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Effect of Vitamin E Supplementation on Plasma Nitric Oxide in Menopausal Women with Hot Flashes: A Cross-Over, Randomized Clinical Trial

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Abstract

Background: Vitamin E serves a major role in increasing nitric oxide (NO) and reducing lipoperoxidation progression during the menopause transition.
Objectives: This study aimed to determine the effect of Vitamin E on plasma nitric oxide in menopausal women with hot flashes (HFs).
Methods: In this double-blind, randomized, cross-over clinical trial performed in a teaching hospital, 83 eligible menopausal women with HFs were randomly block allocated to Vitamin E (n = 42) and placebo (n = 41) groups. In phase I of the intervention, they were administered 400 IU per day of Vitamin E or placebo for 4- weeks. In the phase II, the group receiving Vitamin E was subsequently given placebo and vice versa after wash-out. Primary outcomes were the number and severity of hot flashes per day as determined by the recorded values by the women and using Modified Kupperman Index, respectively. The secondary outcome was plasma nitric oxide measured before and after the intervention in the two phases. Both outcomes were analyzed within and between placebo-vitamin E (P-E) and Vitamin E-placebo (E-P) groups, and in general by gathering Vitamin E and placebo groups in the two phases, separately. Data were analyzed using the Chi-Square test, independent t-test, Wilcoxon test, Mann-Whitney test, and Spearman's correlation coefficient.
Results: The mean number of HFs indicated a significant decreasing trend from week II of the phase I to the end of the phase II within P-E and E-P groups compared to before the intervention. Median (interquartile range [IQR]) of HFs number changes was 2.03 (2.57) in the P-E group and 1.21 (2.21) in the E-P group at the end of the first week of phase II (P = 0.043). There was a very low significant positive correlation between changes in HFs and plasma nitric oxide level in weeks I (r = 0.262, P = 0.029) and II (r = 0.256, P = 0.034) in the Vitamin E group.
Conclusions: Vitamin E and placebo were both effective in reducing HFs in menopausal women. It seems that the subjective effect of placebo contributed to this decline. Vitamin E had no effect on reducing HFs via increasing plasma level of nitric oxide. These results were not in line with our hypothesis. Further research is needed to understand this issue.

Keywords: Antioxidant, Hot Flashes, Menopause, Nitric Oxide, Vitamin E

1. Background

Menopause is a biopsychosocial process occurring in 45 to 55-year-old women and is characterized by the cessation of menstruation for 12 consecutive months (1-3). Reduction of estrogen level is associated with cessation of menstruation, vasomotor instability (for example hot flashes [HFs] and sweating), and psychological problems (4-6). HFs is the most common and annoying symptom of menopause in many countries (7-10). HFs may alter mood and concentration; lead to sleep disturbances and fatigue, and decrease functional ability and social activity by affecting basic body temperature (7, 9-11).

The exact mechanism of skin vasodilatation during HFs is unknown (5, 7, 11, 12). Researchers have shown that the main mechanism of HFs is vasodilatation in response to changes in heat regulation area in the hypothalamus (5,

7). Reduced estrogen levels may interfere with the mechanism of body temperature regulation in the hypothalamus, which can lead to tachycardia, increased skin temperature, vasodilatation, and HFs (3, 7, 9, 13). Kolesnikova et al. quoting Palmieri and Sanchez-Rodriguez wrote that menopause is a risk factor for oxidative stress (OS), which can play an essential role in inflammation and vascular endothelium damage (14).

According to evidence, nitric oxide (NO) with vasodilatation and reduction in heat stress leads to modified HFs and sweating (7, 11, 15, 16). Estrogen drop leads to a decrease in NO concentration and vascular protection. The highest NO reduction is observed between the ages of 40 - 60 years or after menopause (17-19). Regarding the potential effect of HFs on the quality of life of menopausal women, finding a solution with the least side effects is necessary to reduce HFs (5, 10, 11).

Based on the evidence, hormone/estrogen replacement therapy has been effective in reducing HFs; however, this therapy has been limited due to reports of increased risk of stroke, breast cancer, and thromboembolic diseases, which have been confirmed by numerous studies (10, 12, 20). Therefore, researchers are seeking alternative strategies such as exercise, yoga, complementary medicine, dietary plans, non-hormonal drugs, and behavioral therapies to modulate vasomotor symptoms (9, 10, 12). Given the challenges and adverse outcomes of some methods, alternative treatments with fewer complications seem to be necessary (21).

