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Original Article

Effect of Vitamin D Administration on Interleukin–6 (IL-6) Levels in Peritoneal Fluid in Endometrioma Patients

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Abstract

Background : An endometrioma is a cyst that occurs when endometrial tissue grows on the epithelium of the ovary. Vitamin D is a steroid hormone that can be extracted from commonly available foods and can be synthesized by humans when exposed to sunlight. Vitamin D deficiency has been reported to be associated with various pathologies, including endometrioma. Vitamin D has been reported to have anti-angiogenic effects that may inhibit the growth of endometriotic implants.The aimsof this study was to proving the effect of vitamin D administration on interleukin 6 (IL-6) levels in peritoneal fluid in patients with endometrioma.

Methods : Experimental research with two groups, post-test only design with single blind that was carried out at Central General Hospital (RSUP) Dr. Kariadi Semarang. The research subject was 50 patients with endometrioma of the ovary. The definite diagnosis of endometrioma was made based on histopathological examination of tissue samples after the patient went surgery. Patients would be divided into 2 groups, namely the vitamin D3 supplementation and placebo groups. The intervention was carried out by providing vitamin D3 50,000 IU/week for a month. Evaluation of serum vitamin D levels were obtained using venous blood samples immediately when the patient was undergoing surgery. Interlukin-6 levels were obtained using peritoneal fluid samples obtained when the patient underwent surgery. Statistical analysis was carried out using the Independent T Test, Mann-Whitney U, Dependent T Test and Wilcoxon. Results are significant if $p<0.05$. Statistical analysis was carried out using SPSS edition 26.

Results : Peritoneal fluid interleukin-6 (IL-6) levels between the vitamin D supplementation group and the placebo group did not show a significant difference ($p=0.554$).

Conclusion : Vitamin D supplementation in endometrioma patients is not associated with peritoneal fluid interleukin-6 (IL-6) levels.

Keywords : Endometrioma, interleukin 6, peritoneal fluid, vitamin D

INTRODUCTION

Endometrioma is a benign gynecological disease that often occurs in women. It is estimated that 10–15% of women of childbearing age worldwide suffer from endometrioma, and 30–40% of them have the potential to experience infertility.[1–3] Localized pelvic inflammatory disease, accompanied by changes in immune-related cell function and changes in cytokine levels in the abdominal cavity, is associated with the presence of endometrioma. Many studies have shown that the peritoneal fluid of women with endometriomahas significantly increased levels of activated macrophages, produces more cytokines, including IL-6, and increases angiogenesis.[4–6] This has also been proven by several studies reporting increased levels of pro-inflammatory cytokines such as IL-6 in the peritoneal fluid of women with endometrioma.[7,8] It is known that there are several potential stimuli for IL-6 production in the abdominal cavity, one such stimulus being retrograde menstruation, a reported etiology of endometrioma.[6] High levels of IL-6 are associated with the severity of endometrioma and have been used as a marker of endometrioma.[9]

Vitamin D is a steroid hormone that can be extracted from commonly available foods such as egg yolk, marine fish, and liver, which humans can synthesize through exposure to sunlight. Vitamin D comes in different forms, the most common of which is vitamin D3, which is produced in the epidermis by UVB rays. However, it has been reported that more than one billion people suffer from vitamin D deficiency, of which 36–69.9% are of reproductive age. Vitamin D deficiency has been reported to be associated with various pathologies such as musculoskeletal disorders, recurrent miscarriages, and endometrioma.[10,11]

Vitamin D is a powerful antioxidant with anti-inflammatory and immunomodulatory properties that affect both the innate and adaptive immune systems.[12–14] Vitamin D is known to strengthen immunity by suppressing immune reactions. Several studies have shown that vitamin D3 can influence the local activity of immune cells, which have an important pathogenic role in the development of endometrioma. It has been reported that vitamin D administration can significantly reduce IL-1β and IL-8 levels.[15,16] In addition, vitamin D has also been reported to have anti-angiogenic effects that can inhibit the growth of endometriotic implants in mice. These properties support vitamin D as an effective adjuvant therapy with minimal side effects.

