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High dose vitamin D supplementation can improve menstrual problems, dysmenorrhea and premenstrual syndrome in adolescents

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Running title: Vitamin D and PMS

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Abstract

Vitamin D has a crucial role in female reproduction, possibly through its effects on calcium homeostasis, cyclic sex steroid hormone fluctuations, or neurotransmitter function. We have assessed the effects of vitamin D supplementation on dysmenorrhea and premenstrual syndrome (PMS) in adolescents. In this study, 897 adolescent girls living in Mashhad and Sabzevar, Iran, received 9 high-dose vitamin D supplements (as 50000 IU/ week of cholecalciferol) and were followed up over 9 weeks. We evaluated the effect of vitamin D supplementation on individuals in 4 categories: those with only PMS; individuals with only dysmenorrhea; subjects with both PMS and dysmenorrhea and normal subjects. The prevalence of PMS after the intervention fell from 14.9% to 4.8% ($P < 0.001$). Similar results were also found for the prevalence of subjects with dysmenorrhea (35.9% reduced to 32.4%), and in subjects with both PMS and dysmenorrhea (32.7% reduced 25.7%). Vitamin D supplementation was associated with a reduction in the incidence of several symptoms of PMS such as backache and tendency to cry easily as well decrement in pain severity of dysmenorrhea ($P < 0.05$). High dose vitamin D supplementation can reduced the prevalence of PMS and dysmenorrhea as well has positive effects on the physical and psychological symptoms of PMS.

Key Words: Vitamin D supplementation, Menstrual cycle, Premenstrual syndrome, Dysmenorrhea

Introduction:

Adolescence is a time of great physical and psychological change. Menstrual disorders are common and may add further disruption during this difficult stage for adolescents and their families[1]. Immaturity of the hypothalamus-pituitary-ovarian axis and disturbances in menstrual cycle are common through this phase of life. Approximately 75% of late adolescent girls experience some menstrual disorders [2]. Heavy menstrual bleeding, painful, irregular, and delayed are primary reasons for physician office visits, as well as dysmenorrhea known as pelvic pain or cramps is the main reason for school absenteeism among adolescent girls[2, 3]. A variation in mood and physical symptoms can take place several days to 2 weeks before menstruation, restricted to the luteal phase of the menstrual period, and gradually abate following the onset of menses[4, 5]. These symptoms include depression, irritability, tension, fatigue, sleep disturbances, somatic complaints such as abdominal cramping, and headache[5]. This phenomenon is known as the premenstrual syndrome (PMS). Epidemiological studies report that as many as 80% - 90% of women experience PMS[6]. PMS and dysmenorrhea significantly reduce the quality of life in all aspects in adolescent girls during menstruation[7].

Vitamin D has a critical role in female reproduction; the vitamin D receptor is expressed in ovarian tissue, endometrium, fallopian tube epithelial cells, decidua and placenta [8, 9]. It has also been reported that vitamin D reduces the production of prostaglandins [10]. Previous studies have assessed the role of vitamin D in preventing and/ or treating mood and gynecologic disorders that share common features with PMS [11]. In a sub-study within the prospective Nurses' Health Study II (NHSII), a high dietary intake of vitamin D was related to a 41% lower risk of PMS, while high amount vitamin D from food sources only was

related to a significant 31% lower risk [12]. Several studies have reported an inverse association between serum vitamin D levels and risk of depression, fibromyalgia, dysmenorrhea, and uterine fibroids [10, 13-17]. But, it remains unclear whether vitamin D therapy may be useful for preventing and/or treating of dysmenorrhea or PMS.

We have previously reported that vitamin D supplementation has a beneficial effect on cardio-metabolic profile, systemic inflammation and mood/emotional function in adolescents [9, 18, 19]. We have also recently reported that adolescent girls with dysmenorrhea have significantly more depression, aggression and sleep disorders compared with the normal subjects [20]. In this current study we aimed to assess the effect of supplementation with high dose vitamin D on menstrual problems, dysmenorrhea as well as PMS and associated symptom in adolescents.

Methods

Participants

This was a prospective, interventional study with one treatment group over 9 weeks, and was conducted from January through April 2015 in the cities of Mashhad and Sabzevar, in Iran, as previously described [19, 21]. Briefly, participants were recruited from 6 geographical areas of these cities, using a cluster randomization method. Exclusion criteria included: any inflammatory or autoimmune disease, tumor, renal or hepatic failure, cardiovascular disease, chronic lung disorder, diabetes, asthma, multiple sclerosis (MS), polycystic ovary syndrome (PCOS), pelvic organ pathology chronic bronchitis, fibromyalgia, malabsorption, metabolic bone disease and thyroid, or parathyroid illness. Also, those participants who were receiving anti-inflammatory, and anti-diabetic, vitamin D or calcium supplement within the last six

months before intervention were omitted. Of the 940 adolescents met the inclusion criteria,, 897 girls had the menarche one year before the intervention and were entered into the present study. Subjects were assigned to receive 9 capsules containing 50000IU of vitamin D for 9 weeks. The Ethics Committee of Mashhad University of Medical Sciences (MUMS) approved the study, and written informed consent was obtained for all participants and their parents/ guardian.

