Effect of vitamin D vaginal suppository on sexual functioning among postmenopausal women: A three-arm randomized controlled clinical trial

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Objective
Vaginal atrophy, the second most common complication of menopause, can lead to sexual dysfunction. This study evaluated the effect of a vitamin D vaginal suppository on sexual functioning in postmenopausal women.

Methods
This three-arm randomized controlled trial was conducted between August 2019 and August 2020. The sample comprised 105 postmenopausal women who were referred to comprehensive health service centers to receive postmenopausal care. The inclusion criteria were as follows: (i) being menopausal for at least 1 year, (ii) being married, (iii) being sexually active, and (iv) having sexual desire. Participants were randomly assigned to three groups for 8 weeks of treatment: intervention (vaginal suppository containing 1,000 units of vitamin D3), placebo (vaginal suppository placebo), or control (no treatment). The main outcome measure was sexual functioning, which was assessed using the Female Sexual Function Scale (FSFI) 4 times during the study (i.e., 1 month before the intervention, immediately after the intervention, 1 month after the intervention, and 2 months after the intervention).

Results
Immediately and 1 month after the trial, the intervention group had the highest FSFI score, followed by the placebo group, both of which were significantly higher than those of the control group ($P<0.05$). At the 2-month follow-up, the intervention and placebo groups had similar FSFI scores ($P=0.08$), both of which were significantly higher than those in the control group ($P=0.001$ and $P=0.03$, respectively).

Conclusion
Vitamin D vaginal suppositories were more effective at improving sexual functioning among postmenopausal women in the short-term and appeared to prevent aging-related sexual functioning decline in the long term.

Keywords: Sexual function; Postmenopausal period, women; Vaginal suppository; Vitamin D

Introduction

Due to estrogen deficiency, vaginal atrophy is experienced during menopause [1]. Symptoms include vaginal dryness, burning, bleeding after sexual intercourse, and painful intercourse experienced by nearly 30% of women during the postmenopausal period [2]. The standard treatment for vaginal atrophy is continuous low-dose vaginal estrogen therapy [3]. However, there is an increasing need for alternative therapies to relieve the symptoms of menopause including vaginal atrophy. Cumming et al. [4] reported that 95% of postmenopausal women preferred alternative therapies because they considered them more natural.

Therefore, vitamin D3 may be an alternative therapy for relieving vaginal atrophy. Vitamin D is essential for metabolic homeostasis and physiological function [5]. It has also been proposed to lessen or prevent female reproductive disorders [6-12]. Vitamin D3 can be absorbed when applied vaginally, as its receptors are located in the basal and parabasal cellular layers of the vaginal tissue [13]. Physiologically, vaginal epithelial cell proliferation might be regulated by vitamin D [8,9]. However, the expression of vitamin D receptors changes during the menstrual cycle. Consequently, the number of receptors decreases as ovarian activity ceases during menopause [14,15].

Vitamin D exerts its biological effects through nuclear receptors. Vitamin D and its receptors form a complex that affects gene transcription, resulting in restoration of the mucosal tissue of the vagina [16]. Vitamin D also affects specific genes controlling vaginal squamous cell differentiation [17,18]. Vitamin D suppositories effectively improve vaginal dryness, paleness, and cellular proliferation in postmenopausal women [19-21].

The population of postmenopausal women is increasing worldwide owing to decreased average age at menopause and increased life expectancy [22]. Moreover, many women remain in the postmenopausal stage for a considerable number of years. Therefore, finding solutions to eliminate health problems during this period, including those related to sexual health, may increase the strength and durability of marital relationships. Considering the general acceptance of complementary therapies for hormone replacement and the promising effects of vitamin D vaginal suppositories on vaginal health, the present study evaluated the effects of vitamin D suppositories on sexual functioning among postmenopausal women.

Materials and methods

1. Study design and participants

This three-arm blind randomized clinical trial with a negative control group was conducted between August 2019 and August 2020. Participants were postmenopausal women who were referred to comprehensive health service centers in Qazvin Province to receive postmenopausal care. The trial protocol has been peer-reviewed and published [23].

To identify eligible participants, a member of the research team approached comprehensive health service centers in Qazvin, including Buin Zahra, Takestan, and Alborz counties. A total of 105 eligible postmenopausal women were recruited from 35 medical centers using convenience sampling. The inclusion criteria were as follows: (i) being menopausal for at least 1 year, (ii) being married, (iii) being sexually active, and (iv) having sexual desire. The exclusion criteria were: (i) use of intravaginal medications; (ii) moderate to severe utero/genital infection (e.g., vaginitis, cervicitis); (iii) receiving hormone therapy; (iv) having experienced a stressful event during the previous quarter (e.g., death of a loved one); (v) having undergone a hysterectomy or other gynecological surgery; (vi) presence of any other endocrine disease such as Cushing's disease, diabetes, hyperprolactinemia, or hyperparathyroidism; (vii) having experienced premature ovarian failure; (viii) having any organ abnormalities, sarcoidosis, kidney disease, or histoplasmosis; and (ix) spousal disorder that prevents sexual activity (e.g., diabetes).