This research was conducted to prove the effect of vitamin D administration on interleukin 6 (IL-6) levels in peritoneal fluid in patients with endometrioma.

METHODS

This research is an experimental research with two group, post-test only design with single blind that was carried out at Central General Hospital (RSUP) Dr. Kariadi Semarang. The research sample was 50 patients with endometrioma, and the selection of research subjects would be carried out using consecutive sampling, namely the selection of research subjects based on research criteria and the subjects signing an agreement to participate in the research. Patients would be divided into 2 groups, namely the vitamin D3 supplementation group and placebo. The intervention was carried out by providing vitamin D3 50,000 IU/week for a month.

The diagnosis of endometrioma was established in two stages. The first stage was based on the suspicion of endometrioma through physical and ultrasound (USG) examinations. In the second stage, a definite diagnosis was made based on histopathological examination of tissue samples after the patient went surgery. The inclusion criteria in this study were 1) female, 2) aged 15–45 years, 3) patients diagnosed with endometrioma on ovary, 4) vitamin D deficiency with a 25 (OH)D concentration <20 mg/mL, 5) indicated to undergo laparoscopy surgery. The exclusion criteria in this study were 1) patients with a history of consuming vitamin D 6 months before surgery, 2) patients with systemic diseases (hypertension, diabetes, coronary heart disease, kidney and liver disease), 3) patients with malignancies, 4) menopausal patients, 5) patients undergoing hormonal therapy, including oral contraceptives in the last 6 months.

Vitamin D serum levels were obtained using venous blood samples immediately when the patient was undergoing surgery. Interlukin-6 levels were obtained using peritoneal fluid samples obtained when the patient underwent surgery. The data that has been obtained is entered in the form of a master table using the SPSS statistical data processing software program. The data was tested for normality of data distribution using the Shapiro-Wilk test, where the data distribution is said to be normal if $p>0.05$. For numerical variables (ratios and intervals), if the data distribution was normal, the analysis was carried out using the independent T-test (for unpaired data) or dependent T-test (for paired data), but if the data distribution was not normal then the analysis was carried out using the Mann Whitney U test (for unpaired data) or Wilcoxon test (for paired data). Results are significant if $p<0.05$. Before the research was carried out, the researcher submitted an ethical clearance to the Health Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University no 1457/EC/KEPK-RSDK/2023.

RESULT AND DISCUSSION

Endometrioma often occurs in women of reproductive age, but not in postmenopausal women due to the lack of estrogen hormone. Hormonal changes can affect the

TABLE 1
Vitamin D levels based on vitamin D supplementation

Variable	Vitamin D Supplementation		Placebo		p
	n (%)	Mean ± SD; Median (min–max)	n (%)	Mean ± SD; Median (min–max)	
Vitamin D pre		14.54 ± 3.27; 14.6 (8.8–20)		15.73 ± 3.56; 15.7 (10.2–24)	0.224†
Deficiency	24 (96)		21 (84)		
Insufficiency	1 (4)		4 (16)		
Normal	0 (0)		0 (0)		
Vitamin D post		28.54 ± 12.80; 23.9 (12.1–55.5)		14.34 ± 4.32; 13.3 (8.8–28.8)	<0.001†
Deficiency	9 (36)		23 (92)		
Insufficiency	7 (28)		2 (8)		
Normal	9 (36)		0 (0)		
p		<0.001¶		0.034¶	
Δ Vitamin D	–	13.99 ± 12.60; 9.4 (0.7–43)	–	-1.38 ± 3.08; -0.5 (-8.8–4.8)	<0.001†

