

# The omega-3 and Nano-curcumin effects on vascular cell adhesion molecule (VCAM) in episodic migraine patients: a randomized clinical trial

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## Research note

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# Abstract

## Objective

The purpose of this clinical trial was to examine the effect of omega-3 fatty acids (W-3 FAs), nanocurcumin and their combination on serum levels and gene expression of VCAM in patients with episodic migraine.

## Results

In this study, 80 patients were randomly divided in to 4 groups to receive for 2 months. Both serum levels and gene expression of VCAM showed remarkable decreases after single W-3 and after combined W-3 and nanocurcumin interventions. However, a borderline significant change and no remarkable change were observed after single nanocurcumin supplementation and in control group, respectively. While a significant difference between study groups in VCAM concentrations existed, there was no meaningful difference in VCAM gene expression among groups. It appears that the W-3 and combined W-3 and nanocurcumin can relieve VCAM serum level and its gene expression in patients with episodic migraine. Moreover, the combination of W-3 with nanocurcumin might cause more significant declines in VCAM level in the serum of migraine patients than when W-3 is administered alone.

## 1. Introduction

Migraine is a neurovascular disturbance that is often characterized by throbbing, unilateral headaches accompanied by other symptoms such as photophobia, intolerance of light and autonomic features (1). Neurogenic inflammation enhances sensitivity of the as nociceptors (2) which up-regulate the secretion of pro-inflammatory factors which contributes to pain intensification (3, 4). These cytokines trigger the permeability and cell-to-cell interaction in vascular system, suggesting a mechanistic role of them in elevating the expression of endothelium-derived products such as VCAM, ICAM and soluble selections. These molecules increase the migration of microglia and other immune cells across the vessel wall, which amplify headaches severity (5, 6). The pain-alleviating power of most of migraine drugs is due to their anti-inflammatory effect and preventing from expression of endothelium-derived factors. Existing evidence have shown that W-3 FAs and curcumin have the same anti-inflammatory effects as the popular drugs in migraine treatment without any serious side effect (7, 8). There have also been promising findings regarding the efficacy of W-3 FAs and curcumin on ICAM and VCAM reduction (9–12). With regard to chemical instability of curcumin in intestinal environment, its prescription with accompanying nanoparticles (nanocurcumin) is useful for increased bioavailability and solubility (13). Moreover, some scientific documents have reported the elevated anti-inflammatory activity of lower dosages of W-3 FAs and curcumin, when prescribed in combination (14). In this study we aimed to examine the efficacy of supplementation with W-3 FAs, nanocurcumin and their combination on the serum concentrations and mRNA expression of VCAM in patients with episodic migraine disease.

## 2. Method And Materials

### Design and population

Among 285 subjects who attended to Sina hospital neurology clinic in Tehran, 80 men and women were recruited to this double-blind (neither the participants nor the experimenters) randomized controlled trial on July 2015. Participants were adults and had a current diagnosis of episodic migraine in accordance with the IHS criteria ( $\geq 15$  headache days per month for more than 3 months or  $\geq 1$  attack per week) (15). All recruiting criteria are shown in **Table 1**.

### Omega-3 and nanocurcumin supplementation

The stratified randomization method based on sex and BMI was used to assign participants in 4 groups to receive 2 months supplementation of **Group 1**) 2 capsules containing 600 mg EPA + 300 mg DHA + 100 mg other W-3 FAs + 1 placebo capsule of nanocurcumin, **Group 1**) 1 capsule containing 80 mg nanocurcumin + 2 placebo capsule of W-3, 3) 2 capsules containing 600 mg EPA + 300 mg DHA + 100 mg other W-3 FAs + 1 capsule containing 80 mg nanocurcumin (group 3) and 4) 2 placebo capsule of W-3 + 1 placebo capsule of nanocurcumin (group 4 or control group). Placebo capsules were composed of edible paraffin with the same appearance as W-3 and nanocurcumin capsules. Random allocation and enrolling participants to the study were done by trained people who were outside the investigation. Administration of 25–50 mg from three types of cyclic antidepressants (amitriptyline or nortriptyline) and 20–40 mg of  $\beta$ -blockers (eg propranolol) (as a prophylaxis treatment) along with study supplements was given to all groups. In comparison to W-3 FAs and nanocurcumin,  $\beta$ -blockers and cyclic antidepressants have a different mechanism of action in reducing inflammatory state (16). Patients were asked to abstain from altering their dietary habits and physical activity during the intervention. Moreover, we instructed patients to consume NSAIDS in times of severe headache attacks.

### Questionnaires

A predesigned questionnaire was used to consider general characteristics of migraine patients including gender, age, financial perception, migraine duration, educational status, history of other diseases and use of medications and dietary supplements. Severity, numbers and duration of headaches were examined by a trained clinician. Scales for headache severity was within 1–10 in which score 1 and 10 were indicative of minimum and maximum pain of headaches. We also sought dietary information via 3-day dietary recall method to obtain an estimate of energy intake.