2. Sample size estimation

According to the results of a previous study [24], using a type I error of 0.05, test power of 80%, and average effect size of 0.6, the minimum number of participants in each group was 25. Considering a 40% attrition rate, 35 individuals from each group were invited to participate, resulting in a total sample size of 105 individuals.

3. Randomized allocation

The eligible participants were randomly assigned to three groups for 8 weeks of treatment: intervention (vaginal suppository containing 1,000 units of vitamin D3), placebo (vaginal suppository placebo), or control (no treatment). The

allocation sequence was prepared using a random allocation software. Intervention type was then written inside sealed opaque envelopes based on the allocation sequence and coded on the envelope. Questionnaires were coded using the same codes as those used for the intervention. Therefore, participants who received Intervention completed a questionnaire with the same code.

4. Blinding

The suppositories were prepared and packaged in coded forms by a pharmaceutical company. Therefore, the participants, presenters, evaluators, and statisticians did not know whether the suppository contained vitamin D. After the statistical analysis, suppository codes were obtained from the pharmaceutical company.

5. Outcomes and measurements

The primary outcome of the present study was a change in sexual functioning assessed using the Female Sexual Function Scale (FSFI) before, immediately after, 1 month after, and 2 months after the intervention. The FSFI, a 19-item self-reported questionnaire, was designed by Rosen et al. [25] in 2000 to assess female sexual functioning. This scale assesses six aspects of female sexual functioning: desire, arousal, lubrication, orgasm, satisfaction, and pain [25]. Psychometric evaluation of the Iranian version was assessed and confirmed to have good reliability and validity [26].

Another questionnaire was used to record the participants' demographic characteristics, including age, education, menopausal age, spouse's age, spouse's educational level, body mass index, and number of pregnancies and deliveries. All participants were interviewed by a trained research team that was blinded to their study group allocations. The interviewer obtained informed consent from all participants. The questionnaires were completed once they agreed to participate. The interviewer helped those who were illiterate answer the questions by recording their answers.

6. Procedure

Prior to the intervention, sexual performance was assessed using the FSFI. The trial was implemented based on the assigned interventions: vitamin D suppository (group A), placebo (group B), and control (group C).

The methods of preparation and storage of the vitamin D and placebo suppositories were previously outlined in detail

[23]. The vitamin D and placebo suppositories consisted of a common Suppocire AM-15 base and had the same shape, color, and weight. Only one identification code (A or B) was placed on the packaging to match it to the envelope. During the first meeting, envelopes were provided to the participants in groups A and B according to the allocation sequence. They were instructed to insert a suppository 3 cm into their vagina before going to bed. Before inserting the suppository into the vagina, the participants were told to: (i) wash their hands with soap and water, (ii) insert the suppository into the vagina at a depth of two fingertips; and (iii) use only one suppository each time. The treatment schedule was every night in the first 2 weeks and every other day for the following 6 weeks. Follow-up visits were also scheduled. All procedures were conducted under the supervision of a gynocologist who was also a member of the research team.

7. Safety considerations

The risk of vitamin D toxicity occurs with the long-term use of high doses (>10,000 [27,28] to 15,000 [29] units/daily). The total dose of vitamin D in the present study was 32,000 units over 8 weeks. Therefore, the risk of toxicity was considered low and within the safe range considering that it was much lower than the potentially toxic dose based on current evidence [27-30]. The participants were asked not to take any oral vitamin D supplements during the study period. In addition, all symptoms of vitamin D toxicity were explained to the participants for self-monitoring of adverse responses.

8. Follow-up

Sexual functioning was assessed immediately and 1 and 2 months after the completion of the 8-week intervention. Moreover, symptoms of potential vitamin D toxicity and side effects were evaluated at each visit.

9. Ethical considerations

This study was approved by the research committee of the University of Medical Sciences (approval number: IR.QUMS. REC.1397.117). The study protocol was prospectively registered with the Iranian Clinical Trial Registration System (IRCT20180704040346N1) on October 10, 2018. After obtaining the necessary permits, patients were invited to participate in the study. Written informed consent was obtained from all participants. All of the research processes were monitored and verified by an agency selected from the Research

Committee of the University of Medical Sciences.

