Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

The Relationship between Vitamin D and Vasomotor Symptoms During the Postmenopausal Period

Tufan Arslanca [1], Hilal Korkmaz [2], S. Banu Arslanca [3], Bilge Pehlivanoglu [4], Özgül Celikel [5]

[1] *Department of Obstetrics and Gynecology, Ufuk University, Ankara, Turkey*
[2] *Department of Physiology, Gazi University, Ankara, Turkey*
[3] *Department of Obstetrics and Gynecology, Ankara University, Ankara, Turkey*
[4] *Department of Physiology, Hacettepe University, Ankara, Turkey*
[5] *Department of Obstetrics and Gynecology, Yenimahalle Training and Research Hospital, Ankara, Turkey*

SUMMARY

Background: **During the postmenopausal period, hot flashes are frequent symptoms and might impact quality of life. Vitamin D deficiency is commonly seen in this period. This study aims to assess the association between vitamin D deficiency and hot flashes.**
Methods: **Two hundred ten postmenopausal women were recruited. The participants were divided into two groups: Group 1 consisted of postmenopausal women with hot flashes (n = 104), and Group 2 included the participants without hot flashes (n = 106).**
Results: **The comparison of the two groups concerning vitamin D level showed that 52 patients Group 1 had vitamin 25(OH)D levels below 20 ng/mL, whereas only 25 patients in Group 2 ($p < 0.001$). After adjusting for age and menopause duration, there was also a significant difference between groups (21.65 vs. 34.17, respectively, $p < 0.001$). In multiple regression analysis, one unit decrease of vitamin 25(OH)D (1 - 0.941 = 0.059) increased the risk of hot flashes by 5.9%.**
Conclusions: **The decreases of vitamin D levels were significantly associated with hot flashes in postmenopausal women independent of age and menopause duration.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.191116)

Correspondence:
Tufan Arslanca, MD
Ufuk University
Department of Obstetrics and Gynecology Ankara
Kizilirmak, Ufuk Ünv. Cd No:2
06510 Cankaya/Ankara
Turkey
Phone: +90 5324580546
Fax: +90 312 204 40 00
Email: drtufanarslanca@hotmail.com

Manuscript accepted December 18, 2019

KEY WORDS

vitamin D, menopause, hot flashes

INTRODUCTION

Most women experience menopause-related symptoms, which can severely influence their life quality, work performance, and relationships [1,2]. These symptoms include mood disturbances [3], muscle aches, and hot flashes [4]. Hot flashes are among the frequent symptoms with the highest prevalence (i.e., 64%) at the age of 54 in menopausal women [5,6]. They experience it as sensations of internal heat in the whole body, which also cause awakening and sweating during the night. The most reliable mechanism underlying is the alterations in estrogen, norepinephrine, and vitamin tiers at the menopause transient, which causes hot flashes by affecting

the central thermoregulatory centers [7].

Vitamin D (Vit D) could potentially improve menopausal symptoms using a range of mechanisms. Some authors claim that a link between Vit D and menopause-related symptoms is reasonable. For instance, it has been demonstrated that Vit D prevents a decline in serotonin levels [8]. Serotonin is a neurotransmitter with a known impact on thermoregulation and possible contribution to hot flashes [9,10]. As suggested by previous data, Vit D is crucial in serotonin biosynthesis by its differential role in tryptophan hydroxylase activation favoring elevation of central serotonin levels [11]. Also, estrogen affects the activity of the enzyme, which is responsible for activating Vit D [12]. Hence, during the transition period of menopause and the decline in estrogen levels, women exhibit symptoms similar to those associated with Vit D deficiency, including mood disturbances and musculoskeletal complaints [13]. Therefore, considering the role of serotonergic signaling in thermoregulation and the impact of estrogen on Vit D activation, the low estrogen in the menopausal period could be the cause of the cascade ending up with hot flashes.

We are unaware of any previous data on this association in postmenopausal women. However, Vit D deficiency may likely have a crucial role in hot flashes experiences. This study aimed to evaluate the effect of Vit D levels on hot flashes in postmenopausal women.

MATERIALS AND METHODS

Yenimahalle Education and Research Hospital Ethics Committee approved the protocol and the patient informed consent form. The postmenopausal women (n = 210) who applied to the menopausal outpatient clinics for a routine visit between August 2018 to January 2019 were consecutively enrolled in the study.

