

Comparison of Serum Levels of Vitamin D Between Women With and Without Endometriosis

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Background: Endometriosis is a prevalent disease in reproductive aged women causing disabling pain (dyspareunia, dysmenorrhea, pelvic pain) and infertility, many factors have been assessed to find its etiology, but its etiology remains unclear. The serum level of vitamin D is one of the factors that is suspected to be effective in diagnosis or treatment of endometriosis.

Objectives: The aim of this study was to determine if there is any significant difference between serum levels of vitamin D of women with and without endometriosis. The answer to this question may help the diagnosis or treatment of endometriosis.

Patients and Methods: In this cross-sectional analytic study on 145 women (aged 16 - 40 years old) who underwent laparoscopy, we took venous blood samples for serum levels of vitamin D before laparoscopy and then divided the samples into two groups based on findings of laparoscopy with endometriosis and without endometriosis. The data was analyzed to compare serum levels of vitamin D in two groups.

Results: After laparoscopy, we had 40 cases of endometriosis and 105 cases without endometriosis. Mean serum level of vitamin D was 19.38 ± 1.65 ng/mL in endometriosis group and 19.96 ± 1.13 ng/mL in the non-endometriosis group with no significant difference ($P = 0.68$). In 83.4% of samples serum level of vitamin D was less than 30 ng/mL.

Conclusions: To find a more precise data about the role of vitamin D in pathogenesis and treatment of endometriosis, clinical trials are needed to find if the prescription of vitamin D is effective in treatment of endometriosis.

Keywords: Endometriosis; Laparoscopy; Vitamin D

1. Background

Endometriosis is a gynecologic disorder, characterized by the growth of endometrial gland and struma outside the uterine cavity and affects 10% of reproductive aged women. Its most prevalent symptoms include dysmenorrhea, dyspareunia, pelvic pain, and infertility. Endometriosis is diagnosed by visual inspection of pelvis during laparoscopy and confirmed by positive histologic results. Yet, negative histologic results will not rule out its diagnosis (1). As the etiology of endometriosis is not well-known, more than 100 biomarkers have been investigated for diagnosis of endometriosis, like CA125, Cytokines, Interleukin 6, Interleukin 8, Tumor necrosis factor-alpha, vitamin D binding protein (DBP) (2). In some studies the level of DBP has been assessed in serum, urine, or peritoneal fluid and it was found that its polymorphism was different in patients with endometriosis than non-endometriosis patients (3-10). In the past few years, calcium and phosphorus homeostasis regulation and the role of low vitamin D levels in occurrence of various diseases of female and male reproduction system, such as endome-

triosis have been assessed (11-13). In some studies calcium and vitamin D oral intake of normal women has been identified to be higher than women with endometriosis (14, 15) and in some other studies serum levels of vitamin D was higher in women with endometriosis (11). In one study on rat model, vitamin D was approved to regress endometriosis (16).

Recent epidemiologic studies have observed the relationship between vitamin D serum level and various diseases like cancers, diabetes, cardiovascular, autoimmune diseases, pre-eclampsia, preterm birth, and gestational diabetes. Low levels of vitamin D is suggested to play a role in polycystic ovary syndrome, endometriosis, and infertility of men and women and might predict the success rate of IVF (11, 12, 17, 18).

2. Objectives

In spite of a few studies that have considered the role of vitamin D in endometriosis, its role is not yet clear. For this

study, we hypothesized a possible relationship between serum levels of vitamin D and presence of endometriosis.

3. Patients and Methods

In this cross-sectional analytic study conducted between March 2013 and July 2014 in a tertiary university hospital, we measured serum levels of 25-hydroxy vitamin D in women of reproductive age (15 - 40 years old) who underwent laparoscopy due to various indications including: infertility, ovarian cyst, suspicion to endometriosis (pelvic pain, dysmenorrhea, dyspareunia).

Following ethics committee approval and obtaining informed consent from patients, we took 5 mL of patients' peripheral blood sample after at least 10 hours fasting, the day before laparoscopy. The samples were transported immediately to the hospital's laboratory and quantitative amount of 25-hydroxy vitamin D was measured by enzyme-linked immunosorbent assays (ELISA-EUROIMMUN-Germany).

After the operation, the samples were classified as endometriosis (E) and non-endometriosis (NE) group according to laparoscopic findings. The severity of endometriosis was recorded by gynecologic surgeon according to revised classification of American society for reproductive medicine (ASRM).