†Independent T test; ¶Dependent T test; significance $p<0.05$

TABLE 2
IL-6 levels based on vitamin D supplementation

Variable	Vitamin D Supplementation		Placebo		p
	n (%)	Mean ± SD; Median (min–max)	n (%)	Mean ± SD; Median (min–max)	
Interleukin 6	–	19.03 ± 35.93; 1.9 (0.02–105)	–	19.92 ± 36.69; 1.6 (0.02–105)	0.554‡

‡Mann Whitney U; significance $p<0.05$

proliferation of endometrial cells attached to the mesothelium. This supports the idea that endometrioma is an estrogen-dependent condition.[17] In addition, domestic work largely dominates among female employees in the private sector. This contrasts with outdoor workers who are exposed to more sunlight than indoor workers. Vitamin D3 levels were significantly lower in patients with severe endometrioma compared to normal controls and patients with mild endometrioma. Vitamin D is produced in the body from food and supplements and from sunlight on the skin.[18]

Assessments carried out between initial vitamin D and final vitamin D levels showed that there was a significant difference in the vitamin D supplementation group ($p<0.001$) and the placebo group ($p=0.034$), whereas, in the vitamin D supplementation group, there was a significant increase in vitamin D levels whereas in the placebo group experienced a significant decrease in vitamin D levels.

Khodadadiyan A *et al.*, in a systematic review and meta-analysis regarding the effect of vitamin D supplementation on serum vitamin D levels, found a significant change in 25(OH)D (SMD: 2.2, I2: 92.3, 95% confidence interval (CI): 1.38–3.02, *p*-value: 0.048) and 1.25(OH)2D (SMD: 1.23, I2: 86.3, 95% CI: 0.01–2.44, *p* value < 0.010) was affected by vitamin D intervention.[19] Best CM *et al.*, in an experimental study of 161 adults supplemented with 2,000 IU/day of vitamin D3, obtained results that initially showed a positive, non-linear relationship between total vitamin D intake. in serum and total 25(OH)D in serum. concentration. The modulating effect of supplementation was an increase in serum vitamin D to 29.2 (95% CI: 24.3, 34.1) nmol/L and an increase in serum vitamin D 25(OH)D to 33.4 (95% CI: 27.7, 39.2) nmol/L.[20]

The response of 25(OH)D to a given dose of vitamin D varies widely among individuals. A systematic review explained approximately 50% of the interindividual variation in response to body weight, age, type of supplement (vitamin D2 or D3), concomitant use

TABLE 3
Vitamin D levels based on interleukin-6 levels

Variable	IL-6 <0.36 pg/mL		IL-6 > 0.36 pg/mL		p
	n (%)	Mean ± SD; Median (min–max)	n (%)	Mean ± SD; Median (min–max)	
Vitamin D pre		15.85 ± 3.75; 16.45 (10.4–24)		14.74 ± 3.24; 14.75 (8.8–20.5)	0.278†
Deficiency	15 (83.3)		30 (93.8)		
Insufficiency	3 (16.7)		2 (6.3)		
Normal	0 (0)		0 (0)		
Vitamin D post		22.70 ± 9.32; 21.55 (10–43)		20.73 ± 13.17; 16.1 (8.8–55.5)	0.130‡
Deficiency	8 (44.4)		24 (75)		
Insufficiency	6 (33.3)		3 (9.4)		
Normal	4 (22.2)		5 (15.6)		

†Independent T test; ‡Mann Whitney U; significance *p*<0.05

of calcium supplements, and baseline serum 25(OH)D concentrations. More recent evidence emphasizes the contribution of genetic variation.[21] For example, variants of the GC gene encoding the vitamin D binding protein (DBP) are strongly associated with the 25(OH)D response.[22] Although not confirmed, this may be related to genetic differences in serum DBP concentrations, which also vary depending on clinical status, sex, and life stage.