Evaluation of menstrual pattern and menstruation associated symptoms

Menstrual pattern, common menstruation associated psychovegetative symptoms, dysmenorrhea and PMS status were evaluated before and after vitamin D therapy using standard questionnaires, as previously described [20]. In brief, answers to questions regarding menstrual patterns within the last 2 cycles of menstruation were gathered. These included: menstrual cyclicity, cycle duration, quantity of blood loss, and days of flow length. Amount of menstrual flow was defined regarding to the number of pad being suffice for protection during the menstrual periods: Little (less than 1 fully soaked pad or of panty liner), moderate (1-3 fully soaked pad) and Heavy (4 or more fully soaked pads a day for protection) [22].

Dysmenorrhea was characterized by abdominal pain, or cramping associated to menstrual bleeding and individuals rated their pains using a scale that varied from a painless (score of 0) to mild (score of 1), moderate (score of 2), severe (score of 3), very severe (score of 4) and a most severe (score of 5)[23].

Participants were instructed to answer questions about the most frequent physiological and psychological symptoms that occur before the menses including: leg ache, backache, diarrhea, nausea, vomiting, fatigue, irritability, palpitation, appetite changes, poor sleep, energy depletion, decrease interest, feeling sad or blue, loss of concentration and tendency to cry easily.

Those having at least two symptoms, one physical and one psychological symptom were diagnosed as PMS, while those with one symptom or no symptoms were regarded as not having PMS [24]. For exact assessment, participants were also classified into four categories as follows: subjects with only PMS, subjects with only dysmenorrhea, those suffered from both PMS and dysmenorrhea, as well as normal subjects.

Laboratory measurement

An electrochemiluminescence (ECL) method was used for the measurement of serum 25 (OH) vitamin D. Serum 25 (OH) vitamin D was categorized based on the accepted cutoff values (nmol/L): serum 25 (OH) vitamin D levels <50 deficiency, 50-74.9 insufficiency and >75 sufficiency [25].

Statistical analysis

McNemar or Cochran's Q tests and paired sample t-test were used to compare variables before and after supplementation. All data are expressed as frequency, percent and mean±SD. P < 0.05 was considered statistically significant. Statistical analyses were conducted with SPSS version 17 (SPSS Inc, Chicago, Ill., USA).

Results

A total of 897 girls were analyzed. All the study population was unmarried at the time of the study. The mean age of the participants was 14.72 ± 1.50 years (range, 12-18 years) and their mean age at menarche was 12.57 ± 1.19 years, and occurring between 9 and 17 years. No adverse effects were observed in those who taking the high dose vitamin D supplement. Serum levels of 25 (OH) vitamin D increased significantly by the end of intervention period, compared to the baseline levels (22.7 ± 22.6 nmol/L vs. 89.9 ± 38.3 nmol/L, $p < 0.001$). The prevalence of deficiency, insufficient and sufficient serum levels of 25OH vitamin D was 85.3%, 9.7% and 4.9% in participants at the baseline, respectively.

After supplementation, the prevalence of vitamin D deficiency was reduced to 16.6%, while insufficiency and sufficiency levels were 19.9% and 63.5% respectively ($p < 0.001$).

A normal duration of menstrual flow was seen in 81.4% of participants. After intervention this increased to 85.5%. At baseline, 23.6% had short length cycle, 71.8% had normal cycle, and 4.7% had long length cycle. After supplementation, the number of subjects with a normal cycle increased to 78%, while the number of girls with short length cycle and long length cycle decreased to 18.0% and 4.0% respectively ($p = 0.015$). Furthermore, subjects with moderate menstrual flow increased post therapy but this value did not reach statistically significance (Table1).

All 15 individual symptoms of PMS were analyzed to determine the efficacy of intervention. Symptom of backache, nausea, vomiting and diarrhea decreased after intervention, but only the reduction in backache was statistically significant ($P < 0.05$). There were no significant differences in psychological symptoms, except for tendency to cry easily which suggests a statistical trend toward before and after taking supplementation. Tendency to cry easily

before each menses was experienced by 31.8% of woman. After intervention, 22.8% still showed this symptom (Table 1).

At baseline, the prevalence of subjects with PMS alone was 14.9%, while this value after intervention was decreased to 4.8% ($P < 0.001$). Similar results were also found for the prevalence of subjects with only dysmenorrhea (35.9% reduced to 32.4%), both PMS and dysmenorrhea (32.7% reduced to 25.7%), while normal cases increased from 16.5% to 37.1% after therapy ($P < 0.001$).