The exclusion criteria included the following:

- 1- Patients who received vitamin D in the last six months.
- 2- Patients suffering from a known systemic disease including hypertension, diabetes, coronary, renal, and hepatic diseases.
- 3- Patients suffering from a diagnosed malignancy.
- 4- Menopause women.
- 5- Patients who received hormonal treatment, including oral contraceptive pills, in the last three months.

Vitamin D deficiency is defined as serum level of 25-hydroxy vitamin D less than 20 ng/mL. Levels between 21 - 29 ng/mL is considered a relative insufficiency of vitamin D and level higher than 30 ng/mL indicates sufficient serum vitamin D. Vitamin D intoxication is considered when serum level of 25-hydroxy vitamin D is higher than 150 ng/mL (20).

The data was analyzed by SPSS 18, using descriptive statistics: the ks test (one sample Kolmogorov-Smirnov test) was used for checking normality of data distribution, Levene's test for equality of variances and independent samples t-test for equality of means for comparing quantitative normal data between the two groups. We used the Mann-Whitney U test for comparing the non-normal quantitative data between the two groups.

4. Results

In this study, 40 patients enrolled in endometriosis group (E group) and 105 in non-endometriosis group (NE group). The patients ranged in age from 16 - 40 years old. The mean age in E group was 30.32 years and 33.99 in NE

group ($P = 0.001$). In E group, 70% of patients ($n = 28$) had moderate or severe endometriosis (Table 1). Mean number of parity of patients had significant difference between two groups ($P = 0.001$) and patients' BMI was lower in E group (22.60 kg/m^2) than NE group (24.88 kg/m^2) ($P = 0.002$). Table 2 shows the indication of laparoscopy in two groups. The most frequent indication of laparoscopy in E group was ovarian cyst ($n=16$). Mean serum level of vitamin D was $19.38 \pm 1.65 \text{ ng/mL}$ in E group and $19.96 \pm 1.13 \text{ ng/mL}$ in NE group with no significant difference ($P = 0.68$). The serum levels of vitamin D ranged from 1 - 63 ng/mL. Table 3 shows serum levels of vitamin D in two groups.

Serum levels of vitamin D was lower than 30 ng/mL ($n = 121$) in 83.4% of samples and lower than 20 ng/mL in 66.8% ($n = 97$), which was 72.5% in E group and 64.8% in NE group. Table 4 shows the summary of findings in this research.

Table 1. Severity of Endometriosis in Women With Endometriosis ^a

Parameters	Values
Minimal	3 (7.5)
Mild	9 (22.5)
Moderate	18 (45)
Severe	10 (25)
Total	40 (100)

^a Data are presented as No. (%).

Table 2. Indications of Laparoscopy in Two Groups ^a

	Infertility	Dysmenorrhea	Dyspareunia	Pelvic Pain	Ovarian Cyst
E group (n = 40)	9 (22.5)	4 (10)	1 (2.5)	10 (25)	16 (40)
NE group (n = 105)	16 (15.2)	5 (4.8)	2 (1.9)	42 (40)	40 (38.1)

^a Data are presented as No. (%).

Table 3. Serum Levels of Vitamin D in E and NE Groups ^a

Serum Vitamin D, ng/mL	E Group	NE Group
< 10	2 (5)	11 (10.5)
11 - 20	27 (67.5)	57 (54.3)
21 - 29	4 (10)	20 (19)
≥ 30	7 (17.5)	17 (16.2)
Total	40 (100)	105 (100)
Range	8 - 63	1 - 56

^a Data are presented as No. (%).

Table 4. Summary of Findings

Variable	Group	Number	Mean±SE	P Value
Parity, No.	E Group	40	0.55 ± 0.13	0.002 ^a
	NE Group	105	1.66 ± 0.19	
	Total	145	1.36 ± 0.15	
Age, y	E Group	40	30.32 ± 0.81	0.001 ^b
	NE Group	105	33.39 ± 0.72	
	Total	145	32.98 ± 0.59	
BMI, kg/m²	E Group	40	22.60 ± 0.50	0.002 ^b
	NE Group	105	24.88 ± 0.41	
	Total	145	24.25 ± 0.33	
Serum vitamin D, ng/mL	E Group	40	19.38 ± 1.65	0.68 ^a
	NE Group	105	19.96 ± 1.13	
	Total	145	19.80 ± 0.93	

^a Based on Mann-Whitney U test.^b Based on independent t-test.