In this regard, the existing clinical evidence supports the effectiveness and safety of most alternative supplements such as vitamins, for instance, Vitamin E (menopausal vitamin) as an alternative for estrogen therapy (22, 23). Some evidence suggests that all antioxidants, especially Vitamin E, are reduced in menopausal women (4, 14). In the plasma of menopausal women aged 45 - 55 years, who are affected by HFs, antioxidants are lower and oxidative biomarkers are higher than in those who do not have HFs at this age (3). The results of the studies by Victorino et al. and Bonaccorsi et al. illustrated the contrary results of this association and suggested that longitudinal studies regarding HFs and OS in menopausal women are required (24, 25).

Vitamin E is fat soluble and its major role in the body is antioxidant defense (26). Saxena and Jaiswal and Kolesnikova et al. showed that Vitamin E plays an important role in increasing antioxidants including NO and reducing lipoperoxidation progression during menopause transition (14, 19). Saxena and Jaiswal suggested a diet rich in Vitamin E as age increases in order to prevent or delay degenerative diseases such as atherosclerosis and vascular injury (19).

The effectiveness of Vitamin E on HFs has been supported by some studies. In a study by Ziaei et al. 400 IU per day of Vitamin E was administered to healthy menopausal women, and in another study by Barton 800 IU/day of Vitamin E was administered to menopausal women with breast cancer for 4 weeks in a cross-over design, which showed a significant effect on reducing the number and severity of HFs in Vitamin E group compared to a placebo group. However, the impact of Vitamin E on HFs was low and was not clinically significant in the study of Barton (5, 20).

These findings suggest that Vitamin E deficiency during menopause can reduce plasma NO levels, especially in women with HFs. The present study aimed to determine the effect of Vitamin E supplementation on plasma NO level in menopausal women with HFs. The research hypothesis was that Vitamin E supplementation decreases the number and severity of HFs by increasing the plasma level of NO.

2. Methods

2.1. Study Design

This double-blind, randomized, cross-over clinical trial was performed between February and November 2017. Out of 2000 menopausal women with HFs referred to a governmental/referral teaching hospital affiliated to the Guilan University of Medical Sciences, Iran, 83 eligible volunteers were enrolled in this study. The inclusion criteria were natural menopause at the age of 45 - 60 years, at least 12 consecutive months elapsed from the end of the last menstruation (27), and at least one HFs per day based on the HFs/sweating item of Modified Kupperman Index (28). The exclusion criteria were a history of diabetes, hypertension, dyslipidemia, cardiovascular, kidney, and liver diseases, and cancer, body mass index (BMI) of $\geq$ 30, consumption of any medicines or alcohol, smoking, and hormone replacement therapy.

In this study, the primary outcomes were the number (times/day) and severity of HFs. The secondary outcome was plasma NO level (mg/dL). The sample size was calculated based on the primary outcome with 90% power test at a significance level of 5% and considering the mean and standard deviation (SD) of HFs in the study by Ziaei et al. (5). Considering 50% sample attrition on follow-up, the total amount of inclusions was considered to be 46 in each group as follows.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left({S_1}^2 + {S_2}^2\right)}{2(\mu_1 - \mu_2)^2}$$
$$= \frac{(1.96 + 1.28)^2 \left(3.79^2 + 2.43^2\right)}{2(4.03 - 1.88)^2}$$
$$= 23$$

As shown in Figure 1, the participating women were placed in group A (n = 41) and group B (n = 42) according to randomized block allocation. The group allocations were unknown to the women and researchers. Placebo and Vitamin E were produced and packed in containers labeled "A" and "B" by the Daana Pharma Co. In the phase I of the study, one group was given 400 IU/day Vitamin E capsule (gelatin soft) and another group was given 400 IU/day placebo capsule (including oral paraffin, with the same color, and shape of Vitamin E) for 4- weeks.

In the phase II, the group receiving Vitamin E was subsequently given placebo and vice versa for 4- weeks after an eight-day wash-out. The wash-out period was determined based on the half-life of Vitamin E/alpha-tocopherol (44 hours) (29) and considering that more than 90% of Vitamin E was eliminated after four half-lives (30) in order to control carry-over effect. Before and after each phase, plasma NO levels were determined in the laboratory of the hospital after 12 hours of intermittent fasting. In both phases, women were advised to record the number of HFs/day in a set of cards. Weekly follow-up via phone calls was carried out to ensure that the capsules were used regularly and correctly, that HFs were recorded correctly, and that whether the capsules had any side effects including a headache, nausea, anorexia, abdominal pain, diarrhea, dizziness, weakness, and fatigue.