10. Statistical analysis

SPSS version 24 (IBM SPSS Statistics, Armonk, NY, USA) was used for the data analysis. The normal distributions of the variables (total sexual functioning scores and subscale scores) were examined using the Shapiro-Wilk test for the indicators of central tendency, dispersion, and histogram diagram. Intergroup comparisons were performed to evaluate the distribution of variables based on the proposed Imbens and Rubin [31] method considering the standardized mean difference (MD) criteria of <0.25 for continuous quantitative variables and <10% risk difference index for categorical variables. Owing to the normal distribution of the data, one-way repeated-measures analysis of variance (ANOVA) was used to compare the changes in mean FSFI scores between the study groups at different time points. In each case, the prerequisites for performing repeated-measures ANOVA (including the sphericity test and homogeneity of variance) were checked. Owing to the lack of sphericity default, the results were reported with the Greenhouse-Geisser correction. In cases in which the ANOVA test was significant for repeated measures, the Sidak *post hoc* test was used to determine the differences. Moreover, an analysis of covariance was performed to compare groups according to the corrected sexual functioning score to control for the mean baseline score. Bonferroni correction was used for multiple comparisons. The effect sizes of the MD and the standardized MD (SMD) were calculated based on Cohen's *d*. A Cohen's *d* effect size of 0.2-0.5 was considered a small effect size; 0.8-0.5 was considered a medium effect size; and >0.8 was considered a large effect size [32].

The MD was examined to assess the minimal clinically important differences (MCID). The MCID for outcomes can be defined via three methods based on evidence from previous studies and calculated using anchor- and distribution-based methods [33]. Two approaches were used to define the MCID for FSFI scores. First, based on a literature review, Krychman et al. [34] reported an MCID of 4.2 for the total FSFI score. As the MCID of patient-reported measures might differ among patients and clinical contexts [35], the second approach of calculating the MCID based on the distribution-based method was used. According to Jacobson et al. [36], the MCID was calculated using the following for $MCID = 1.96 \times SD_{base} \times \sqrt{2 \times (1\text{-ICC})}$.

Considering an standard deviation$_{base}$ of 4.82 for the intervention group and an intraclass correlation coefficient of 0.908 from the first and last measurements (for a more conservative approach), the calculated MCID of 4.05 was used to assess the clinical significance of the intervention and placebo. If the MD was higher than the calculated MCID at any follow-up point, it was considered clinically significant. The significance level for all tests was *P*<0.05.

Results

A CONsolidated standards of reporting trials diagram illustrates the study procedure (Fig. 1). A total of 105 participants were enrolled and randomly assigned to one of three study groups. Table 1 shows the balanced distribution of demographic variables (except for spousal age) among the three groups. Table 2 presents the effects of vitamin D vaginal suppositories on the FSFI scores. Table 3 presents the MD and SMD of the FSFI mean scores among study groups. Table 4 presents the effect of the vitamin D vaginal suppository on the mean FSFI subscale scores.

1. Comparison of intragroup changes over the follow-up time

As shown in Fig. 2 and Tables 2, 3, in the intervention group using the vitamin D suppository, significant improvement in sexual functioning was observed immediately after and 1 month after the intervention (*P*<0.001 vs. baseline). Examining the MD between these two time points (adjusted mean, 26.36 and 23.69 immediately after and 1 month after the intervention, respectively) with the baseline (mean, 19.16) value also confirming that a minimal clinically important difference was achieved (7.17% and 4.53%, respectively, compared to the MCID of 4.05). However, at 2 months after the intervention, no difference in sexual functioning scores was noted compared to baseline (*P*=0.65; adjusted mean, 20.54; MD, 1.38).