The number of subjects with dysmenorrhea fell post supplementation and pain severity of dysmenorrhea had relief after the 9-week intervention. Furthermore, the number of cases who used medication for pain relief fell significantly following supplementation (Table 3).

Discussion

To our knowledge, this is the first study investigating the effect of a supplementation with very high doses of vitamin D on menstrual pattern, primary dysmenorrhea and PMS concomitantly in large population of adolescents. Taking a high-dose of vitamin D3 (50000 IU/ weekly) is recommended for preventing and treatment of vitamin D deficiency [26]. In the current study, we administrated nine high-dose vitamin D pearls (50000 IU cholecalciferol /weekly) during a period of nine weeks. We have demonstrated a significant association between vitamin D therapy and improvement of PMS and dysmenorrhea among adolescents. In line with our observation, a placebo-controlled study of calcium (500 mg) plus vitamin D (200 mg) supplementation and PMS, showed that supplementation was associated with a reduced severity of symptom of PMS [27]. Bertone-Johnson in 2009 discussed this issue and claimed that intake of low dietary vitamin D has been related to the development of PMS [14]. Additionally high dietary intake of vitamin D may decrease the risk of PMS possibly by

affecting calcium levels, fluctuation of cyclic sex steroid hormone, and/or neurotransmitter function [14, 28, 29]. Vitamin D fluctuations across the menstrual cycle along with alterations in estradiol at ovulation and through the luteal phase have been reported in a number of but not all studies [29]. Thys-Jacobs and Alvir observed serum 25(OH) vitamin D concentrations to be significantly lower in PMS subjects across 3 phases of the menstrual cycle, although 1,25(OH) vitamin D concentrations were non-significantly higher in PMS subjects at all phases compared to control[30]. Results from another larger study showed that non-significantly higher use of vitamin D supplements (41% vs. 30%) among women with premenstrual dysphoric disorder compared to symptom-free controls[30]. Over 100 different menstrual symptoms are associated with PMS[31], and the unique symptoms affected women may differ considerably. Similarly, associations of vitamin D with specific symptoms may vary. In the present analysis, vitamin D supplementation can significantly decrease incidence of backache and tendency to cry easily ($P < 0.05$) and possibly nausea, loss of concentration, and lack of energy ($P \leq 0.2$). Despite the low rates of symptoms reduction in our study, the reduction in PMS incidence is both statistically and clinically significant. Regarding to the importance of PMS in the overall quality of life for each women, even low reduction rate can be considered important.

The majority of adolescents with dysmenorrhea use self-medication; few consult health care providers [32]. Based on our finding, high dose vitamin D supplementation can reduce the prevalence and pain intensity of dysmenorrhea. Consistent with our result, trials in Iran [33] and Italy [10] have demonstrated that the use of a single dose oral cholecalciferol(300000 units) five days before the beginning of menstrual bleeding significantly decreased the pain

of severe primary dysmenorrhea, however this effect was non-significant where the pain intensity was moderate [33].

In another clinical trial on 60 women with primary dysmenorrhea administration of 50000 unit oral vitamin D for 8 weeks decreased significantly pain severity versus control group[34]. But, Zarei et al found that supplementation with combination of calcium-vitamin D did not significantly decrease pain severity [35]. It may be due to the absence of vitamin D-alone group and its assignment may have no further effect when there is a sufficient calcium supplementation.

Prolonged menstrual bleeding usually occurs early after the onset of menarche because of anovulatory cycles. In the present study, a reduction in the number of cases with a long menstrual cycle was associated with vitamin D supplementation, but this effect was not significant.

We have demonstrated that vitamin D supplementation significantly affects the duration of menstrual cycle among participants. The prevalence of cycles longer than 35 days or oligomenorrhea ranged from 8% to 22% in various studies. Although infrequent cycling is not generally associated with adverse health outcomes, irregular cycles or amenorrhea may be associated with infertility, which puts the social burden of childlessness on women specially in developing countries [36]. Jukic et al have reported that low plasma levels of 25(OH) D are associated with a high risk of having irregular cycles [37]. In another study, an increase in 25(OH)D was related with a low odds of long menstrual cycles [38]. This discrepancy between these two reports may be as a result of different population samples; long and irregular cycles may be different in younger compared to older women.

Menorrhagia or excessive heavy or prolonged menstrual blood loss, affect 10–15% of female during their lifetime [36] which may be caused by anatomical gynecological pathology, hormonal variations and medical disorders such as hypothyroidism[39]. Congenital and acquired disorders of homeostasis, thrombocytopenia and inherited bleeding disorders, such as von Willebrand Disease, platelet function defects are the common causes of menorrhagia in adolescents[40]. Whilst we found that vitamin D supplementation reduced the number of subjects with heavy menstrual flow, this was not statistically significant.

