A Novel Combination of ω-3 Fatty Acids and Nano-Curcumin Modulates Interleukin-6 Gene Expression and High Sensitivity C-reactive Protein Serum Levels in Patients with Migraine: A Randomized Clinical Trial Study

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Abstract: Background: Migraine is a disabling neuroinflammatory condition characterized by increasing the levels of interleukin (IL)-6, a proinflammatory cytokine and C-reactive protein (CRP) which considered as a vascular inflammatory mediator, disrupting the integrity of blood-brain barrier and contributing to neurogenic inflammation, and disease progression. Curcumin and ω-3 fatty acids can exert neuroprotective effects through modulation of IL-6 gene expression and CRP levels. The aim of present study is the evaluation of combined effects of ω-3 fatty acids and nano-curcumin supplementation on IL-6 gene expression and serum level and hs-CRP levels in migraine patients.

Methods: Eighty episodic migraine patients enrolled in the trial and were divided into four groups as 1) combination of ω-3 fatty acids (2500 mg) plus nano-curcumin (80 mg), 2) ω-3 (2500 mg), 3) nano-curcumin (80 mg), and 4) the control (ω-3 and nano-curcumin placebo included oral paraffin oil) over a two-month period. At the beginning and the end of the study, the expression of IL-6 from peripheral blood mononuclear cells and IL-6 and hs-CRP serum levels were measured, using a real-time PCR and ELISA methods, respectively.

Results: The results showed that both of ω-3 and nano-curcumin down-regulated IL-6 mRAN and significantly decreased the serum concentration. hs-CRP serum levels significantly decrease in combination and nano-curcumin within groups (P<0.05). An additive greater reduction of IL-6 and hs-CRP was observed in the combination group suggested a possible synergetic relation.

Conclusion: It seems that ω-3 fatty acids and curcumin supplementation can be considered a new promising target in migraine prevention.

Keywords: Curcumin, ω-3 fatty acids, interleukin-6, High sensitivity C-reactive protein, migraine.

1. INTRODUCTION

Migraine is a chronic, disabling neurovascular disorder characterized by episodic painful attacks leading to a decreased quality of life [1, 2]. The aetiology of migraine is not clearly understood, but it appears to have a gene-environment interaction basis [3, 4]. Recent findings have shown that neuroinflammation plays a key role in migraine neuropathogenesis [5]. Neuroinflammation derived from the glia cells activation included microglia, astrocytes and other proinflammatory mediators. The activated microglia and astrocytes lead to enhanced neuronal activity as a result of release of pro-inflammatory peptides such as IL-1β, tumor necrosis factor (TNF)-α and IL-6 from perivascular neuronal...
In migraine patients, adhesion of molecules, and dilatation of the intracranial and extracranial vessels [6]. Subsequently, recurrent vascular inflammation leads to endothelial injury [7]. Cytokines and their receptors have a wide distribution in the central nervous system and neurons [8]. Proinflammatory cytokines increase the permeability and cell-to-cell interaction, thus playing an important role in inflammation, pain, and migraine progression [9, 10]. As a result, the levels of IL-1β, TNF-α and IL-6 in migraine patients increase, in turn disrupting the blood-brain barrier (BBB), neuroinflammation, and vascular disorders [11]. In addition, a strong correlation between migraine and increased serum CRP levels has been proven [12]. CRP plays a key role in vascular and ischemic disorders and increases in migraine patients [13].

Numerous studies have shown that some compounds or combination of some nutrients have a modulatory effect [14, 15]. In this context, curcumin and ω-3 fatty acids exert neuroprotective and anti-inflammatory effects on disorders with neuroinflammatory and neurodegenerative pathogenesis [16-18]. Moreover, both curcumin and ω-3 fatty acids have been shown to synergistically amplify the effects of NSAIDs and valproate sodium, drugs used in migraine management [19, 20].

Curcumin (diferuloylmethane) is an active polyphenol of turmeric, derived from the rhizome of Curcuma longa [21]. Several reports have shown that curcumin is able to suppress the nuclear factor-kappa B (NF-kB) and its gene products, thus playing a pivotal role in inflammation-mediated proinflammatory molecular synthesis [21]. Curcumin also inhibits several proinflammatory mediators such as CRP, TNF-α, IL-1β, IL-6, IL-8, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [22]. In addition, curcumin relieves neurogenic pain by down-regulating the inflammatory mediator expression [23].