The daily hot-flash questionnaire asked about the numbers and the severity of hot flashes per day (24 hours period). Descriptions of hot flash definitions from women who had taken part in previous studies were provided to each patient in the questionnaire booklet [14].

Based on the hot flash complaints, two groups were created; Group 1 comprised postmenopausal women with hot flashes (n = 104), and Group 2 was composed of participants with no experience of hot flashes (n = 106). The two groups were compared for demographic and laboratory findings. All women provided a signed informed consent form.

Exclusion criteria were as follows: prior hormone replacement therapy of more than ten years, current usage of vitamin D supplements or taking any medication, smoking, and artificial menopause. Also, women who had any systemic disorders such as chronic renal impairment, chronic hypertension, diabetes mellitus, and thyroid diseases were excluded. Women who had a feeling of rapid heartbeat accompanied by intense heat and sweating, with each occurrence lasting from 2 to 30 minutes, were identified as hot flash. Anthropometric measurements such as weight and height in light clothing without shoes were obtained. BMI was calculated as the weight (in kilograms) divided by height (in meters squared).

All biochemical tests were performed by a single biochemist in an external standardized biochemistry laboratory via commonly used current techniques. Venous blood samples were collected after 12 hours of overnight fasting. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and fasting plasma glucose were measured using standard enzymatic analytical methods. Lipoprotein a [Lp(a)] levels were calculated with immunonephelometry by an automated assay (Immage; Beckman-Coulter, Brea, CA, USA). Lp(a) levels vary from 0.5 to 100 mg/dL, and the normal reference value was less than 33 mg/dL. TSH (0.34 - 6 µIU/mL), FT3 (2.3 - 4.2 pg/mL), and FT4 (0.58 - 1.25 ng/dL) tiers were calculated with chemiluminescence microparticle immunoassay methods on an Abbott Architect 2000 device (Abbott Molecular Inc., Des Plaines, IL, USA).

25-Hydroxyvitamin D [25(OH)D] concentration is an accepted marker for Vit D status in humans. The serum samples of the venous blood collected were processed and stored at -80°C until 25(OH)D concentrations (mmol/L) were measured using the DiaSorin LIAISON chemiluminescence method (DiaSorin Laboratories, Stillwater, MN, USA). There are no cut points on optimal 25(OH)D concentrations; levels $\leq$ 20, 21 - 29, and $\geq$ 30 ng/mL were defined as deficient, insufficient, and sufficient, respectively, based on the current clinical definitions in a recent review [15].

Collected data were analyzed by SPSS (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, NY, USA), and all the statistical tests were performed by a medical statistician. In the literature, there was no study evaluating the direct association between vitamin D deficiency and hot flashes in postmenopausal women. Therefore, we did not calculate the sample size. Nevertheless, Post hoc power analysis was performed with G* Power programs at the end of the study. Student's *t*-test was used to compare continuous variables for independent samples, categorical variables were compared by Pearson's chi-square test. Covariance of analysis (ANCOVA) was used to compare the corrected data. The factors that may be related to hot flashes are included in the univariate logistic regression. In the univariate analysis, variables with $p < 0.25$ were included in the multivariate analysis. In multivariate analysis, the backward logistic regression method was applied. ROC analysis was used to determine the predictive values and calculate cutoffs. A p-value of < 0.05 was accepted as statistically significant.

Table 1. Comparison of clinical and laboratory findings of groups.

	Group 1 (n = 104)		Group 2 (n = 106)		p
Age (year)	53.75	± 7.30	56.75	± 7.64	0.001
BMI (kg/m^2)	28.29	± 3.51	27.81	± 3.10	0.294
Duration of menopause (year)	6.03	± 5.08	8.12	± 5.88	0.002
Fasting blood glucose (mg/dL)	90.35	± 6.68	90.06	± 6.85	0.795
LDL (mg/dL)	140.12	± 36.34	143.54	± 40.20	0.519
HDL (mg/dL)	54.46	± 16.97	54.40	± 11.86	0.572
Total cholesterol (mg/dL)	226.13	± 42.40	230.99	± 44.50	0.436
TG (mg/dL)	161.00	± 90.95	158.46	± 78.49	0.886
Lipoprotein-a (mg/dL)	23.04	± 26.43	32.39	± 45.29	0.199
TSH (µIU/mL)	2.12	± 0.84	2.19	± 0.92	0.753
FT3 (pg/mL)	2.84	± 0.33	2.78	± 0.41	0.216
Free T4 (ng/dL)	1.23	± 0.17	1.22	± 0.18	0.699

Continuous variables are expressed as either the mean ± standard deviation (SD) and categorical variables are expressed as either frequency or percentage. Continuous variables were compared with Student's *t*-test and categorical variables were compared using Pearson's chi-square test. Statistically significant p-values are underlined.