2.2. Data Collection

2.2.1. Socio-Demographic and Reproductive Characteristics

Socio-demographic and reproductive data based on the statements of women were obtained. Height and weight were measured by the main researcher (NE). The women were weighed in light indoor clothing with Beurer MS 50 mechanical scale (measuring accuracy 1 kilogram), which was calibrated daily at the beginning of each working day. Height was measured with roll-up measuring body meter tape with wall attachment (measuring accuracy +/-1 millimeter). The person being measured stood under the meter and measuring tongue was lowered onto her head. In order to evaluate intra-observer reliability, the height and weight of 10 women were measured by the main researcher at an interval of one hour and interclass correlation coefficient obtained was 0.92, which indicated a high reliability. BMI (kg/m^2) was calculated using weight (kg) divided by height (m) squared.

2.2.2. Menopausal HFs

The number of HFs/day was evaluated based on the recorded values in the cards by the women. The severity of HFs was determined using the Modified Kupperman Index. The severity of HFs included three scales, including 1 (HFs number less than 3 times/day), 2 (HFs number 3-9 times/day), and 3 (HFs number $\geq$ 10 times/day) (28). The number and severity of HFs were recorded in a follow-up form.

2.2.3. Nitric Oxide Measurement

A standard NO measurement kit was purchased from a research-based company named Cib Biotech Co. Two packages of this kit were delivered to the laboratory with preservation of the cold chain according to the company's instructions. The basis for NO measurement was the grease method by enzyme-linked immunosorbent assay (ELISA). In this method, the amount of NO in the supernatant of cell culture or serum is determined by grease reaction. During the first step of grease reaction, NO reacts with sulfanilic acid and produces diazonium ion. In the second step, this ion couples with a compound named N-(1-naphthyl) ethylenediamine and produces a mixture of pink azo derivatives. Plasma NO levels were measured by an expert (head of the laboratory of the center) with more than ten years of work experience to perform various experiments. The results from four requested fasting blood samples were recorded in the follow-up form by the main researcher.

2.3. Statistical Analysis

Data analysis was performed using SPSS, Statistics Software for Windows version 22.0 (IBM Corp., Armonk, N.Y., USA). Normality of the data was examined using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Socio-demographic/reproductive characteristics data were analyzed and compared between the intervention groups with the parametric analytical tests, Chi-Square test, and independent t-test. The primary and secondary outcomes were non-normal and analyzed with the non-parametric tests, including Wilcoxon Signed Rank test and Mann-Whitney U test. Correlation between HFs changes and plasma NO was estimated by Spearman's correlation coefficient. The primary and secondary outcomes were analyzed within and between Placebo-Vitamin E (P-E) and Vitamin E-Placebo (E-P) groups, and in general by gathering Vitamin E and placebo groups in the two phases, separately. The results were presented as frequency (%), mean $\pm$ SD/SE, median, interquartile range (IQR), and/or mean rank. A P-value less than 0.05 was considered statistically significant.

As we indicated in Figure 1, data analysis was conducted separately based on the number of women who participated in each phase of the study. Therefore, sample attrition in the second phase did not affect the analyses in the first phase of the study. Accordingly, we used the intention-to-treat method for analysis.

2.4. Ethical Consideration

We obtained approval of the Ethics Committee and Institutional/Ethical Review Board of Guilan University of Medical Sciences, Rasht, Iran (Ref.: 1395.189). This study was registered at the Clinical Trials Registry on 20 February, 2017 with the IRCT code: IRCT2016063028717N1. Written informed consents were obtained from all the participants