In the placebo group, only at the first follow-up (immediately after the intervention; adjusted mean, 23.10) was sexual functioning significantly better than at baseline (mean, 17.93; MD, 5.17; *P*=0.001; Fig. 2). At 1 and 2 months after the intervention, the sexual functioning score was not significantly or clinically different from that baseline (adjusted mean at 1 month after the intervention, 21.22; *P*=0.35; MD, 3.29;
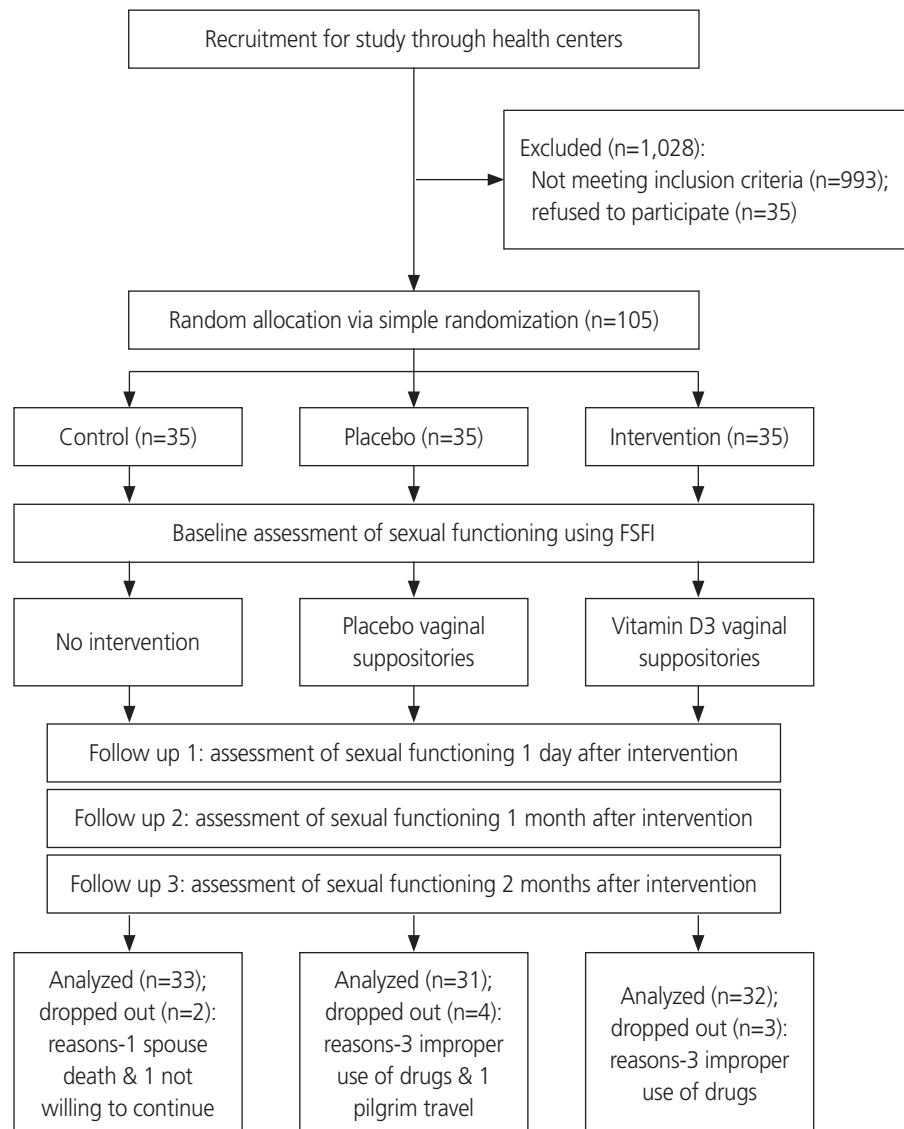
Fig. 1. CONsolidated standards of reporting trials diagram of the study. FSFI, Female Sexual Function Scale.

adjusted mean at 2 months after the intervention, 19.30; P=1.00; MD, 1.37; Fig. 2). In the control group, a decrease in sexual functioning score was observed over time, which is expected in such individuals. This decline was statistically significant at 2 months (adjusted mean, 16.16) compared with baseline (mean, 20.03). However, it had not yet reached an MCID of 4.05 ($P<0.001$; MD, -3.87) (Table 2).

2. Comparison of intergroup differences
The intergroup differences are presented as pairwise comparisons between the study groups at each time point in Tables 2, 3 and Fig. 2. The FSFI subscales are compared among groups in Table 4. Comparing the intervention and placebo groups

based on the adjusted value using the baseline score of the FSFI and the spouse's age immediately and 1 month after the intervention, the sexual functioning score in the intervention group was significantly higher than that in the placebo group based on Cohen's d (immediately after: MD, 3.25; Cohen's d=0.85; 1 month after: MD, 2.48; Cohen's d=0.51). Over time, the effect size of the intervention decreased; therefore, the effect size of the intervention group was not significantly different from that of the placebo group at the third follow-up (2 months post-intervention).

The sexual functioning score was higher in the intervention versus control group at all three follow-up points (MD between the two groups at the three time points post-inter-

Table 1. Distribution of qualitative and quantitative variables of demographic characteristics of participants by study groups