5. Discussion

The major finding in this study was that the mean level of vitamin D was 19.38 in E group and 19.96 in NE group ($P = 0.68$). More than 66% of patients had vitamin D deficiency (< 20 ng/mL) and lower than 30 ng/mL in 83.4%.

Vitamin D, a fat-soluble vitamin is essential for human's health and its main source is food, supplements, and cutaneous sun exposure. Vitamin D deficiency is a public health concern, even in sunny areas. At least 20 ng/mL serum level of 25-hydroxy vitamin D is essential for bone's health (19).

Prevalence of vitamin D deficiency is high worldwide. In a systematic review on 195 studies conducted in 44 countries and more than 168,000 participants, mean serum levels of vitamin D was less than 20 ng/mL in 37.3% of the studies (20). Prevalence of vitamin D deficiency was estimated at 42.4% in African-American women (21), 81.4% in Turkish pregnant women (22), 79.7% in some areas of China in women (23), and 87.3% in Saudi Arabian men (24).

In a study on 1047 high school students in Iran, the mean serum level of vitamin D was reported 14.7 ± 9.4 ng/mL (25). In another study in Isfahan (Iran) on 1111 adults, the prevalence of vitamin D deficiency was estimated at 50.8% (26). In another study on university students in Shiraz (Iran), 51.2% of female students had low serum level of vitamin D (27). In other study on 993 adults in Zahedan (Iran), 85.2% of adults had serum vitamin D level less than 20 ng/mL (28).

In our study more than 83.4% of patients had serum level of vitamin D lower than 30 ng/mL and less than 20 ng/mL in 72.5% of women with endometriosis and 64.8% of women without endometriosis.

Different results have been reported in comparison of serum level of vitamin D between women with and without endometriosis. But the role of vitamin D in endometriosis is not clear yet. In a prospective study on dairy food, calcium, magnesium and vitamin D intake in women with and without endometriosis, women without endometriosis had higher intake (15). In a study on experimentally induced endometriosis in mice, treatment with a selective vitamin D receptor agonist (Elocalcitol) for three weeks reduced total lesion weight up to 70% (29) and also in another rat model, treatment with vitamin D induced fibrosis and apoptosis in the struma (16). On the contrary, some studies found higher serum levels of vitamin D in patients with endometriosis. In one study on 87 women with endometriosis and 53 controls, mean level of vitamin D was 24.9 ± 14.8 ng/mL in women with endometriosis and 20.4 ± 11.8 in women without endometriosis ($P = 0.05$) (6). In a study comparing DBP in the serum and peritoneal fluid of women with endometriosis ($n = 26$) and women without endometriosis ($n = 17$), no difference was found (7). In 1385 cases of endometriosis diagnosed by laparoscopy, total dairy food consuming were 18% lower than people without endometriosis (13).

There may be an association between vitamin D and pathogenesis of endometriosis, but our study did not support this. To determine the role of vitamin D in pathogenesis of endometriosis, it is necessary to compare the transcription of vitamin D with placebo in clinical trials.

Authors' Contributions

Fariba Almassinokiani: preparing the proposal, collecting data and drafting the manuscript. Sanaz Emadi: preparing the proposal and collecting data and drafting the manuscript. Sepideh Khodaverdi: collecting data and corresponding author. Hamid Salehiniya: statistical analysis.