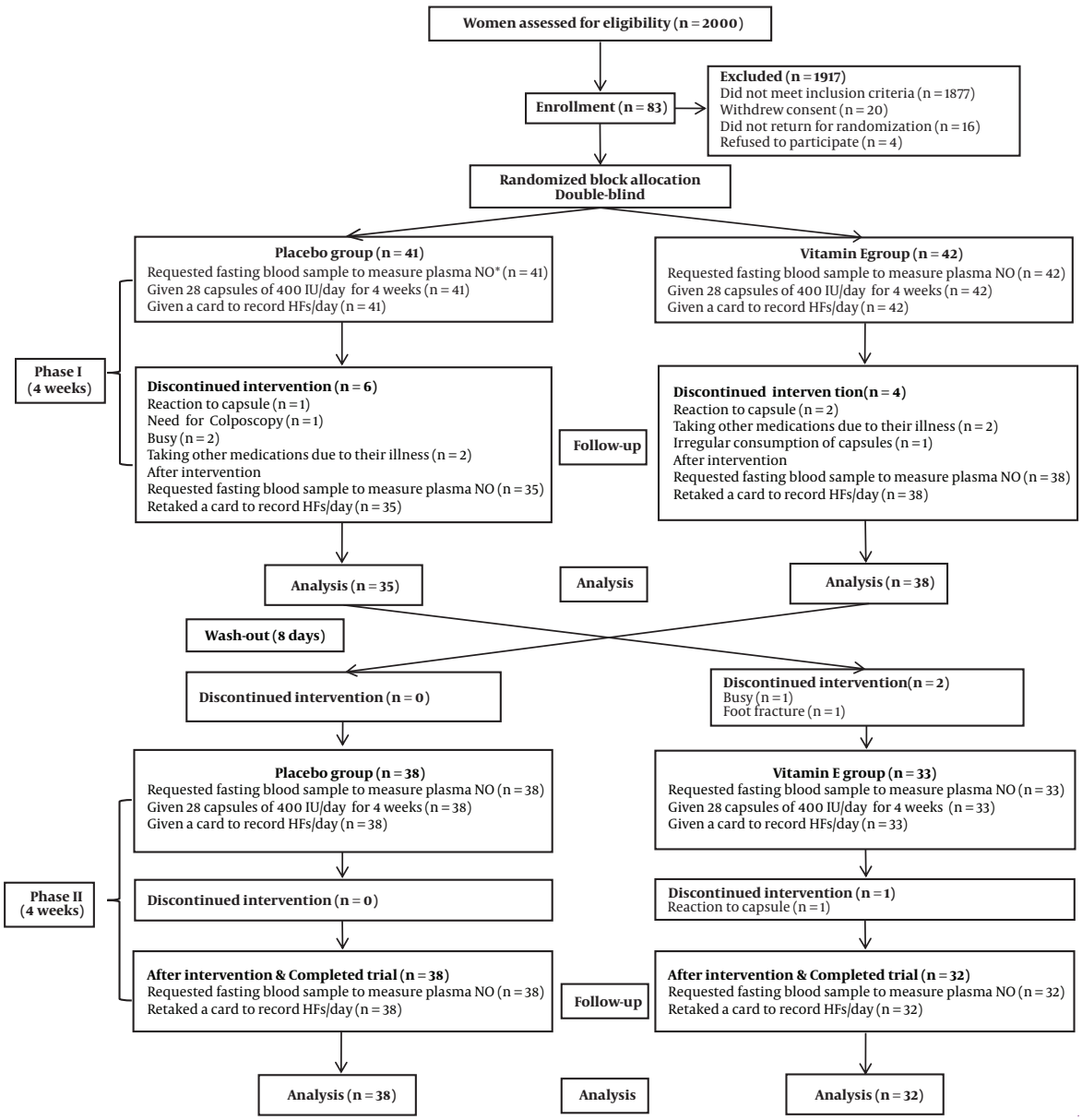

Figure 1. Flaw chart of the study (* Nitric Oxide); HF, Hot Flash.

included in the study. The study protocol is consistent with the ethical guidelines of the Declaration of Helsinki.

3. Results

Various parameters were compared between P-E and E-P groups before the intervention (Table 1), which showed the groups were homogeneous. The difference in median (IQR) of HFs number between the P-E and E-P groups before the intervention was not statistically significant (3.50 [4.00] and 2.50 [2.00], respectively; P = 0.064). Also, the difference in median (IQR) of plasma NO between the P-E and E-P groups before the intervention was not statistically significant (18.70 mg/dL [6.20] and 20.85 mg/dL [7.43], respectively; P = 0.063).