	Intervention (n=32)	Placebo (n=31)	Control (n=33)
Participant's educational status			
Illiterate	12 (34.3)	13 (37.1)	14 (40.0)
Below highschool diploma	20 (57.1)	21 (60.0)	20 (57.1)
Highschool diploma & higher	3 (8.6)	1 (2.9)	1 (2.9)
Spouse's educational status			
Illiterate	7 (20.0)	10 (28.6)	7 (20.0)
Below highschool diploma	24 (68.6)	22 (62.9)	26 (74.3)
Highschool diploma & higher	4 (11.4)	3 (8.6)	2 (5.7)
Perceived family economic status by the particians			
Poor	2 (5.7)	1 (2.9)	5 (14.3)
Moderate	27 (77.1)	31 (88.6)	26 (74.3)
Good	6 (17.1)	3 (8.6)	4 (11.4)
History of spouse's chronic disease			
No	28 (80.0)	27 (77.14)	26 (74.3)
Yes	7 (20.0)	8 (22.86)	9 (22.9)
Participant's age, yr	52.89±5.17	52.71±4.23	53.06±3.69
Menopause age, yr	46.77±4.78	47.74±4.55	47.51±4.22
Spouse's age, yr	56.03±5.96	58.23±6.18	56.60±7.02
Marriage duration, yr	32.06±8.61	34.54±7.51	29.54±10.13
Gravida (no)	4.71±2.81	4.86±2.49	5.03±2.37
Parity (no)	3.77±2.21	4.23±2.07	4.46±1.99
Body mass index	28.85±4.52	26.89±4.20	27.81±4.33

Values are presented as mean±standard deviation or number (%).

vention: 7.19, 6.59, and 4.38, respectively, greater than the MCID of 4.05). The vitamin D intervention had a significant effect on sexual functioning versus control (Cohen's d=1.87, 1.37, and 0.77, respectively, for the three follow-up points).

The sexual functioning score of the placebo group was significantly higher than that of the control group at all three follow-up points, but the difference was not clinically important (MD between the two groups at all three time points post-intervention was less than MCID of 4.05).

3. Side effects

No side effects were reported during the intervention or follow-up periods.

Discussion

The main findings of the present study were that: (i) vita-min D vaginal suppositories can markedly improve sexual functioning in postmenopausal women immediately after use; and (ii) the effect can last for 1 month with continuous intervention. Interestingly, oil-based placebo suppositories also improved sexual functioning. However, this was limited to immediately after the intervention, with no effect at 1 month of follow-up. In contrast, sexual functioning declined in the control group, which represented natural age-related physiological changes and indicated the sensitivity of the trial participants.

Vitamin D vaginal suppositories were statistically and clinically more effective than placebo suppositories at improving sexual functioning in postmenopausal women. Overall, because of the effect size in the corrected models at all time points, vitamin D had a larger effect size, approximately 1.5 to 2 times larger than the placebo suppositories and no intervention. In addition, the clinical significance of the improvement in sexual functioning benchmarked by the MCID

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Table 2. Statistical analysis of the effect of vitamin D vaginal suppository on the female sexual function index score

Model	Intervention (n=32)	Placebo (n=31)	Control (n=33)	Statistical results			
				Effect	F	P	Partial η^2
Crude[a]							
Baseline	19.16 (4.82)	17.93 (6.08)	20.03 (4.69)				
Follow up 1	26.33 (2.29)	22.76 (2.75)	19.51 (6.46)	Time	20.01	<0.001	0.179
Follow up 2	23.72 (3.31)	20.81(3.07)	17.44 (7.60)	Group	9.64	<0.001	0.173
Follow up 3	20.51 (5.15)	19.09 (4.38)	16.38 (6.95)	Time[a] group	6.93	0.001	0.131
Adjusted for baseline score of the FSFI[b]							
Follow up 1	26.50 (4.02)	22.94 (4.01)	19.18 (3.96)	Time	0.397	0.67	0.004
Follow up 2	23.88 (4.98)	20.99 (5.19)	17.12 (4.99)	Group	24.71	<0.001	0.177
Follow up 3	20.62 (5.72)	19.20 (5.63)	16.17 (5.57)	Time[a] group	1.23	0.30	0.026
Adjusted for baseline score of the FSFI and spouse's age[c]							
Follow up 1	26.36 (3.85)	23.10 (3.84)	19.17 (3.85)	Time	0.882	0.39	0.10
Follow up 2	23.69 (4.81)	21.22 (4.90)	17.10 (4.82)	Group	26.01	<0.001	0.366
Follow up 3	20.54 (5.66)	19.30 (5.68)	16.16 (5.68)	Time[a] group	1.15	0.33	0.025

FSFI, Female Sexual Function Scale; RM-ANOVA, analysis of variances for repeated measures; ANCOVA, analysis of variance- covariance.

[a]The crude model was analyzed using RM-ANOVA.

[b]The adjusted models were analyzed using RM-ANOVA ANCOVA. Statistical results for baseline score of the FSFI: F=19.57, P<0.001, partial η^2=0.177.

[c]The adjusted models were analyzed using RM-ANOVA ANCOVA. Statistical results for baseline score of the FSFI: F=16.04, P<0.001, partial η^2=0.511 and statistical results for spouse's age: F=8.75, P=0.004, partial η^2=0.89.