Additionally, a large body of in vivo and in vitro evidence has demonstrated the neuroprotective effects of polyunsaturated fatty acids in the central nervous system (CNS) and neuroinflammatory disorder progression [24]. ω-3 fatty acids block the NFkB signalling pathway, CRP production and proinflammatory cytokine (IL-1β, TNF-α and IL-6) as well as iNOS and COX-2 activity [25]. The consumption of fish oil, which is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), improves endothelial dysfunction [26]. Observations also showed that ω-3 fatty acids reduced inflammatory and neurogenic pain as COX-2 inhibitor drugs [27]. In migraine patients, ω-3 fatty acid supplementation can significantly reduce the frequencies of headache [28].

Recent in vitro and in vivo studies have provided evidence suggestive of the synergistic properties of curcumin and ω-3 fatty acids in neurogenic disorders [29, 30]. In this context, in an attempt to investigate new targets for the management of neuroinflammation in migraine disorder at nutrigenomic levels, the present study evaluated the synergistic effects of ω-3 and nano-curcumin supplementation on IL-6 gene expression and serum level as well as hs-CRP levels in migraine patients.

2. MATERIALS AND METHODS

2.1. Patients and Supplementation

The present study was done as a randomized, double-blind placebo-controlled clinical trial. With 5% significance level, a power of 90% and 20% missing of patients (probability of missing of patients during the study), the sample size was calculated 20 subjects in each group. Eighty eligible patients with an episodic migraine enrolled in this survey (including 64 females and 16 males aged 20-50) in 1:1 ratio. Six patients withdrew from the study because of altered medication (Fig. 1). Sampling was conducted in Iranian centre of Neurology Research located in Imam Khomeini Hospital in Tehran.

At the beginning of the study, written informed consent, approved by the Ethics Committee of the Tehran University of Medical Sciences (TUMS), was obtained from each patient. The participants were informed about the aim and possible benefits or risks of the present study. The patients were free to leave the clinical trial at any time during study. The study was approved by the Ethics Committee of TUMS (ID: IR.TUMS.REC.1394.462) and identified in ClinicalTrials.gov as ID: NCT02532023.

All the patients were considered to have an episodic migraine as per the International Headache Society (IHS) criteria used as the standard by neurologists. All participants received three cyclic antidepressants plus β-blocker. None of the patients had any other diseases (such as diabetes, renal and heart disease, thyroid disorder, cancer, inflammatory disorders) and the exclusion criteria included pregnancy, drug alternation, or adverse reaction to ω-3 or curcumin compounds during the study.

Permuted block randomization was used for the study design and all patients were divided into the four following allocated groups: A) a group receiving ω-3 and nano-curcumin supplementation, B) group receiving ω-3 supplementation and nano-curcumin placebo, C) group receiving nano-curcumin supplementation and ω-3 placebo, and D) group receiving ω-3 and nano-curcumin placebo as control group. The capsules containing ω-3, nano-curcumin or placebo were coded by a third person. Group A received 2500 mg ω-3 fatty acids (two capsules daily, each containing EPA 600 mg and DHA 300 mg) and 80 mg nano-curcumin (one capsule daily). Group B received 2500 mg ω-3 fatty acids and nano-curcumin placebo (paraffin oil). Group C received 80 mg nano-curcumin (one capsule daily) and ω-3 fatty acid placebo (paraffin oil, two capsules daily), and Group D received ω-3 fatty acids placebo (two capsules daily) and nano-curcumin placebo (one capsule daily). Each of the ω-3 or nanocorcinoma placebo were similar in color, shape and size and were synthetized by the related pharmaceutical company. The duration of treatment was two months [31].

2.2. Blood Sample Collection and Peripheral Blood Mononuclear Cell (PBMC) Isolation

About 15-20 cc blood samples collected from patients; 8 cc for PBMC isolation in heparinized sterile falcon, and the rest for serum obtained in tube without any anticoagulant. PBMCs were isolated from heparinized peripheral blood
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According to the standard Ficoll-Hypaque density-gradient centrifugation. Serum collected after 10 minutes of centrifugation at 3000 RPM and transferred into microtubes was stored at -80°C in order to measure IL-6 and hs-CRP levels with an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Mediagnost, Germany).