Based on Figure 2, the mean of numbers of HFs in weeks I to VIII after the intervention showed a decreasing trend within P-E and E-P groups in phases I and II. As it is

Table 1. Comparison of Socio-Demographic and Reproductive Characteristics Between the Two Groups[a]

	Placebo-Vitamin E	Vitamin E-Placebo	P Value
Education			0.016[c]
Illiterate	9 (25.7)	1 (2.6)	
Under ND[b]	19 (54.3)	26 (68.4)	
ND and academic	7 (20.0)	11 (29.0)	
Total	35	38	
Occupation			0.152[c]
Householder	32 (91.4)	28 (73.6)	
Employed	1 (2.9)	5 (13.2)	
Retired	2 (5.7)	5 (13.2)	
Total	35	38	
Place of residence			–
City	35 (100.0)	38 (100.0)	
Village	0 (0)	0 (0)	
Total	35	38	
Marital status			0.431[c]
Married	28 (80.0)	33 (86.8)	
Single	0 (0)	0 (0)	
Widow	7 (20.0)	5 (13.2)	
Total	35	38	
Husband's education			0.750[c]
Illiterate	2 (7.1)	4 (12.1)	
Under ND	15 (53.6)	19 (57.6)	
ND and academic	11 (39.3)	10 (30.3)	
Total	28	33	
Husband's job			0.115[c]
Free	15 (53.6)	13 (39.4)	
Employed	12 (42.8)	13 (39.4)	
Unemployed	1 (3.6)	7 (21.2)	
Total	28	33	
Type of delivery			0.130[c]
Vaginal	30 (85.7)	27 (71.1)	
Section	5 (14.3)	11 (28.9)	
Total	35	38	
Age, y	52.00 ± 4.32	52.82 ± 4.34	0.426[d]
Weight, kg	69.44 ± 6.74	69.86 ± 6.63	0.793[d]
Height, cm	160.54 ± 5.58	159.50 ± 5.53	0.425[d]
BMI, kg/m^2	26.92 ± 2.41	27.45 ± 2.11	0.372[d]
Husband's age, y	57.18 ± 5.94	57.72 ± 7.03	0.583[d]
Family income, Rials	8951428.60 ± 5021739.30	11368421.10 ± 7641179.50	0.118[d]
Menopausal age, y	52.00 ± 4.30	52.80 ± 4.30	0.585[d]
Duration of amenorrhea, mo	54.66 ± 52.30	64.68 ± 47.66	0.394[d]
Duration of experiencing HFs, mo	54.77 ± 56.36	64.97 ± 49.88	0.415[d]
Number of gravidity	3.74 ± 1.65	3.47 ± 1.45	0.760[d]
Number of parity	3.26 ± 1.44	2.97 ± 1.17	0.358[d]
Number of children	3.11 ± 1.37	2.76 ± 1.26	0.258[d]

[a] Qualitative variables are presented as frequencies with percentages, No. (%); Quantitative variables are presented as means with standard deviations, mean ± SD.
[b] National Diploma.
[c] Based on Chi-Square test.
[d] Based on Independent *t*-test.

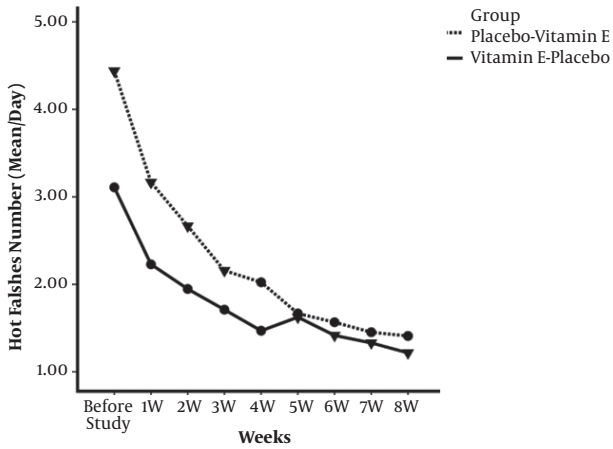

Figure 2. Trend of changes in the mean number of hot flashes during the studied weeks within the two groups

demonstrated in Table 2, pairwise comparisons reflected statistically significant differences in the mean number of HFs at the end of phases I and II compared to it before the intervention within P-E and E-P groups. Mean difference of HFs number at the end of phase II compared to it at the end of phase I was not statistically significant within P-E and E-P groups. Mean ranks of severity of HFs at the end of phases I and II compared to it before the intervention were significantly different within P-E and E-P groups. Mean rank of severity of HFs at the end of phase II compared to the end of phase I was not significantly different within P-E and E-P groups.

