was performed using the SPSS version 29 software. Outcomes are considered significant when the p value is less than 0.05. Ethical approval was secured from The Health Research Ethics Committee RSUP dr. Kariadi Semarang no. 1473/EC/KEPK-RSDK/2023.

## RESULTS

This study found significant differences in several demographic characteristics of the subjects. The average age of patients with endometriosis ( $33.7 \pm 6.77$  years) was significantly higher than that of the normal women group ( $26.87 \pm 5.45$  years;  $p < 0.001$ ), indicating that endometriosis is more common in the older age group. Height also showed a significant difference, where endometriosis patients had a lower average height ( $155.7 \pm 5.52$  cm) than normal women ( $159.17 \pm 4.14$  cm;  $p = 0.008$ ). In addition, a history of infertility was significantly more common in endometriosis patients (46.7%) compared to the normal women group, all of whom had no history of infertility ( $p < 0.001$ ), which strengthens the relationship between endometriosis and infertility.

Analysis of vitamin D levels between the endometriosis group and normal women showed the following results.

In the endometriosis group, Vitamin D levels had an average of 13.23 ng/mL and a standard deviation of 5.39 ng/mL, with a median of 12.45 ng/mL; the minimum value was 5.8 ng/mL and the maximum was 33 ng/mL. In the cohort of typical women, the average was 15.83 ng/mL with a standard deviation of 5.30 ng/mL, the median was 14.65 ng/mL, the minimum was 10.4 ng/mL, and the maximum was 30.9 ng/mL. A

**Table 1.** Subject characteristic between study groups

Variable	Endometriosis (n=30)	Normal Women (n=30)	p
Age, years	$33.7 \pm 6.77$ ; 33.5 (20-45)	$26.87 \pm 5.45$ ; 26.5 (20-39)	$<0.001^{\ddagger}$
Height, cm	$155.7 \pm 5.52$ ; 155 46-170)	$159.17 \pm 4.14$ ; 158 (53-167)	$0.008^{\dagger}$
History of infertility			$<0.001^f$
• Yes	14 (46.7)	0 (0)	
• No	16 (53.3)	30 (100)	
Vitamin D level, ng/mL	$13.23 \pm 5.39$ ; 12.45 (5.8-33)	$15.83 \pm 5.30$ ; 14.65 (10.4-30.9)	$0.048^{\ddagger}$

<sup>††</sup>Independent T-test; <sup>‡</sup>Mann Whitney U; <sup>f</sup>Fischer exact

**Table 2.** The risk of endometriosis based on vitamin D levels

Variable	Endometriosis		p	OR (CI95%)
	Yes	No		
Vitamin D level, ng/mL				
< 13.45 ng/mL	19	9	0.010	4.03
> 13.45 ng/mL	11	21		(1.37-11.83)

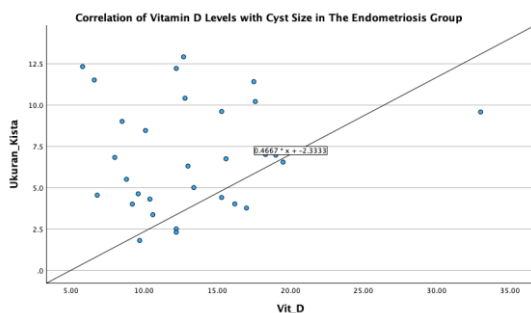
Chi-square; significant  $p < 0.05$

notable difference existed in the distribution of vitamin D among the study groups ( $p=0.048$ ). The endometriosis group exhibited notably lower average vitamin D levels.

In research subjects with vitamin D levels  $<13.45$  ng/mL, there were 19 subjects suffering from endometriosis and 9 subjects without endometriosis. In subjects with vitamin D levels  $>13.45$  ng/mL, there were 11 subjects suffering from endometriosis and 21 subjects without endometriosis. There is a relationship between vitamin D levels and the incidence of endometriosis ( $p=0.010$ ). Subjects with vitamin D levels  $<13.45$  had a 4.03x (OR 4.03; CI95% 1.37-11.83) higher risk of experiencing endometriosis compared to subjects with vitamin D levels  $>13.45$  ng/mL

**Table 3.** Endometriosis cyst size

Variable	Endometriosis
Cyst size, mm	$69.3 \pm 32.9$ ; 66.4 (20-130)



**Figure 1.** Scatter plot between vitamin D levels with cyst size

Cyst size, in the endometriosis group, the mean was 69.3 mm with a standard deviation of 32.9 mm, the median value was 66.4 mm with the smallest value being 20 mm and the largest value being 130 mm.