Table 3. Mean difference (MD) and standardized mean difference (SMD) of the female sexual function index mean scores among study groups

Model[a]	Intervention vs. placebo		Intervention vs. control		Placebo vs. control	
	MD (95% CI)	SMD (95% CI)	MD (95% CI)	SMD (95% CI)	MD (95% CI)	SMD (95% CI)
Crude						
Baseline	1.23 (−1.39 to 3.84)	0.01 (−0.48 to 0.51)	−0.87 (−3.14 to 1.40)	−0.29 (−0.78 to 0.20)	2.1 (−4.69 to 0.49)	−0.27 (−0.76 to −0.22)
Follow up 1	3.56 (2.29 to 4.83)	1.41 (0.87 to 1.98)	6.81 (4.39 to 9.23)	1.40 (0.86 to 1.94)	3.25 (0.74 to 5.76)	0.65 (0.15 to 1.15)
Follow up 2	2.91 (1.28 to 4.53)	0.91 (0.39 to 1.43)	6.28 (3.36 to 9.20)	1.07 (0.55 to 1.59)	3.37 (0.40 to 6.35)	0.58 (0.08 to 1.08)
Follow up 3	1.41 (−1.00 to 3.48)	0.30 (−0.20 to 0.79)	4.12 (1.10 to 7.15)	0.67 (0.17 to 1.17)	2.71 (−0.22 to 5.63)	0.46 (−0.04 to 0.96)
Adjusted for baseline score of the FSFI						
Follow up 1	3.57 (1.18 to 5.96)	0.89 (0.37 to 1.40)	7.32 (4.95 to 9.69)	1.84 (1.26 to 2.41)	3.75 (1.37 to 6.14)	0.94 (0.43 to 1.46)
Follow up 2	2.89 (−0.13 to 5.90)	0.57 (0.07 to 1.07)	6.76 (3.79 to 9.72)	1.36 (0.82 to 1.90)	3.87 (0.85 to 6.89)	0.76 (0.36 to 1.26)
Follow up 3	1.42 (−1.97 to 4.80)	0.25 (−0.25 to 0.75)	4.45 (1.1 to 7.80)	0.79 (0.28 to 1.29)	3.03 (−0.35 to 6.41)	0.54 (0.05 to 1.04)
Adjusted for baseline score of the FSFI and spouse's age						
Follow up 1	3.25 (0.93 to 5.57)	0.85 (0.33 to 1.36)	7.19 (4.91 to 9.48)	1.87 (1.29 to 2.45)	3.94 (1.64 to 6.25)	1.02 (0.51 to 1.54)
Follow up 2	2.48 (−0.45 to 5.40)	0.51 (0.01 to 1.01)	6.59 (3.73 to 9.45)	1.37 (0.83 to 1.91)	4.12 (1.21 to 7.03)	0.85 (0.34 to 1.36)
Follow up 3	1.24 (−2.17 to 4.65)	0.22 (−0.28 to 0.71)	4.38 (1.03 to 7.73)	0.77 (0.27 to 1.28)	3.14 (−0.25 to 6.53)	0.57 (0.07 to 1.06)

CI, confidence interval; FSFI, Female Sexual Function Scale; RM-ANOVA, analysis of variances for repeated measures; ANCOVA, analysis of variance- covariance.
[a]The crude model was analyzed using RM-ANOVA. The adjusted models were analyzed using RM-ANOVA ANCOVA.

was observed with vitamin D suppository treatment. Moreover, correction of the results by considering baseline scores as covariates was associated with a significant increase in the effect size. However, spousal age was not a confounding variable in the present study because the differences in effect size after correction were only ~1%. Therefore, the results suggest that vitamin D vaginal suppositories are both statistically and clinically more effective than placebo suppositories at improving sexual functioning in postmenopausal women.

The total sexual functioning score on the FSFI in the control group showed a functional decline during follow-up. This most likely reflects the physiological changes in response to aging. Vaginal dryness is among the most common problems in postmenopausal women owing to estrogen deficiency, a significant reduction in the number of superficial and intermediate cells, and an increase in parabasal cells [37]. Vaginal dryness is associated with decreased sexual desire and functioning as well as decreased quality of life [38].

To the best of our knowledge, no previous study has compared the effects of vitamin D with those of standard topical estrogen therapy. Therefore, the results of the present study were compared to those of other studies that used vaginal or oral vitamin D supplements. Our findings are consistent with those of a previous study by Rad et al. [21] in which vitamin D vaginal suppositories were associated with a significant reduction in parabasal cells in the vaginal mucosa compared with placebo suppositories at the end of 8-week treatment. Therefore, using vitamin D vaginal suppositories can rapidly improve vaginal dryness and paleness in the mucosa and increase the proliferation of superficial cells in the vaginal mucosa among postmenopausal women. In the present study, vitamin D suppositories immediately improved female sexual functioning. However, since longer-term follow-up was not performed by Rad et al. [21], the long-term effect on the vaginal mucosa and cellular function is unknown, as is the longer-term effect on sexual functioning beyond 2 months.