### Anthropometric indices

Measured anthropometric indices included: i) weight (through 803, Seca Clara, Germany with 0.1 kg accuracy with light clothing and no shoes); ii) height (by a stadiometer (Seca) with 0.1 cm accuracy); and iii) WC (with a common tape after normal exhalation).

### Statistical analysis

Sample size was computed based on the VCAM-1 variable considering the difference of 1.817 ng / dl between the intervention and control groups via the following formula (If  $\alpha = 0.05$ ,  $1 - \beta = 1.28$  (Power = 90%)) (17)

$$n = \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{d} \right)^2$$

N = 17 was obtained for each study group. By considering 20% missing in study sample size during intervention, 20 subjects were selected for each study group. Data normality was checked through Kolmogorov–Smirnov distribution. For comparison of normal quantitative data means among groups and in each group, ANOVA paired t-tests were conducted, respectively. We used Kruskal-Wallis and Wilcoxon to compare abnormal quantitative variables between groups and in each single group, respectively. We also conducted ANCOVA test to compare study outcomes between groups with adjustment of confounding factors (BMI, energy intake and age). The data were finally analyzed by SPSS version 22, in which P-value  $\leq 0.05$  was considered as significant.

### Measurement of serum concentration and gene expression of VCAM

Laboratory measurements were done at the laboratory of School of Public Health, Tehran University of Medical Sciences. At baseline and after 2-month intervention, two blood samples from subjects were collected including 10 ml in tubes containing heparin for PBMCs separation and 5 ml that was centrifuged quickly (10min) at 3500 RPM for serum separation. Heparin-containing tubes were diluted in a 1:1 ratio in PBS. Afterwards, lymphocytes and monocytes were separated in Ficoll gradient-gel by centrifugation at 800 RPM for 40 min at 4°C. Obtained cells were washed in PBS for two times and then centrifuged at 600 RPM for 10 min at 4°C for the second time. Total RNA from cells were obtained using RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA). For RNA quality and quantity assessment, a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was applied, in which RNAs were considered as pure, if had absorbance ratios 260/ 280 nm between 1.9–2.1. The purified RNA was reverse transcribed to result in the synthesis of DNA using QuantiTect Reverse Transcription Kit (Qiagen, Germany). In the PCR step, appropriate primer for VCAM was designed using Primer Express 3 software (Applied Biosystems). Whole genome sequences of VCAM primer were forward: 5'-TAGCGTGTACCCCTTGACC-3' and reverse: 3'-AACTTAGCCTGACAAACAAGAGC-5'. PCR Analysis were performed through the StepOne system (Applied Biosystems, Foster City, CA, USA) using 7 µL of Enzymes (Taq polymerase, SYBR Green, etc.) (Applied Biosystems, Foster City, CA, USA), 2 µL of cDNA, 0.5 µL of each forward and reverse primers in an ultimate volume of 20 µL (18). Finally, the calculation of VCAM gene expression was conducted by Ct (2-ΔΔCt) equation. Blood serums were stored in -80 ° C until serum VCAM analysis via ELISA method (Bioassay Technology Laboratory, Chain).

## 3. Results

## Basic patient information

From the 80 individuals (20 participants in each group), 6 subjects (1 woman in group 1, 1 woman in group 2 and 1 woman and 2 men in group 3 and 1 woman in group 4) did not continue with the trial because of changing their treatment trends or drugs (Fig. 1). This minor attrition was considered as random and did not enter bias to the analysis. No serious harms or unintended effects were reported from study supplements and compliance rate of the patients was 100 %. A summary of baseline characteristics of participants is demonstrated in **Table 2**. No significant between-group differences in general, biochemical and clinical characteristics was detected ( $P > 0.05$ ).

## Outcome assessment

**Table 3** summarizes the results of this study trials. 2-month supplementation in group 1 and group 3 caused statistically significant reductions of both gene expression and serum levels of VCAM in the participants. Additionally, there were near to significant changes in VCAM serum levels and gene expression after supplementation in group 2. No meaningful change in study outcomes was observed in control group. Moreover, significant difference was presented in VCAM serum concentrations among all groups. Although, VCAM gene expression difference between 4 groups was not significant.

## 4 Discussion

Our study for the first time, evaluates whether W-3 FAs, nanocurcumin and their combination could reduce serum levels and mRNA expression of VCAM in episodic migraine. This research showed that single W-3 and accompanied W-3 and nanocurcumin supplementation for 2 months led to significant decreases in both gene expression and blood levels of VCAM in our participants. Furthermore, our results presented that these outcomes had near to significant declines and no significant change in response to single nanocurcumin supplementation and placebo, respectively. As we expected, the change of both outcomes in group 3 was greater than group 1. The changes of VCAM serum concentrations was statically significant within 4 study groups. However, VCAM gene expression was not statistically significant between the 4 groups during the trial, perhaps due to small sample size. In our previous article in the present population, we indicated that treatment with combined W-3 and nanocurcumin caused meaningful reductions in severity and frequency of migraine headaches (19). We suspect that greater reductions in serum concentrations when accompanied W-3 and nanocurcumin are administered in migraine patients might be one of the underlying mechanisms for the significant alleviation in headaches severity and frequency in comparison to when they are administered alone might. The findings of the current intervention contradict with Poreba et al's study where W-3 supplementation had no effect on serum VCAM in obese population (20). Moreover, a meta-analysis reported no meaningful effect of W-3 supplementation on serum VCAM (21). These contradicted results could be due to the reported disturbance in the W-3 DPA-derived lipid factors synthesis in Poreba's study, which serve as a potent mediator for W-3 anti-inflammatory functions as well as differences in study duration, target population and W-3 dosage. To date, there is no study on the investigation of nanocurcumin or accompanied W-3