The figure 1 shows the relationship between vitamin D levels and cyst size in a group of endometriosis patients. The graph shows a positive correlation between vitamin D levels and cyst size, as indicated by a regression line with the equation  $y = 0.4667x - 2.3333$ . This indicates that increasing vitamin D levels tend to be followed by increasing cyst size. However, the wide distribution of data points around the regression line indicates that the strength of this relationship is relatively weak to moderate, suggesting that other factors may play a role in influencing cyst size in endometriosis patients.

**Table 4.** Correlation of vitamin D levels with cyst size in the endometriosis group

Variable	Cyst size	
	p	r
Vitamin D level	0.720	0.068

Spearman; significant  $p < 0.05$

Analysis carried out only in the endometriosis group showed that there was no correlation between vitamin D levels and the size of endometriosis cysts ( $p=0.720$ ).

## DISCUSSION

In the endometriosis group, the average age was much higher compared to the group of women without the condition. Earlier research shows that endometriosis can only develop during a woman's reproductive years. Studies have found that the highest risk is at age 44, with other high-risk periods between 25 and 29 years old. After 44, the risk goes down in all these studies. In this study, the average age when the disease first started was 33.7 years. There is usually a delay between when the first symptoms appear and when the disease is diagnosed through surgery. It's also known that when a woman has her first surgery for endometriosis depends on factors like her education level, how easy it is to get specialized medical care, and when she first started having symptoms.<sup>6,7</sup> These various factors can lead to a delay in the surgical diagnosis of endometriosis.

The average age of 33 falls within the productive age range. This connects to the idea that endometriosis is more common in women who are able to have children and less common in women who have gone through menopause and no longer make estrogen. Research shows that changes in hormones influence how endometrial cells develop when they stick to the mesothelium, and the body's immune system does not clear these cells. This supports the view that endometriosis is a disease that depends on estrogen. Estrogen levels drop quickly around age 50, which is why endometriosis is mainly a concern before menopause.<sup>8</sup>

Women with endometriosis were more likely to have a history of infertility compared to those without the condition. A study by Radhika AG and others found that 73% of endometriosis patients had moderate to severe cases, and 31.46% had infertility issues.<sup>9</sup>

The way endometriosis affects fertility is complicated. It involves interactions between different types of endometriosis, pain, inflammation, changes in the pelvic area, adhesions, reduced ovarian function, and problems with the lining of the uterus. Additionally, the disease has effects beyond the pelvis that also affect fertility.<sup>10</sup>

Pain can play a role in infertility related to endometriosis. When superficial dyspareunia, which is pain near the entrance of the vagina, makes sex difficult to start or keep, it can lead to avoiding sexual activity. However, not many studies have looked at how superficial dyspareunia is connected to endometriosis, and it often happens along with deep dyspareunia. Endometriosis is linked to a nine times higher risk of dyspareunia. This is mainly because of more severe forms of the disease that affect areas like the back of the vagina, the pouch of Douglas, the uterosacral ligaments, and the rectum. The ongoing pelvic pain that isn't tied to menstruation can affect sexual life by lowering desire, frequency, arousal, or the ability to have an orgasm. This can lead to major problems in relationships, emotional health, and overall quality of life.<sup>11,12</sup>

Anastasia E, et al., who studied vitamin D concentrations in endometriosis patients, found that women with this condition had an average serum 25-OH-vitamin D level of  $21.3 \pm 8.9$  ng/mL. A deficiency

of 25-OH-vitamin D exists in 48% of women, insufficiency affects 32% of women, and only 20% have adequate levels of vitamin D. Overall, vitamin D deficiency or insufficiency occurs in 80% of cases. In the healthy group, the mean concentration of 25-OH-vitamin D was  $32.3 \pm 2.67$  ng/mL. The prevalence of 25-OH-vitamin D deficiency/insufficiency was significantly greater in women with endometriosis than in the control group (80% vs. 33.3%;  $p < 0.001$ ). There is a notable connection between 25-OH-vitamin D levels and the incidence of endometriosis ( $p < 0.001$ ).<sup>13</sup>

Low levels of vitamin D are linked to endometriosis. Vitamin D plays an essential role in various pathogenetic mechanisms, such as cellular proliferation, invasion, degradation, and inflammation, by modulating cytokines such as interleukin-6 and interleukin-8, prostaglandin activity, and matrix metalloproteinases.<sup>14</sup>

Vitamin D plays a role in multiple functions of the reproductive system, particularly the endometrium, as it serves as a location for vitamin D production beyond the kidneys, potentially affecting alterations in its cycle. Vitamin D production can also take place in ectopic endometrium, potentially exerting a paracrine influence.<sup>15</sup> Vitamin D exhibits properties that are antiproliferative, anti-inflammatory, and immunomodulatory. Vitamin D engages with the vitamin D receptor (VDR), functioning as a ligand-activated transcription factor. VDR is present in different cell types and is expressed in reproductive tissues, such as the endometrium. Moreover, studies by Miyashita et al. indicated that 1,25(OH) vitamin D supplementation in endometrioma tissue culture markedly diminished prostaglandin production, inflammatory responses induced by IL-1 or TNF- $\alpha$ , and expression levels of matrix metalloproteinase (MMP)-2 and MMP-9.<sup>16</sup>