The high dose vitamin D supplementation improved serum concentration of 25(OH) vitamin D in our study subjects. Taking 50000 IU-vitamin D for 8 weeks has been recommended for the treatment of vitamin D deficiency [26]. Similar results were observed for the effect of vitamin D supplementation on serum 25 OH) vitamin D in previous studies [41-43].

No side effects related to the vitamin D supplement was observed among participants. Some limitations need to be considered in the explanation of our finding. We were unable to include a control group (non-supplemented) in the current study because this was not approved by our Ethics Committee. Furthermore, there is a potential for recall bias, as all of the variables related to menstruation issue were taken using a recall method. According to the studies conducted on supplementation to treat PMS symptoms, several confounding factors may influence the results. These factors include age, education level, and stress, although we adjusted their possible effects. Moreover, we were unable to assess the long term effect of vitamin D supplementation. The available evidence suggest that vitamin D supplementation can have positive effects on dysmenorrhea and its pain severity as well as PMS and related symptoms. Therefore these positive findings have renewed optimism that vitamin D supplementation can be a potential treatment for dysmenorrhea and PMS symptoms. Other

large-scale randomized controlled trials are needed to confirm our finding and evaluate the longer term effect of supplementation.

Geolocation information

Iran also known as Persia is a sovereign state in Western Asia. It is bordered to the northwest by Armenia, the Republic of Azerbaijan, and the Azerbaijani exclave of Nakhchivan; to the north by the Caspian Sea; to the northeast by Turkmenistan; to the east by Afghanistan and Pakistan; to the south by the Persian Gulf and the Gulf of Oman; and to the west by Turkey and Iraq. Mashhad is the second most populous city in Iran and capital of Razavi Khorasan Province. It is located in the northeast of the country.

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Table 1. Effects of vitamin D supplementation on menstrual pattern and common menstruation associated psychovegetative symptoms in adolescent girls			
	Before supplementation	After supplementation	P value
Average days of bleeding, n (%)			
Short bleeding periods(4 days)	46(5.1)	40(4.5)	0.549
Normal periods (4-7 days)	730(81.4)	767(85.5)	
Long periods(>7 day)	121(13.5)	90(10.0)	
Duration of the menstruation cycle, n (%)			
<21 days	212(23.6)	161(18.0)	0.015
21-35 days	643(71.7)	700(78.0)	
>35 days	42(4.7)	36(4.0)	
Amount of menstrual flow, n (%)			
Little	208(23.2)	198(22.1)	0.434
Moderate	675(75.2)	688(76.7)	
Heavy	14(1.6)	11(1.2)	
Menstruation-associated symptoms, n (%)			
Leg ache	172(19.2)	174(19.5)	0.99
Backache	538(60.0)	492(54.8)	0.002
Nausea	73(8.1)	63(7.0)	0.185
Vomiting	26 (2.9)	20(2.2)	0.210
Diarrhea	28(3.1)	25(2.8)	0.70
Appetite changes	4(0.4)	5(0.5)	0.99
Irritability	57(6.4)	54(6.0)	0.99
Fatigue	86(9.6)	67(7.5)	0.219
Palpitation	84(9.4)	85(9.5)	0.99
Energy depletion	89(9.9)	73(8.1)	0.185
Poor sleep	11(1.2)	5(0.5)	0.90
Feeling sad or blue	74(8.2)	66(7.4)	0.693
Decrease interest	111(12.4)	108(12.0)	0.937
Loss of concentration	139(15.5)	117(13.1)	0.146
Tendency to cry easily	285(31.8)	205(22.8)	0.001
*Using McNemar test or Cochran's Q test			

Table 2. Effects of vitamin D supplementation on dysmenorrhea and PMS subjects			
Subjects	Before supplementation n(%)	After supplementation n(%)	P value
PMS+Dysmenorrhea	293(32.7)	231(25.7)	<0.001
Only dysmenorrhea	322(35.9)	291(32.4)	
Only PMS	134(14.9)	43(4.8)	
Normal	148(16.5)	332(37.1)	
Using Cochran's Q test			

Table 3. Effects of vitamin D supplementation on dysmenorrhea and its severity in adolescent girls			
	Before supplementation n(%)	After supplementation n(%)	P value
Dysmenorrhea, yes	617(68.8)	565(63.0)	0.002
Mild	85(9.5)	99(11)	0.043
Moderate	187(20.8)	170(19.0)	
Severe	162(18.1)	145(16.2)	
Very severe	110(12.3)	81(9.0)	
Worst	73(8.1)	70(7.8)	
Use of medication for relief pain	316(35.2)	258(28.8)	0.009
Using McNemar or Cochran's Q tests			