Table 2. Comparison of groups according to vitamin D level.

Vitamin D (ng/mL)	Group 1 (n = 104)	Control Group (n = 106)	p
	n (%)	n (%)	
≤ 20	52 (50.0%)	25 (23.6%)	< 0.001
21 - 29	37 (35.6%)	35 (33.0%)	
≥ 30	15 (14.4%)	46 (43.4%)	
Mean ± SD	21.64 ± 11.78	34.16±20.09	

Continuous variables are expressed as either the mean ± standard deviation (SD) and categorical variables are expressed as either frequency or percentage. Categorical variables were compared using Pearson's chi-square test. Statistically significant p-values are underlined.

RESULTS

According to the retrospective power analysis results, based on the standard deviation of Vit D levels of the two groups conducted at the end of the study, the study power was calculated at 95%; the effect size was calculated for Vit D and revealed a Cohen's D of 0.76 (CI 0.48 - 1.04), which demonstrates a high efficiency. This result shows that the difference between the two groups is a significant difference that can be considered clinically meaningful.

The mean age of the participants was 55.3 ± 7.6 years, BMI was 28.7 ± 9.2 kg/m^2, and 25(OH) Vit D levels were 27.9 ± 17.6 ng/mL. The mean number of hot flashes during the day was 6.56 ± 3.87 times, and the duration of hot flashes was 7.53 ± 5.26 minutes. The occurrence time of hot flashes was predominantly during daytime in 49 patients (47.1%) and at night in 55 patients (52.9%). The comparison of the groups revealed a significant difference between age (53.75 vs. 56.75, respectively, p = 0.001) and menopause duration (6.03 vs. 8.12, respectively, p = 0.002) (Table 1).

When the patients were compared according to Vit D levels, the result showed that in the study group, 52 patients (50%) had vitamin 25(OH) D levels below 20 ng/mL. The levels for 37 patients (35.6%) and 15 patients (14.4%) were 21 - 29 ng/mL and above 30 ng/mL, respectively (p < 0.001) (Table 2) for the comparison of the group 1 and group 2 according to the vitamin 25(OH)D levels. When age and menopause duration were taken as a covariate and tested by ANCOVA, the significant difference between Group 1 and Group 2 persisted (21.65 vs. 34.17, respectively, p < 0.001) after age and menopause duration.

Table 3. Univariate and multivariate analysis of the factors affecting hot flashes.

	Univariate logistic				Multivariate logistic			
	Regression analysis				Regression analysis (backward LR 4 Step)			
	Wald statistic	OR	95% CI Lower - Upper bound	p-value	Wald Statistic	OR	95% CI Lower - Upper bound	p-value
Age (year)	7.888	0.947	0.911 - 0.984	0.005	6.794	0.945	0.905 - 0.986	0.009
BMI (kg/m^2)	1.105	1.045	0.962 - 1.136	0.293				
Vitamin D (ng/mL)	21.953	0.945	0.923 - 0.968	< 0.001	21.765	0.941	0.917 - 0.965	< 0.001
Duration of menopause (year)	7.06	0.931	0.884 - 0.981	0.008				
Fasting blood glucose (mg/dL)	0.094	1.006	0.967 - 1.048	0.759				
LDL (mg/dL)	0.42	0.998	0.991 - 1.005	0.517				
HDL (mg/dL)	0.001	1	0.982 - 1.019	0.978				
Cholesterol (mg/dL)	0.657	0.997	0.991 - 1.004	0.418				
TG (mg/dL)	0.048	1	0.997 - 1.004	0.827				
Lipoprotein-a (mg/dL)	2.994	0.992	0.984 - 1.001	0.084	3.648	0.989	0.978 - 1.000	0.056
TSH (µIU/mL)	0.304	0.917	0.673 - 1.249	0.581				
FT3 (pg/mL)	1.529	1.587	0.763 - 3.301	0.216	4.786	2.705	1.109 - 6.596	0.029
Free T4 (ng/dL)	0.152	1.362	0.287 - 6.458	0.697				

Variable excluded from multivariate analysis since presented p > 0.25 in univariate analysis. Nagelkerke R square:0.357 Hosmer vs. Lemeshow p - 0.739.
OR - odds radio, CI (95%) - confidence interval, Statistically significant p-values are underlined.