2.3. RNA Extraction and cDNA Synthesis

RNA was extracted and purified by the RNeasy Plus Mini Kit (Qiagen, Valencia, Calif., USA) based on the manufacturer’s protocol. In order to evaluate the purity and quantity of the extracted RNA, the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA) was used. A ratio of 260:280 between 1.9-2.1 accounted for pure RNA. A single strand of the cDNA from the extracted RNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) and stored at -20°C.

2.4. Real-Time Polymerase Chain Reaction for (PCR) Gene Expression

Real-time polymerase chain reaction (PCR) was performed in the StepOne system (Applied Biosystems, Foster City, Calif., USA) using the SYBR Green detection method. PCR primers for IL-6 and β-actin as housekeeping were designed by Primer Express 3 software (Applied Biosystems) (Table 1). The PCR reactions were carried out in special optical tubes in 48-well reaction plates (MicroAmp Optical, ABI, Foster City, Calif., USA) containing 20 µl reaction mixture (0.5 µl forward primer, 0.5 µl reverse primer, 10 µl SYBR Green PCR Master Mix (Applied Biosystems), 7 µl DEPC-treated water and 2 µl cDNA). The wells were covered with an optical adhesive film (Applied Biosystems). Amplification was done by the standard two-step run protocol: step one, 10 seconds at 95°C; and step two, 40 cycles of 15 second at 95°C plus 1 second at 60°C. After the PCR amplification cycles, the melt curve was used in order to confirm that a single gene product had been amplified. Subsequently, the IL-6 mRNA expression level was normalized to the mRNA expression level of β-actin. The fold changes of the IL-6 gene expression were calculated by the comparative Ct (2^(-∆∆Ct)) method.

2.5. Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software. Data was expressed as the mean±SE. The Kolmogorov-Smirnov distribution test was used for checking the

Fig. (1). Flow diagram of double-blind clinical trial study. A total of 80 patients were screened, consented and enrolled in the trial. Seventy-four patients received the full study intervention and completed the trial at 2-months follow up.
normality of the data. For normal data, a Paired t-test was used for the comparison of IL-6 gene expression and IL-6 and hs-CRP the serum levels before and after within group. Statistical differences among the groups were evaluated using the ANOVA test. Nonparametric tests were used for data lacking normal distribution after log transformation. Two related sample tests (Wilcoxon) and K independent sample tests (Kruskal-Wallis test) were used for comparison between before-and-after variables within groups and between groups respectively. In order to eliminate confounder effects, the ANCOVA test was used. The test level for statistical significance of the differences between the four treatment arms was defined as $P \leq 0.05$ for all tests.

3. RESULTS

3.1. Patient Information

The demographic and clinical characteristics of patients in the four groups studied have been illustrated in Table 2. As shown, there were no statistically significant differences in age, sex, height, weight, BMI, WC and frequency of attacks among patients in the four groups of the study. Also, analysis of dietary intake of total fat, PUFA and MUFA of participant (three 24 hours recall included two typical day of a holiday) showed no significant differences in all of groups (Table 2).

3.2. IL-6 Gene Expression in Freshly Isolated PBMCs

As shown in Fig. 2, the post-hoc analysis of the mRNA expression for IL-6 indicated a significant decrease in the case of the $\omega$-3/ nano-curcumin combined treated group ($P < 0.05$) as compared to control group. These differences are significant between groups (Fig. 2). These results indicate a possible additive effect of $\omega$-3 and nano-curcumin in a decrease of IL-6 gene expression.

3.3. IL-6 and hs-CRP Serum Levels

As for the relative gene expression of IL-6, a significant difference was observed in the serum level of this interleukin among patients in three groups but not in the control group. Patients who took a combination of $\omega$-3 + nano-curcumin, $\omega$-3 alone, and nano-curcumin alone showed a significant reduction in serum levels of IL-6 which was greater in combination group. As shown in Table 3, also the differ-

Table 1. Sequencing and information of primers.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Sequence</th>
<th>Length</th>
<th>Tm</th>
<th>CG%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Forward: 5’- CAGTACCCGCAGAGAGATT-3’</td>
<td>21</td>
<td>58.52</td>
<td>52.38</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’- GCAAGTCTCATTGATGACCAT-3’</td>
<td>22</td>
<td>58.39</td>
<td>45.45</td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward: 5’- TGGCCAGCGACAAATGAAG-3’</td>
<td>20</td>
<td>61.18</td>
<td>55.00</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’- AGTCATAGTGCCTAGAAG-3’</td>
<td>21</td>
<td>59.04</td>
<td>52.38</td>
</tr>
</tbody>
</table>