In another study by Schulte-Uebbing et al. [13] that did not include a control group, an estradiol suppository (0.5 mg) with topical vitamin D (12,500 international unit, three nights a week for 6 weeks) showed a significant effect on stage I and stage II stress incontinence. The conditions of more than two-thirds of the participants in the study improved. This finding suggests that the local administration of vitamin D may improve pelvic muscle function. However, the study did not assess the participants' sexual functioning;

Table 4. Results of the effect of vitamin D vaginal suppository on the female sexual function index subscale mean scores

FSFI subscales mean scores	Intervention (n=32)	Placebo (n=31)	Control (n=33)	Effect	F	P	Partial η²
Desire							
Baseline	2.36 (0.90)	2.54 (1.09)	2.82 (0.97)				
Follow up 1	4.56 (0.61)	3.52 (0.87)	3.07 (1.09)	Time	3.93	<0.001	0.295
Follow up 2	3.73 (0.71)	3.14 (0.86)	2.73 (1.23)	Group	6.56	0.002	0.124
Follow up 3	3.06 (0.99)	2.69 (0.79)	2.71 (1.24)	Time[a] group	9.55	<0.001	0.17
Arousal							
Baseline	2.67 (1.13)	2.84 (1.29)	3.4 (1.14)				
Follow up 1	4.79 (0.79)	3.76 (0.86)	3.00 (1.5)	Time	19.10	<0.001	0.17
Follow up 2	4.07 (0.94)	3.43 (0.88)	2.65 (1.60)	Group	7.69	0.001	0.142
Follow up 3	3.47 (1.23)	2.86 (1.08)	2.52 (1.65)	Time[a] group	11.20	<0.001	0.194
Lubrication							
Baseline	2.82 (1.43)	3.02 (1.63)	3.67 (1.12)				
Follow up 1	5.47 (0.46)	4.59 (0.78)	3.28 (1.47)	Time	31.76	<0.001	0.255
Follow up 2	4.74 (0.81)	3.99 (0.9)	2.85 (1.71)	Group	18.60	0.001	0.286
Follow up 3	3.96 (1.19)	3.28 (0.03)	2.24 (1.61)	Time[a] group	14.35	<0.001	0.236
Orgasm							
Baseline	3.31 (1.17)	3.21 (1.47)	3.64 (1.09)				
Follow up 1	5.03 (0.61)	4.37 (0.9)	3.37 (1.51)	Time	16.98	<0.001	0.156
Follow up 2	4.54 (0.8)	3.72 (1.03)	2.96 (1.66)	Group	12.03	0.001	0.207
Follow up 3	3.91 (1.08)	3.47 (1.2)	2.62 (1.59)	Time[a] group	7.73	<0.001	0.144
Satisfaction							
Baseline	3.51 (1.11)	3.38 (1.50)	3.81 (1.44)				
Follow up 1	5.1 (1.03)	4.63 (0.8)	3.7 (1.52)	Time	24.8	<0.001	0.211
Follow up 2	4.61 (0.9)	3.92 (1.03)	3.08 (1.59)	Group	7.73	0.01	0.142
Follow up 3	3.7 (1.29)	3.46 (0.99)	2.75 (1.55)	Time[a] group	6.37	<0.001	0.121
Pain							
Baseline	3.98 (1.44)	3.68 (1.55)	2.68 (1.25)				
Follow up 1	1.39 (0.67)	1.88 (0.86)	3.1 (1.34)	Time	27.58	<0.001	0.229
Follow up 2	2.03 (1.1)	2.68 (1.07)	3.18 (1.75)	Group	3.46	0.04	0.069
Follow up 3	2.41 (1.34)	3.32 (1.34)	3.55 (2.1)	Time[a] group	13.74	<0.001	0.228

FSFI, Female Sexual Function Scale; RM-ANOVA, analysis of variances for repeated measures.
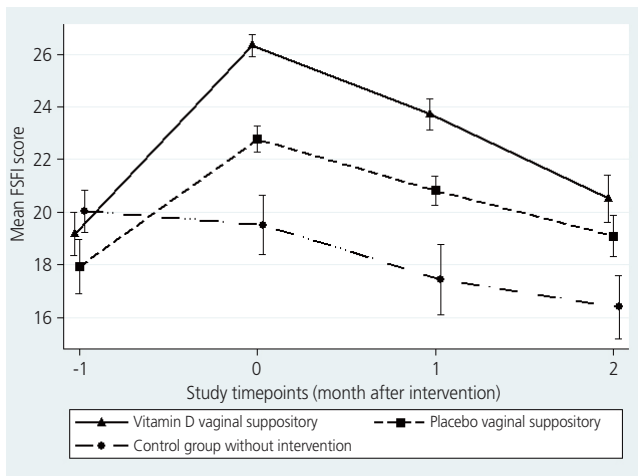[a] Analyzed using RM-ANOVA.