Based on the Mann-Whitney U test, the median (IQR) of HFs number changes showed a significant (P = 0.043) reduction in the E-P group (1.21 [2.21]) compared to the P-E group (2.03 [2.57]) at the end of week I of the phase II. This test revealed a significant difference in mean ranks of HFs severity only in week I of phase I between the two groups (42.67 and 30.66, respectively; P = 0.003). In Table 3, HFs number and its changes during the studied weeks were compared between placebo and Vitamin E groups in general, showing no statistically significant difference between them.

Wilcoxon signed-rank test showed that mean and median (IQR) of plasma NO decreased significantly after the intervention compared to before the intervention within P-E group (16.75 ± 4.35, 16.60 [5.20] and 19.40 ± 4.60, 18.70 [6.20], respectively; P = 0.010) and E-P group (18.62 ± 5.08, 17.20 [4.20] and 21.84 ± 5.89, 20.85 [7.40], respectively; P > 0.001) in phase I. There was no significant difference in mean and median (IQR) of plasma NO before and after the intervention in phase II within the P-E and E-P groups. Table 4 indicates that there was no significant difference in plasma NO changes in phases I and II between the P-E and E-P groups and in general between placebo and Vitamin E groups.

In the Vitamin E group, there was a very low significant positive correlation between HFs changes and plasma NO

Table 2. Mean Difference of HFs[a] Number and Mean Rank of HFs Severity Before and After Intervention in Phases I and II Within the Two Groups

	Placebo-Vitamin E			P Value (Within Group)	Vitamin E-Placebo			P Value (Within Group)
	Mean Difference	SE[b]	N		Mean Difference	SE	N	
HFs Number								
End of phase I than before the intervention	2.41	0.50	35	0.002[i]	1.64	0.34	38	0.001[i]
End of phase II than before the intervention	3.03	0.48	32	0.001[i]	1.89	0.32	37	< 0.001[i]
End of phase II than end of phase I	0.61	0.54	32	0.999[i]	0.25	0.17	37	0.999[i]
	NR[d]	PR[e]	Tice[f]		NR	PR	Tice	
HFs severity[c]								
End of phase I than before the intervention	18[g]	1[g]	16[g]	< 0.001[j]	22[g]	0[g]	16[g]	< 0.001[j]
	10.11	8.00			11.50	0.00		
End of phase II than before the intervention	20[g]	0[g]	12[g]	< 0.001[j]	19[g]	0[g]	18[g]	< 0.001[j]
	10.50	0.00			10.00	0.00		
End of phase II than end of phase I	7[g]	3[g]	22[g]	0.166[j]	3[g]	7[g]	27g	0.206[j]
	5.71	5.00			5.50	5.50		

[a] HF; Hot flashes.
[b] SE; Standard error.
[c] Values are mean rank.
[d] Negative Rank: The severity of HFs decreased.
[e] Positive Rank: The severity of HFs increased.
[f] Tice: The severity HFs did not change.
[g] Values are n.
[i] Based on Bonferroni correction adjustment for multiple comparisons.
[j] Based on Wilcoxon Signed Ranks test.

Table 3. Comparison of HFs[a] Number and Its Changes in the Studied Weeks Between the Placebo and Vitamin E Groups[b]

	Placebo (N = 73)				Vitamin E (N = 70)				P Value[d] (Between Groups)
	Mean	SD[c]	Median	IQR	Mean	SD	Median	IQR	
Mean of HFs number/day in week I	2.33	2.61	1.57	2.58	1.90	1.90	1.29	2.00	0.329
Mean of HFs number/day in week II	1.98	2.44	1.29	2.43	1.71	1.66	1.29	2.14	0.614
Mean of HFs number/day in week III	1.80	2.27	1.14	2.14	1.58	1.40	1.21	2.07	0.884
Mean of HFs number/day in week IV	1.67	2.26	1.00	2.00	1.40	1.36	0.86	2.14	0.833
Changes in HFs number by the end of week I	1.25	2.19	1.07	2.21	1.65	2.25	1.07	2.00	0.357
Changes in HFs number by the end of week II	1.57	2.15	1.36	1.72	1.87	2.24	1.36	1.72	0.854
Changes in HFs number by the end of week III	1.84	2.28	1.50	1.85	2.05	2.36	1.57	2.15	0.958
Changes in HFs number by the end of week IV	1.88	2.41	1.71	2.00	2.17	2.39	1.50	1.85	0.977