Table 2. Patient clinical data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Omega 3+ Nano-curcumin Group (n=17)</th>
<th>Omega 3 Group (n=19)</th>
<th>Nano-curcumin Group (n=19)</th>
<th>Control Group (n=19)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.82±1.99</td>
<td>36.15±1.99</td>
<td>37.36±1.95</td>
<td>36.57±1.87</td>
<td>0.95</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>3/14</td>
<td>4/15</td>
<td>4/15</td>
<td>4/15</td>
<td>0.99</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.41±2.72</td>
<td>69.26±2.89</td>
<td>72.63±3.87</td>
<td>75.05±2.43</td>
<td>0.77</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.41±2.16</td>
<td>162.68±2.02</td>
<td>161.47±1.84</td>
<td>162.84±1.45</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.02±0.98</td>
<td>26.16±0.98</td>
<td>27.59±1.05</td>
<td>26.94±0.89</td>
<td>0.65</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>80.52±1.84</td>
<td>80.00±1.65</td>
<td>83.89±2.23</td>
<td>81.94±1.74</td>
<td>0.37</td>
</tr>
<tr>
<td>Attack frequency (number/week)</td>
<td>2.72±0.40</td>
<td>2.77±0.26</td>
<td>2.81±0.31</td>
<td>2.76±0.24</td>
<td>0.99</td>
</tr>
<tr>
<td>Total fat intake (gr)</td>
<td>58.46±6.56</td>
<td>60.77±5.79</td>
<td>52.40±9.45</td>
<td>58.05±6.95</td>
<td>0.23</td>
</tr>
<tr>
<td>MUFA intake (gr)</td>
<td>15.82±1.61</td>
<td>16.58±1.63</td>
<td>13.10±2.14</td>
<td>19.40±2.55</td>
<td>0.09</td>
</tr>
<tr>
<td>PUFA intake (gr)</td>
<td>14.90±1.74</td>
<td>17.83±1.58</td>
<td>15.21±3.07</td>
<td>16.92±2.40</td>
<td>0.26</td>
</tr>
</tbody>
</table>

BMI: Body Mass Index; WC: Waist Circumference. All values are expressed as means ± SE or numbers. *ANOVA. **Kruskal-Wallis test.
ences were significant between groups after adjusting for IL-6 baseline levels (P=0.03).

Fig. (2). The fold change of IL-6 gene expression in PBMC extracted mRNA. A significant decrease was found only in combination group. Kruskal-Wallis test among groups shows significant differences in IL-6 fold Change. * P value<0.05.

In the context of hs-CRP, at the end of the study, a significant within-group reduction of hs-CRP serum levels was observed in combination and nano-curcumin groups (P<0.05). The results showed a greater reduction in combination group compared to ω-3 or nano-curcumin treatment groups. However, there was no statistically significant differences between group (P>0.05).

Table 3. Serum level of IL-6 and hs-CRP.

| Serum levels | Omega 3+ Nano-curcumin Group (n=17) | Omega 3 Group (n=19) | Nano-curcumin Group (n=19) | Control Group (n=19) | P Value *
|---------------|------------------------------------|----------------------|----------------------------|----------------------|----------
| IL-6 serum levels | Before | 48.97±7.61 | 46.02±7.52 | 46.61±7.62 | 44.11±7.13 | 0.92 |
|                | After  | 34.67±5.82 | 33.83±5.58 | 36.76±5.31 | 44.00±6.58 | 0.03 |
|                | Difference | -14.30±4.87 | -12.18±3.72 | -9.84±3.52 | 0.11±4.29 | 0.03 |
|                | P value | 0.01 | 0.006 | 0.02 | 0.86 |
| hs-CRP Serum levels | Before | 4.05±0.70 | 4.16±0.60 | 3.64±0.70 | 3.87±0.69 | 0.86 |
|                | After  | 2.81±0.55 | 3.10±0.48 | 2.53±0.46 | 3.44±0.55 | 0.56 |
|                | Difference | -1.46±0.56 | -1.05±0.62 | -1.17±0.42 | -0.45±0.36 | 0.61 |
|                | P value | 0.01 | 0.08 | 0.01 | 0.29 |

Data are reported as means±SE.