Endometriosis shares features with malignant tumors, such as decreased apoptosis, infiltration of endometrial cells into adjacent organs (bowel, bladder), increased angiogenesis, recurrence, and the need for several surgical interventions. In preclinical models, 1,25(OH)<sub>2</sub>D has demonstrated considerable antineoplastic activity, functioning as a transcription factor that impacts key processes involved in tumor development, growth, cell differentiation, and apoptosis. The inhibition of tumor invasion by 1,25(OH)<sub>2</sub>D occurs via the suppression of serine proteinases, metalloproteinases, and angiogenesis.<sup>17</sup>

The reasons for vitamin D deficiency in cases of endometriosis are still under investigation. Diet may play a significant role, especially due to the low consumption of dairy products. A large prospective study revealed that women with higher plasma 25-OH vitamin D levels and increased dairy consumption had significantly lower rates of laparoscopically confirmed endometriosis.<sup>17</sup>

The conducted analysis revealed no correlation between vitamin D levels and the dimensions of endometriosis cysts. Conversely, a study by Ciavattini A, et al yielded distinct findings regarding the connection between serum vitamin D levels and endometrioma size, revealing a significant linear relationship between serum 25-OH-D3 levels and the

diameter of ovarian endometriomas ( $r = -0.3$ ,  $p = 0.03$ ). The average serum 25-OH-D3 concentration among endometrioma patients was  $22.0 \pm 8.9$  ng/ml, with 42 of them (85.7%) being diagnosed with hypovitaminosis D. In “hypovitaminosis D women,” the average size of ovarian endometrioma was  $40.2 \pm 22.6$  mm, while in “women with adequate serum vitamin D levels” it measured  $26.7 \pm 12.1$  mm.<sup>18</sup>

In women suffering from endometriosis, immune system function is altered, showing a reduction in cell-mediated immunity and an elevation in the humoral immune response. Consequently, Vitamin D might affect the growth and development of endometriosis by helping to lower proinflammatory levels.<sup>18</sup>

A recent in vitro study showed that administering 1,25(OH)<sub>2</sub>D3 to endometrial stromal cells significantly reduced inflammatory responses induced by IL-1 $\beta$  or TNF- $\alpha$ , including IL-8 mRNA levels, prostaglandin activity, and matrix metalloproteinases expression.<sup>19</sup> Abbas et al. discovered that administering vitamin D led to a decrease in the size of endometriosis cysts and caused fibrosis and apoptosis in the stroma, indicating that vitamin D treatment might offer positive effects for managing endometriosis.

The analysis that has been carried out did not find any correlation between vitamin D levels and the size of endometriosis cysts. Endometriosis is a complex disease with many factors, including hormonal, genetic, immunological, and environmental factors. A systematic review found that while most studies did find lower vitamin D levels in endometriosis patients, many did not show a consistent association with the size or stage of the disease.<sup>20</sup> Studies evaluating the correlation between vitamin D and cyst size often have limited subject numbers and high variability. For example, a study in 49 women with endometriomas found a negative linear correlation ( $r = -0.3$ ,  $p = 0.03$ ) between serum 25-OH-D and cyst diameter. However,  $r = -0.3$  indicates a weak correlation, and  $p = 0.03$  is only slightly below the usual significance level (0.05), so its practical impact may be limited.<sup>21</sup> Measurements of vitamin D levels and cyst size are often performed cross-sectionally (at a single point in time), and therefore do not reflect the dynamic changes in the disease or the duration of the cyst's presence. If the cyst size has been established for a long time or is stable, the influence of variations in vitamin D levels may be minimal.

The research conducted has several limitations that can affect the results, including 1) the evaluation of the size of endometriosis cysts in this study was carried out using ultrasound which is highly operator dependent. The expertise of the ultrasound examiner greatly determines the results of the existing examination, 2) it is suspected that there are still other factors that affect vitamin D levels but cannot be controlled in this study, including the use of closed clothing, sunscreen, and the duration of daily sun exposure and 3) the ultrasound examination was performed by 1 specialist doctor so that the Cohen's Kappa consistency test could not be carried out in this study.

## CONCLUSION

Endometriosis patients have significantly lower serum vitamin D levels than normal women. Subjects with vitamin D levels <13.45 had a 4.03x (OR 4.03; CI95% 1.37-11.83) higher risk of experiencing endometriosis compared to subjects with vitamin D levels > 13.45 ng/mL.

## ACKNOWLEDGMENTS

The authors would like to thank RSUP dr. Kariadi Semarang, Faculty of Medicine Universitas Diponegoro for allowing us to conduct this research.

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