and nanocurcumin supplementation on endothelial factors in human. Although, in agreement with present study, Pan et al., reported that curcumin analog in diabetic rats had no effect on VCAM-1 gene expression (22). However, our findings were inconsistent with Liu et al.'s study in mice that showed the significant decreasing effect of curcumin supplementation on VCAM gene expression (23). This controversy could be due to differences between human and animal studies. Evidences of experimental studies have demonstrated that W-3 FAs resulted in suppression of VCAM-1 expression by inhibition of PPAR- $\gamma$  (24), IRF-1 and miR-126 expression (25), sirtuin-1 production (26) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I $\kappa$ B $\alpha$ ) and NF $\kappa$ B phosphorylation (27) which are involved in the formation of vascular adhesion molecules. In addition, these FAs inhibit macrophage activity and concomitantly decrease the levels of VCAM-1 and other markers of endothelial dysfunction which in turn may attenuate severity of migraine headaches (28). The next mechanism has been proposed to explain the protective effect of W-3 FAs against VCAM level elevation might be their up-regulating influence of them on the TREK1 potassium channels as maintainers of the blood-brain barrier integrity which has been to block VCAM and ICAM transition from the vessel wall (29). The assessing of the possible mechanisms of the inhibitory property of nano-curcumin in experimental studies also showed that curcumin may reduce the levels of VCAM by down-regulating the phosphorylation of PI3-kinase / Akt, p38 MAPK and JNK (30), the expression of NF $\kappa$ B (22), the migration and proliferation T-cells (23, 31) as well as induction of anti-oxidative enzymes (32). Supplementation with curcumin can also reduce the expression and transcriptional activity of histone acetyltransferases including AP-1 and p300 which can effectively prevent VCAM production (33, 34).

Our results showed the decline in VCAM serum level was significantly larger after accompanied W-3 and nanocurcumin supplementation than other study groups. Based on the results of our current and previous studies, we suspect that more reduction in VCAM serum level might be one of the mechanisms underlying the greater alleviations in severity and frequency of headaches observed in episodic migraine patients after combined W-3 and nanocurcumin interventions than single W-3 or nanocurcumin supplementations. Further researches with different dosages and duration of supplementation are needed to confirm the evidence of the current investigation.

## 5. Limitations

There were some limitation including small sample size and short duration in the present study. Additionally, the mechanistic explanations in the experimental studies mentioned in this study should be attributed to human with caution due to the different conditions exist in cell cultures and animal models (20).

## Abbreviations

MIDs: mitochondrial disorders, VNs: vasoactive neuropeptides, VCAM: vascular cell adhesion molecule, ICAM: intercellular adhesion molecule, NSAIDs: non-steroidal anti-inflammatory drugs, W-3 FAs: omega-3 fatty acids, NF $\kappa$ B: Nuclear factor- $\kappa$ B, HIS: International Headache Society, PCR: polymerase chain

reaction, BMI: body mass index, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, PBMCs: peripheral blood mononuclear cells, WC: waist circumference, SPSS: Statistical Package for Social Sciences, ANOVA: Analysis of variance, ANCOVA: Analysis of covariance, IRF-1: interferon regulatory factor, PPAR- $\gamma$ : Peroxisome proliferator-activated receptor gamma, miR-126: microRNA 126, I $\kappa$ B $\alpha$ : nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, PI3-kinase: Phosphoinositide 3-kinases, Akt or PKB: protein kinase B, p38 MAPK: p38 MAP Kinase, JNK: c-Jun N-terminal kinases and AP-1: Activator protein 1.

## Declarations

**Funding:** None

### Authors' contributions

**MA, MS, AT:** Conceptualization, investigation, Writing, review and editing, **EK, PS, GS BA:** investigation, writing the original draft, methodology; **MB, AS, NY:** methodology, Software, formal analysis; **MJ, HA, HA:** Validation, supervision, project administration, and funding acquisition.

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### Availability of data and material

"The datasets supporting this article results are included within the manuscript". More information could be available by contact with this email address: Elmira.karimii1994@gmail.com.

### Ethics approval and consent to participate

Present investigation was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (with ID number: IR.TUMS.REC.1394.462) and registered in the ClinicalTrials.gov (ID: NCT02532023). The Participants completed a written consent prior of including into the study and being informed about study protocol.

### Consent for publication

Written consent forms were signed by participants for publication of this study before participation.

### Competing interests

The authors in this study declare no conflict of interest.

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