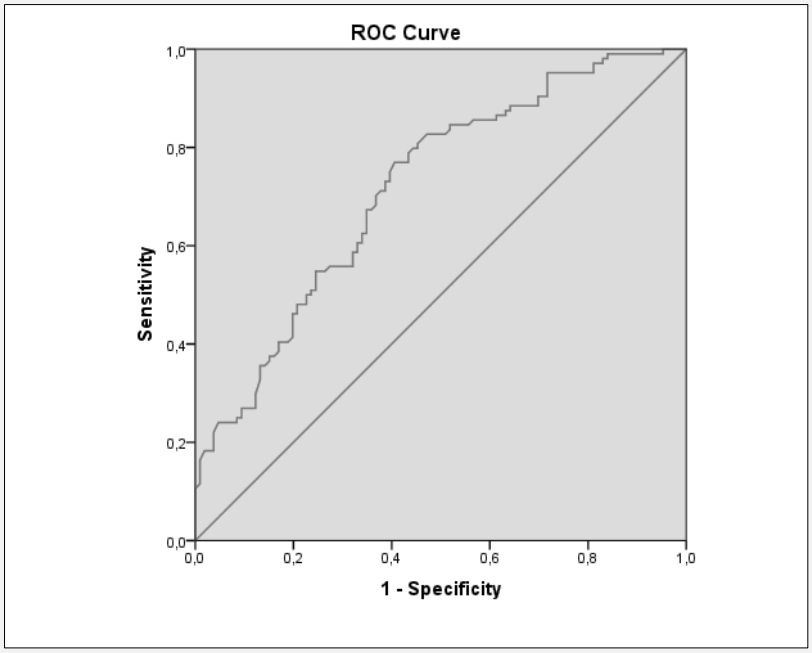

Figure 1. Predictive value of serum vitamin D level (p < 0.001).

In the regression analysis, the control group was taken as the reference value, and the factors associated with hot flash were included in the univariate logistic regression. As a consequence of univariate analysis, p-value of less than 0.25 for age ($p = 0.009$), vitamin 25(OH)D ($p < 0.001$), and FT3 ($p = 0.029$) variables was included in the regression analysis. In multivariate analysis, a backward logistic regression method was applied, and Step 4 results were given. As the results between Nagelkerke R square were from 0.20 to 0.40, the model was significant. Furthermore, it was discovered that the model was compatible with the data because of $p > 0.05$ in Hosmer and Lemeshow tests. Thus, a one unit reduction of age (1 - 0.945 = 0.055) increases the risk of hot flashes by 5.5%. One unit decrease of vitamin 25(OH)D levels (1 - 0.941 = 0.059) increases the risk of hot flashes by 5.9%. A one-unit improvement in FT3 increases the risk of hot flashes by 2.7% (univariate and multivariate regression analysis results are shown in Table 3). In the ROC analysis, when the optimal cutoff value for vitamin 25(OH)D level was 26.21 ng/mL, hot flashes were predicted with a sensitivity of 76.9% and a specificity of 59.4% ($p = 0.001$, AUC: 0.718, Figure 1).

DISCUSSION

Hot flash in the postmenopausal period is a severe symptom, which is characterized as an extreme response to changes in the chemical dependency regulatory control system [9]. Estrogen is the most commonly implicated neurochemical related to hot flashes. Several reports in the literature suggest that the alterations in estrogen production at the time of transient menopause period may influence the central thermoregulatory centers and lead to hot flashes [16]. Fentiman et al. have shown that high estrogen levels were correlated with a decline in the severity of hot flashes. There are many circumstances influencing estrogen production, such as exposure to sunlight, thyroid hormones, and catecholamines [17]. Thus, we may speculate that Vit D deficiency may affect estrogen biosynthesis and contribute to hot flashes in postmenopausal women.