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References

- D'Hooghe TM. Endometriosis. In: Berek JS editor. *Berek and Novak's gynecology*. 15 ed. Philadelphia: Lippincott Williams and Wilkins; 2012. pp. 505-57.
- May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. *Hum Reprod Update*. 2010;**16**(6):651-74.
- Speeckaert MM, Speeckaert R, van Geel N, Delanghe JR. Vitamin D binding protein: a multifunctional protein of clinical importance. *Adv Clin Chem*. 2014;**63**:1-57.
- Cho S, Choi YS, Yim SY, Yang HI, Jeon YE, Lee KE, et al. Urinary vitamin D-binding protein is elevated in patients with endometriosis. *Hum Reprod*. 2012;**27**(2):515-22.
- Faserl K, Golderer G, Kremser L, Lindner H, Sarg B, Wildt L, et al. Polymorphism in vitamin D-binding protein as a genetic risk factor in the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. 2011;**96**(1):E233-41.
- Somigliana E, Panina-Bordignon P, Murone S, Di Lucia P, Vercellini P, Vigano P. Vitamin D reserve is higher in women with endometriosis. *Hum Reprod*. 2007;**22**(8):2273-8.
- Borkowski J, Gmyrek GB, Madej JP, Nowacki W, Goluda M, Gabrys M, et al. Serum and peritoneal evaluation of vitamin D-binding protein in women with endometriosis. *Postepy Hig Med Dosw (Online)*. 2008;**62**:103-9.
- Ametzazurra A, Matorras R, Garcia-Velasco JA, Prieto B, Simon L, Martinez A, et al. Endometrial fluid is a specific and non-invasive biological sample for protein biomarker identification in endometriosis. *Hum Reprod*. 2009;**24**(4):954-65.
- Hwang JH, Wang T, Lee KS, Joo JK, Lee HG. Vitamin D binding protein plays an important role in the progression of endometriosis. *Int J Mol Med*. 2013;**32**(6):1394-400.
- Ferrero S, Gillott DJ, Anserini P, Remorgida V, Price KM, Ragni N, et al. Vitamin D binding protein in endometriosis. *J Soc Gynecol Investig*. 2005;**12**(4):272-7.
- Grundmann M, von Versen-Hoynck F. Vitamin D - roles in women's reproductive health? *Reprod Biol Endocrinol*. 2011;**9**:146.
- Anagnostis P, Karras S, Goulis DG. Vitamin D in human reproduction: a narrative review. *Int J Clin Pract*. 2013;**67**(3):225-35.
- Sayegh L, Fuleihan Gel H, Nassar AH. Vitamin D in endometriosis: a causative or confounding factor? *Metabolism*. 2014;**63**(1):32-41.
- Harris HR, Chavarro JE, Malspeis S, Willett WC, Missmer SA. Dairy-food, calcium, magnesium, and vitamin D intake and endometriosis: a prospective cohort study. *Am J Epidemiol*. 2013;**177**(5):420-30.
- Mesrine S, Clavel-Chapelon F, Boutron-Ruault MC. Re: "Dairy-food, calcium, magnesium, and vitamin D intake and endometriosis: a prospective cohort study". *Am J Epidemiol*. 2013;**178**(4):664-5.
- Abbas MA, Taha MO, Disi AM, Shomaf M. Regression of endometrial implants treated with vitamin D3 in a rat model of endometriosis. *Eur J Pharmacol*. 2013;**715**(1-3):72-5.
- Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. *Eur J Endocrinol*. 2012;**166**(5):765-78.
- Paffoni A, Ferrari S, Vigano P, Pagliardini L, Papaleo E, Candiani M, et al. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. *J Clin Endocrinol Metab*. 2014;**99**(11):E2372-6.
- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;**357**(3):266-81.
- Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. *Br J Nutr*. 2014;**111**(1):23-45.
- Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*. 2002;**76**(1):187-92.
- Ergur AT, Berberoglu M, Atasay B, Siklar Z, Bilir P, Arsan S, et al. Vitamin D deficiency in Turkish mothers and their neonates and in women of reproductive age. *J Clin Res Pediatr Endocrinol*. 2009;**1**(6):266-9.
- Zhen D, Liu L, Guan C, Zhao N, Tang X. High prevalence of vitamin D deficiency among middle-aged and elderly individuals in northwestern China: its relationship to osteoporosis and lifestyle factors. *Bone*. 2015;**71**:1-6.
- Ardawi MS, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. *Osteoporos Int*. 2012;**23**(2):675-86.
- Ebrahimi M, Khashayar P, Keshtkar A, Etemad K, Dini M, Mohammadi Z, et al. Prevalence of vitamin D deficiency among Iranian adolescents. *J Pediatr Endocrinol Metab*. 2014;**27**(7-8):595-602.
- Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. *J Health Popul Nutr*. 2011;**29**(2):149-55.
- Faghih S, Abdolazadeh M, Mohammadi M, Hasanzadeh J. Prevalence of vitamin D deficiency and its related factors among university students in Shiraz, Iran. *Int J Prev Med*. 2014;**5**(6):796-9.
- Kaykhaei MA, Hashemi M, Narouie B, Shikhzadeh A, Rashidi H, Moulaei N, et al. High prevalence of vitamin D deficiency in Zahedan, southeast Iran. *Ann Nutr Metab*. 2011;**58**(1):37-41.
- Mariani M, Vigano P, Gentilini D, Camisa B, Caporizzo E, Di Lucia P, et al. The selective vitamin D receptor agonist, elocalcitol, reduces endometriosis development in a mouse model by inhibiting peritoneal inflammation. *Hum Reprod*. 2012;**27**(7):2010-9.