In the vitamin D supplementation group, the mean IL-6 level was 19.03 pg/mL with a standard deviation of 35.93 pg/mL, the median value was 1.9 pg/mL with the smallest value being 0.02 pg/mL, and the largest value being 105 pg/mL. In the placebo group, the mean IL-6 level was 19.92 pg/mL with a standard deviation of 36.69 pg/mL, the median value was 1.6 pg/mL with the smallest value being 0.02 pg/mL and the largest value being 105 pg/mL. There was no significant difference in the distribution of interleukin-6 levels between study groups (*p*=0.554).

Ashtari F, *et al* in their research found the same thing that there was no correlation between vitamin D levels and IL-6 levels (*p*>0.05).[15] El-Hajj C, *et al* who conducted an RCT study by providing cholecalciferol supplementation of 30,000 IU/week obtained similar results that there was no significant difference (*p*=0.1) in IL-6 levels between the vitamin D supplementation group and the control group.[23]

IL-6 is a pleiotropic cytokine that plays a role in stimulating inflammation. IL-6 is produced by lymphocytes and non-lymphocytes and activates the innate immune system during infection or trauma.[24] Through membrane receptors, IL-6 activates the non-receptor tyrosine kinase JAK2, which induces the JAK2/STAT3 cascade, leading to angiogenesis and tumor mass growth as it regulates cell cycle progression. This mechanism is very important in cachexia, tumor cell migration and cancer development.[25,26] Chronic inflammation is also found in a quarter of cancer cases.[27]

In this study, there was no correlation between vitamin D supplementation and IL-6 levels, presumably due to the dose of vitamin D given to research subjects was too low so it could not have a significant effect on the body's pro-inflammatory cytokines. Another factor that is thought to be able to cause this was too fast a period of vitamin D supplementation. That was too fast, thus those was no time for the immune system to adjust to the factors produced during the inflammatory process so that there were no significant changes in IL-6 levels in research subjects.

Evaluation of serum vitamin D levels showed that there was no significant difference in pre (*p*=0.278) and post vitamin D levels (*p*=0.130) between endometrioma patients who had peritoneal fluid IL-6 levels <0.36 ng/mL versus >0.36 ng/ mL.

Vitamin D is the main regulator of the immune system and inflammation.[28] Numerous studies demonstrate the impact of vitamin D on innate and adaptive immune system cells. Various lines of evidence indicate that vitamin D levels are important for an optimal anti-inflammatory response of monocytes. By lowering the expression of many pro-inflammatory cytokines, such as tumour necrosis factor (TNF) and IL-6, vitamin D exerts an anti-inflammatory effect on monocytes. Numerous immune system cells, including human regulatory T cells, B cells, neutrophils, lymphocytes, and macrophages, have been discovered to contain vitamin D (VDR). By attaching VDR to the immune system, vitamin D can lower inflammatory and immunological responses.[29]

It is believed that vitamin D's inhibition of cell expression is the biochemical mechanism behind the relationship between vitamin D and IL-6.[30] This mechanism involves the pro-inflammatory transcription factors NFkB and MAPK phosphatase-1 (MKP-1). The last target of NF-KB activation, the pro-inflammatory cytokine IL-6, has been found to be regulated by vitamin D. Animal studies indicate that VDR deletion may contribute to increased NFkB transcriptional activity and increased circulating IL-6. This indicates that binding of vitamin D to VDR in the immune system may cause a decrease in circulating IL-6 levels.

The absence of a relationship between serum vitamin D levels and peritoneal fluid IL-6 levels in this study is thought to be due to other factors related to endometriomas that cannot be assessed but can influence IL-6 levels, including the number of endometriomas and endometrioma size. An increase in the number of endometriomas and endometrioma size is thought to be related to an increase in the degree of inflammation that occurs in patients. However, in this study it was not possible to evaluate these two factors due to limited data.

CONCLUSION

Vitamin D supplementation is not associated with peritoneal fluid interleukin-6 (IL-6) levels in endometrioma patients.

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