Most of the previous studies that investigated Vit D deficiency established a link with various complications, such as metabolic syndrome, osteoporosis, type 2 diabetes mellitus, and hypertension [18,19]. Evidence in the literature recommends Vit D replacement therapy to prevent these complications [20]. However, very few studies in the literature have evaluated the relationship between Vit D deficiency and postmenopausal hot flashes. Leblanc et al., for example, did not report a significant association between 25(OH)D status and menopause-related symptoms [21]. However, this study was a post hoc analysis of women who had vitamin D testing performed as part of three nested case-control studies of fracture, breast cancer, and colorectal cancer. Besides, the vast majority of their participants had more than ten years of menopause experience, and women in this study who were still experiencing the symptoms may not be representative of the general population of women who experience symptoms during the transition through menopause.

In contrast, this study showed that one unit decrease of vitamin 25(OH) D increases the risk of hot flashes by 5.9%. Moreover, our study concluded with 95% confidence that there is a significant clinical difference in Vit D levels among the groups. Notwithstanding this, from a different angle, our findings suggested that Vit D deficiency has an association with hot flashes. In support of our results, another study reported a tremendous prevalence of vasomotor symptoms in breast cancer patients taking aromatase inhibitors that decrease estrogen levels and have an impact on associated low Vit D levels [22]. The usage of estrogen replacement therapy (ERT) in postmenopausal women is under scrutiny. The side effects and indications of replacement therapy are reviewed, and recommendations regarding its use are offered. Atrophy of the vaginal epithelium, hot flashes, and prevention of osteoporosis have been established indications for ERT [23]. However, ERT has restricted use due to venous thromboembolism, cardiovascular disease complications [24], endometrial cancer, breast cancer [25], hypertension [23], and stroke [26]. Therefore, it is conceivable that Vit D replacement might reduce the need for ERT. Cianci et al. have shown that after the combination of calcium, inulin, soy isoflavones, and Vit D replacement, the mean number of hot flashes declined to 2.8 number/day in the study group compared with the control group [27]. Therefore, they suggested that Vit D replacement had a beneficial effect on hot flashes. In light of this data, we had thought that Vit D replacement for postmenopausal women with hot flashes would decrease the symptoms and increase life quality; however, future studies examining this issue may benefit from our results.

CONCLUSION

In women with postmenopausal hot flashes, Vit D levels are significantly reduced independently of age and menopause duration. Vit D deficiency may be one of the factors that contribute to the development of hot flashes. In the future, these findings should be supported by results from large-scale studies.

Acknowledgment:
None.

Ethics Approval:
Ethics committee approval was received from Yenimahalle Education and Research Hospital for this study.

Financial Support/Funding Source:
No financial support of any kind was received for this study. The authors have no funding to declare.

Declaration of Interest:
The authors of this study have no conflicts of interest with regard to the research conducted and the authorship of the article.

References:

1. Sarrel PM. Women, work, and menopause. Menopause 2012;19: 250-2 (PMID: 22228323).

2. Conde DM, Pinto-Neto AM, Santos-Sa D, Costa-Paiva L, Martinez EZ. Factors associated with quality of life in a cohort of postmenopausal women. Gynecol Endocrinol 2006;22:441-6 (PMID: 17012106).

3. McKinlay SM, Brambilla DJ, Posner JG. The normal menopause transition. Maturitas 2008;61:4-16 (PMID: 19434875).

4. Freeman EW, Sammel MD, Lin H, et al. Symptoms associated with menopausal transition and reproductive hormones in midlife women. Obstet Gynecol 2007;110:230-40 (PMID: 17666595).

5. Rödström K, Bengtsson C, Lissner L, Milsom I, Sundh V, Björkelund C. A longitudinal study of the treatment of hot flushes: the population study of women in Gothenburg during a quarter of a century. Menopause 2002;9:156-61 (PMID: 11973438).

6. Haines CJ, Xing SM, Park KH, et al. Prevalence of menopausal symptoms in different ethnic groups of Asian women and responsiveness to therapy with three doses of conjugated estrogens/ medroxyprogesterone acetate: the Pan-Asia Menopause (PAM) study. Maturitas 2005;52:264-76 (PMID: 15921865).