* Two related sample tests (Wilcoxon).

5. DISCUSSION

In the present study, 74 patients with an episodic migraine were enrolled in a randomized clinical trial of a ω-3, nano-curcumin, and the combination of them or placebo supplementation for two months to evaluate the synergistic effects of ω-3 and nano-curcumin on IL-6 gene expression and IL-6 serum levels.

Findings in the past decade have suggested that the neuroinflammatory-immunity pathway plays a key role in the immunopathogenesis, signs of disorder and the progression of the migraine disease [32, 33]. Meningeal afferents activation, neurogenic inflammation and release of neuropeptide (Substance P, neurokinin A, and Calcitonin Gene-Related Peptide) resulted in mast cell activation and proinflammatory mediator secretions such as IL-6 and TNF-α, which play a pivotal role in the generation of pain in migraine headache [11, 34]. Migraine disorder is associated with frequent neurovascular inflammation. In this disorder, a special pattern of circulating inflammatory markers can be seen, including an increase in CRP, proinflammatory cytokines such as IL-1, IL-6 and TNF-α [35]. Observations have shown that in patients with migraine the pro-inflammatory cytokine (IL-1β, IL-6) increases and the levels of anti-inflammatory cytokines (IL-10) decreases [36] reciprocally. Wang et al. found that during and in periods of interval of migraine attacks, the serum levels of IL-6 in patients was significantly higher than the healthy control group [37]. Additionally, in vivo studies have shown an increase in the IL-6 mRNA in migraine models [38]. Moreover, it has recently been reported that the risk of cardiovascular disease and stroke is significantly increased in patients with migraine. In this context, CRP has a

4. SAFETY

During this study, all subjects did not disclose any side effects.
stronger association with various thrombotic and ischemic disorders [13]. CRP levels also increase in various type of migraine even at free attacks intervals [12]. These documents confirm the role of proinflammatory mediators such as IL-6 and CRP in neuroinflammatory pathogenesis of migraine.

The current study showed that combination of ω-3 and nano-curcumin can significantly reduce the expression and serum levels of IL-6, which is proinflammatory and play an important role in migraine pathogenesis.

There are numerous instances of *in vitro* and *in vivo* evidence in neuroprotective and anti-inflammatory effects of ω-3 fatty acids in neurodegenerative and neuroinflammatory disorders [39]. ZZ *et al.*, demonstrated that ω-3 acid metabolites can suppress the gene expression of TNF-α, IL-6 and IL-1 in an experimental model as well as nociceptors such as the transient receptor potential cation channel subfamily V, member 1 (TrpV1), which play an important role in neuroinflammatory pain production [40]. Moreover, in migraine patients, ω-3 fatty acids significantly reduced frequencies, duration, and the severity of headache [20, 41].

In addition, curcumin is able to suppress IL-6 production in astrocytes located in CNS and downregulate proinflammatory mediators mRNA such as Monocyte Chemoattractant Protein-1 (MCP-1) [42]. Based on a study by Zaky *et al.*, a combination of curcumin with valproic acid could synergistically potentiate neuroprotection effects in neuroinflammatory condition induced lipopolysaccharide in rat and improve recovery [43]. Additionally, curcumin can suppress the TNF-α, IL-6 and IL-1β gene expression, contributing to inflammatory and neurogenic pain [44]. Zanjani *et al.* also reported that, in the model of chronic neuropathy pain, curcumin can reduce pain and serum level of COX-2 [45]. These observations indicated the role of ω-3 fatty acids and curcumin in the suppression of neuroinflammation and improvement of neurogenic by blocking the proinflammatory mediators such as IL-6. However, majority of these studies are cellular and animal studies but it seems that in human, further studies are needed to discover appropriate dosage. The results of present study show an additive relation between omega3 and nano-curcumin. Moreover, since the bioavailability of curcumin is too poor to exhibit efficacy in clinical trials even in high doses, the nano-curcumin was used in this study. Nano-curcumin is nanoparticle curcumin that increases that safely increases the absorption of curcumin 27-fold than that of curcumin powder [46, 47].