[a] Hot flashes.
[b] In overall by gathering Vitamin E and placebo groups in the two phases, separately: 35 and 38 participants in placebo group, and 38 and 32 participants in Vitamin E group, respectively in phase I and II analyzed, as mentioned in Figure 1).
[c] Standard deviation.
[d] Based on Mann-Whitney U test.

changes in weeks I (R^2 Linear = 0.068, r = 0.262, P = 0.029) and II (R^2 Linear = 0.065, r = 0.256, P = 0.034).

In terms of side effects in phase I, one woman reported numbness and excessive bone pain and another reported inflammation and itching of the skin in the Vitamin E group, while one woman reported difficulty in breathing

Table 4. Comparison of Plasma NO[a] Changes Between the Two Groups at the End of Phases I and II of the Study and in Overall

	Placebo-Vitamin E					Vitamin E-Placebo					P Value[c] (Between Groups)
	Mean	SD[b]	Median (IQR)	Mean Rank	n	Mean	SD[b]	Median	Mean Rank	n	
NO changes											
Phase I	2.65	5.62	0.90 (8.88)	35.80	35	3.22	5.58	1.10 (8.58)	38.11	38	0.643
Phase II	-0.88	4.62	-1.50 (5.08)	38.83	32	-2.44	5.83	-2.60 (6.45)	32.70	38	0.236
	Placebo					**Vitamin E**					
NO changes[d]	0.004	6.23	-0.50 (30.10)	67.62	73	1.34	5.52	0.25 (6.93)	76.10	70	0.197

[a] Nitric oxide (mg/dL).
[b] Standard deviation.
[c] Based on Mann-Whitney U test.
[d] In overall by gathering Vitamin E and placebo groups in the two phases, separately.

in the placebo group. In phase II, one woman reported inflammation and itching of the skin in the Vitamin E group. Knowing the above symptoms, the capsules were immediately discontinued, and the patients were followed until complete recovery. In phase I, ten women in the placebo group and 23 women in the Vitamin E group, and in phase II, 25 women in the Vitamin E group and 13 women in the placebo group tended to continue using the capsules in the future.

4. Discussion

The findings of this study showed that both Vitamin E supplementation and placebo could be effective in reducing the number and severity of HFs in menopausal women. In this study, changes in HFs in the P-E group showed a positive/upward continuous trend towards reducing the number of HFs, while in the E-P group, this positive continuous trend of variations in week I of the phase II showed a downward fluctuation compared to week IV of the phase I. The latter group received placebo during the first week of phase II. In other words, the mean number of HFs increased in the first week with the onset of placebo after wash-out, and then the upward trend of HFs changes continued again in the E-P group in the following weeks.

In this study, HFs number in both groups in phase I of the intervention had a further decrease compared to phase II. However, no statistically significant difference existed in the trend of reduction of HFs between the two groups in both phases. Hence, in justifying the same effects of Vitamin E and placebo, it seems that the subjective effect of placebo contributed to reducing the number and severity of HFs in menopausal women.

In line with the above results, Barton et al. showed that Vitamin E (800 IU/day) compared to placebo significantly reduced the number of HFs in women with breast cancer using tamoxifen, although the severity of this effect was very slight (20). Ziaei et al. in a cross-over, single-blind experimental study in Iran on 60 healthy menopausal women reported different results from the present findings and those of the study by Barton. These researchers showed that both Vitamin E (400 IU/day) and placebo were effective in reducing the number and severity of HFs in menopausal women, although Vitamin E was significantly more effective (5). The present study was different from the study by Barton in terms of Vitamin E dosage and participants. In addition, Barton et al. and Ziaei et al. determined the baseline number of HFs/day in a particular time before the onset of the study, while in the present study; the number of HFs/day was recorded based on the statements of the women before the intervention.

We found that plasma NO levels in phase I after placebo and Vitamin E administration were significantly lower than before within P-E and E-P groups. These levels increased more in phase II (after wash-out) with placebo administration within E-P group compared to Vitamin E administration within P-E group, but these differences were not significant. No significant difference existed in plasma NO level changes in phases I and II of the intervention between the P-E and E-P groups. This implies that the effects of Vitamin E and placebo on NO were the same. In general, the changes in NO were lower in all the individuals who received placebo compared to those who received Vitamin E. It means that plasma NO levels increased with placebo administration and decreased with Vitamin E administration.