7. Shanafelt TD, Barton DL, Adjei AA, Loprinzi CL. Pathophysiology and treatment of hot flushes. Mayo Clin Proc 2002;77:1207-18 (PMID: 12440557).

8. Cass WA, Smith MP, Peters LE. Calcitriol protects against the dopamine- and serotonin-depleting effects of neurotoxic doses of methamphetamine. Ann N Y Acad Sci 2006;1074:261-71 (PMID: 17105922).

9. Rossmanith WG, Ruebberdt W. What causes hot flushes? The neuroendocrine origin of vasomotor symptoms in the menopause. Gynecol Endocrinol 2009;25:303-14 (PMID: 19903037).

10. Pinkerton JV, Zion AS. Vasomotor symptoms in menopause: where we've been and where we're going. J Womens Health (Larchmt) 2006;15:135-45 (PMID: 16536677).

11. Lambert GW, Reid C, Kaye DM, Jennings GL, Esler MD. Effect of sunlight and season on serotonin turnover in the brain. Lancet. 2002 Dec 7;360(9348):1840-2 (PMID: 12480364).

12. Buchanan JR, Santen R, Cauffman S, Cavaliere A, Greer RB, Demers LM. The effect of endogenous estrogen fluctuation on metabolism of 25-hydroxyvitamin D. Calcif Tissue Int 1986;39: 139-44 (PMID: 3093024).

13. Brunner RL, Aragaki A, Barnabei V, et al. Menopausal symptom experience before and after stopping estrogen therapy in the Women's Health Initiative randomized, placebo-controlled trial. Menopause 2010;17:946-54 (PMID: 20505547).

14. Finck G, Barton DL, Loprinzi CL, Quella SK, Sloan JA. Definitions of hot flashes in breast cancer survivors. J Pain Symptom Manage 1998 Nov;16(5):327-33 (PMID: 9846028).

15. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30 (PMID: 21646368).

16. Kronenberg F. Hot flushes: phenomenology, quality of life, and search for treatment options. Exp Gerontol 1994;29:319-36 (PMID: 7925752).

17. Schilling C, Gallicchio L, Miller SR, Langenberg P, Zacur H, Flaws JA. Genetic polymorphisms, hormone levels, and hot flushes in midlife women. Maturitas 2007;57:120-31 (PMID: 171 87946).

18. Srimani S, Saha I, Chaudhuri D. Prevalence and association of metabolic syndrome and vitamin D deficiency among postmenopausal women in a rural block of West Bengal, India. PLoS One 2017;12:e0188331 (PMID: 29190744).

19. Lerchbaum E. Vitamin D and menopause a narrative review. Maturitas 2014;79:3-7 (PMID: 24993517).

20. Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci 2009;338:40-4 (PMID: 195931 02).

21. LeBlanc ES, Desai M, Perrin N, et al. Vitamin D levels and menopause-related symptoms. Menopause 2014;21:1197-203 (PMID: 24736200).

22. Khan QJ, Reddy PS, Kimler BF, et al. Effect of vitamin D supplementation on serum 25-hydroxy vitamin D levels, joint pain, and fatigue in women starting adjuvant letrozole treatment for breast cancer. Breast Cancer Res Treat 2010;119:111-8 (PMID: 19655244).

23. Judd HL, Cleary RE, Creasman WT, et al. Estrogen replacement therapy. Obstet Gynecol 1981;58:267-75 (PMID: 7022279).

24. Simon JA, Laliberté F, Duh MS, et al. Venous thromboembolism and cardiovascular disease complications in menopausal women using transdermal versus oral estrogen therapy. Menopause 2016; 23:600-10 (PMID: 26953655).

25. Antoine C, Ameye L, Paesmans M, Rozenberg S. Systematic review about breast cancer incidence in relation to hormone replacement therapy use. Climacteric 2014;17:116-32 (PMID: 2390 9434).

26. Carrasquilla GD, Frumento P, Berglund A, et al. Postmenopausal hormone therapy and risk of stroke: a pooled analysis of data from population-based cohort studies. PLoS Med 2017;14: e1002445 (PMID: 29149179).

27. Cianci A, Colacurci N, Paoletti AM, et al. Soy isoflavones, inulin, calcium, and vitamin D3 in post-menopausal hot flushes: an observational study. Clin Exp Obstet Gynecol. 2015;42:743-5 (PMID: 26753476).