Fig. 2. Female sexual function index scores of the study groups. The results are expressed as mean±standard error of the mean (n=96). The data were analyzed using one-way repeated-measures analysis of variance *P*<0.05. FSFI, Female Sexual Function Scale.

therefore, it is unclear whether improvement in pelvic muscle function might improve sexual functioning.

In a longitudinal observational study, Yildirim et al. [20] examined the effect of oral vitamin D on vaginal mucosal atrophy for 1 year. In the treatment group, the vaginal maturation index (increase in superficial cells compared to basal and parabasal cells) was significantly higher. However, the symptoms of vaginal atrophy did not significantly improve [20]. This finding suggests that local administration is more effective than systemic administration. Zeyneloglu et al. [39] studied the combined effect of raloxifene (60 mg; a selective estrogen receptor modulator for postmenopausal osteoporosis) and vitamin D (400 units) for 3 months on the vaginal maturation index and urogenital symptoms in postmenopausal women with osteoporosis. The control group received a combination of risedronate (5 mg), calcium (600 mg), and vitamin D (400 units) daily for 3 months. Combined treatment with raloxifene and vitamin D improved the vaginal maturation index and pH but not dyspareunia, pruritus, urinary symptoms, vaginal burning, or flushing. This finding suggests that a lower dose of vitamin D does not improve symptoms related to postmenopausal vaginal integrity.

Overall, vitamin D appears to play a key role in preventing vaginal atrophy by regulating growth and differentiation of the vaginal epithelium. However, high doses are required [7,8,20]. A possible mechanism is that vitamin D increases the proliferation of epithelial cells in the vagina via its recep-

tors in the basal and parabasal cellular layers of the vaginal tissue [8,9]. Therefore, vitamin D effectively regulates squamous cell differentiation [17,18] and restores the mucosal tissue of the vagina to regulate its function during sexual intercourse [16].

Although the aforementioned studies reported the effect of vitamin D on vaginal cellular parameters, their results differed in terms of its effect on improving clinical symptoms. Therefore, the dose of medication, duration of treatment, and administration method can influence treatment efficacy. In the present study, the largest effect of the vitamin D suppository was observed immediately after and 1 month after treatment. By 2 months, its clinical effects had decreased, yet it still prevented age-related decline in sexual functioning. It should be noted that the administration was daily for the first 2 weeks and then every other day for the remaining 6 weeks, when the effects started to decline. Future research should investigate whether such treatments should be performed daily to maintain the drug concentrations in the local area.

1. Research strengths and limitations

The strength of the present study is its use of a randomized clinical trial design with three groups (intervention, placebo, and negative control). However, this study has some limitations. First, the participants were healthy postmenopausal women, which may limit the generalizability of our results to postmenopausal women with underlying diseases. A self-reporting method was used to assess the key outcome variables. Moreover, the spouse's sexual functioning was not assessed, which may have affected the outcomes. Some contributing factors, including psychological and hormonal factors, were not assessed. In addition, only 2 months of follow-up was performed after the intervention; therefore, further studies are required to examine the longer-term effects. The study did not examine vaginal cytology or measure serum vitamin D levels due to funding constraints, which could also be collected in future studies. Because the standard treatment for vaginal atrophy in menopausal women is low-dose topical estrogen, future studies should compare vitamin D interventions with various standard therapies.

Clinical improvement in sexual functioning (based on clinically important differences) was observed only in the intervention group administered vitamin D (versus control). Although the scores increased in the placebo versus control

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group, they were not clinically important. The present study showed that vaginal vitamin D suppositories improved the sexual functioning of postmenopausal women in the short term with no side effects. These short-term effects may limit the use of this protocol. Future studies are required to assess the effects of a daily administration regimen that may yield long-lasting improvements in sexual functioning. Moreover, psychological and hormonal factors as well as serum levels of vitamin D should be assessed both during and after treatment.

Conflict of interest

Authors have no conflict of interest to declare.

Ethical approval

The study protocol was prospectively registered in the Iranian clinical Trial Registration system (ID: IRCT20180704 040346N1) on October 10, 2018.

Patient consent

Written informed consent was obtained from all participants.

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