These results indicate that the effect of Vitamin E supplementation on plasma NO level was not consistent with our hypothesis. These results do not support the results of the study by Saxena and Jaiswal. These researchers suggested that the administration of Vitamin E supplement, 200 mg daily for three months, results in an increase in

plasma NO level and a reduction in oxidative markers and lipoperoxidation with age transition in women (19). In contrast, the results of a study in Iran aimed at determining the effect of short-term Vitamin E supplementation on plasma NO response following a resistance training session in men showed that plasma NO level reduced with Vitamin E supplementation (26).

Considering the results of the above studies and the present study, it seems that there is ambiguity regarding the effect of Vitamin E supplementation on plasma NO changes in menopausal women. On the other hand, with regard to the duration of receiving Vitamin E supplement in the study of Saxena and Jaiswal (three months), extending the duration of receiving the supplement may increase plasma NO level. Therefore, further experimental studies are required to achieve more conclusive results.

In the present study, a very low positive correlation was noted between decreasing HFs and decreasing NO level in weeks I and II in the Vitamin E group. It means that based on the results, 6.8% of HFs changes in week I and 6.5% of these changes in the week II could be predicted based on NO changes. It can be stated that the changes of NO in the Vitamin E group in the mentioned weeks were parallel to the HFs changes in menopausal women, which was unexpected. This is to say that Vitamin E was not effective in reducing HFs via increase in plasma NO level in menopausal women. Few studies have yet been conducted on the effects of Vitamin E on HFs due to its impact on NO. In this regard, the studies of McNamara et al. and Kimberly et al. regarding the effect of plasma NO on the reduction of heat stress and HFs in menopausal women reported that plasma NO level can be effective in modifying HFs by increasing skin blood flow (11, 16). Accordingly, we suggest further studies should be conducted to determine the correlation between HFs changes and NO variations through the administration of Vitamin E supplementation.

A limitation of the present study was that the number of daily HFs before the onset of intervention was recorded by the researcher based on a verbal report of the individuals. In other words, it would have been more accurate if the number of HFs had been recorded by individuals before entering the study during a certain time. It seems that this issue should be considered by researchers in future studies. The strength of this study was the low drop-out. The main researcher attempted to recruit women who were more likely to finish the study. She interacted with them constantly and precisely and followed them up. Therefore, sample attrition was 16% in this study, while it was considered 50% while determining the sample size.

4.1. Conclusion

According to the present study, both Vitamin E supplementation and placebo resulted in a reduction in the number and severity of HFs in menopausal women. In this study, Vitamin E supplement had no superiority to placebo. In addition, Vitamin E supplement had a very poor effect on reducing plasma NO changes and had no effect on declining HFs via increasing plasma NO level. It can be stated that the subjective/mental effect of placebo on HFs in menopausal women was strong and was equal to the effect of Vitamin E supplement. Therefore, caregivers, experts, and health-care professionals including midwives and physicians should have a comprehensive approach in practice and plan further counseling sessions for women with HFs in order to improve individuals subjectively and promote their awareness in addition to administering supplements/vitamin E. In this way, they would be able to better manage their HFs and related consequences. Finally, we suggest further comparative studies on the effect of Vitamin E or other supplements on plasma NO level and HFs in menopausal women.

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Footnotes

Authors' Contribution: Parvaneh Rezasoltani, Nahid Elliyoun, Tahereh Ziaie, Ehsan Kazemnezhjad Leyli, Abdolrasoul Sobhani designed the study. Parvaneh Rezasoltani, Nahid Elliyoun, Tahereh Ziaie, Ehsan Kazemnezhjad Leyli directed the implementation and data collection. Nahid Elliyoun collected the data and Soudabeh Kazemi Aski provided support and assisted with data collection. Ehsan Kazemnezhjad Leyli conducted the data analysis. Parvaneh Rezasoltani, Nahid Elliyoun, Ehsan Kazemnezhjad Leyli interpreted the data. Parvaneh Rezasoltani, Nahid Elliyoun drafted the manuscript. Parvaneh Rezasoltani, Abdolrasoul Sobhani revised the manuscript. All the authors approved the final version of the manuscript and agreed on all aspects of the work.

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