We observed that combination of ω-3 and nano-curcumin has a significant effect on the reduction of IL-6 gene expression and serum levels (p value<0.05). Our results showed a greater decrease in IL-6 gene expression in fresh PBMCs obtained from patients with migraine who were treated with a combination of ω-3 and nano-curcumin versus groups having ω-3 or nano-curcumin only. These results demonstrated a synergistic relationship between ω-3 and nano-curcumin,

![Image](image_url)

*Fig. (3).* The activated glia cells (microglia and astrocytes) produce proinflammatory factors, chemokines and cytokine such as IL-6, TNF-α *etc.* lead to enhanced neuronal activity, more neuroinflammatory response and progression of disease. The combination of ω-3 and nano-curcumin significantly can down-regulated IL-6 and TNF-α expression and improve headache attacks in migraine [52].
which is parallel with IL-6 serum levels results (14.30±4.87 reduction in combination patients versus 12.18±3.72, 9.84±3.52 and 0.11±4.29 decreases in the ω-3, nano-curcumin and control patients). Several cellular and animal studies have demonstrated the synergistic effects of ω-3 fatty acids and curcumin. Neuroscience research findings confirm these observations. In this context, Mirza et al reported that a combined supplementation therapy of curcumin-DHA in a neurodegenerative model are able to modulate microglia activity and improve retina degeneration [30]. A combination of curcumin and fish oil can also significantly suppress proinflammatory mediators such as NFκB compared to curcumin or fish oil alone [30]. Similarly, Saw et al. showed curcumin plus with DHA or curcumin plus EPA to synergistically decrease proinflammatory gene expression and exert anti-oxidative effects on macrophage cells [29].

In the present study, 2 months supplementation of ω-3 could not significantly reduce hs-CRP levels in patients with migraine. Parallel with our results, Freund-Levi et al found no significant changes in hs-CRP serum levels following ω-3 supplementation in Alzheimer patients [48]. It seems that receiving doses of less than 3 gr ω-3 and supplementation less than 6 months does not affect CRP levels [49]. Contrary to ω-3 results, at the end of the study, a significant decrease of hs-CRP levels was found in group receiving nano-curcumin. The effect of curcumin on hs-CRP levels depends on the bioavailability of curcumin and the duration of the supplementation. In this regard, a meta-analysis by Sahebkar showed that curcumin can significantly reduce CRP levels in trials that used higher absorption curcumin and had supplementation period of ≥4 weeks [50].

We also observed a greater reduction of hs-CRP levels in patients who received a combination of ω-3 and nano-curcumin. However, these differences were not significant between groups. There is currently no study on the effects of ω-3 and curcumin on hs-CRP, but evidence suggests that the synergistic potential of these compounds has recently been considered in human studies [51].

In addition, ω-3 and curcumin can significantly reduce the number of headache attacks in a synergistic manner. In the previous work, we found that combination of these compound reduced the number of attacks, gene expression and serum levels of TNF-α more than each other alone [52].

A significant point is that how much ω-3 fatty acids and nano-curcumin blood levels can exert effective clinical impacts in migraine. However, in this study, the serum levels of ω-3 or nano-curcumin were not evaluated which was one of the limitations of the current study. It suggested these measurements to be considered in future studies to research the effective dose of ω-3 or nano-curcumin. Generally, considering the reported results regarding the effect of ω-3 and curcumin on the down-regulation of proinflammatory molecules in neurogenic models and the results of this study, it can be concluded that ω-3 and curcumin may have an additive effect on suppressing of IL-6 and hs-CRP and could exert an anti-inflammation influence in case of migraine disorder. Therefore, these nutritional factors, whiteout potential side effects, can be used as an adjuvant therapy to slow down the progress and enhance the management of the disease.

CONCLUSION
To our knowledge, the present study is the first clinical trial of the synergistic effects of ω-3 and nano-curcumin supplementation on IL-6 gene expression and serum levels of IL-6 and hs-CRP in migraine patients. In this study, we postulated that a combination of ω-3 fatty acids and nano-curcumin have synergistic effects and induce an anti-inflammatory response via the down-regulation of inflammatory factors more than ω-3 or no-curcumin acting by themselves. Therefore, these supplementations can be targeted to decrease disease progression and, thus, headache frequency in migraine patients (Fig. 3).

LIST OF ABBREVIATIONS
- COX = Cyclooxygenase
- CRP = C-reactive Protein
- DHA = Docosahexaenoic Acid
- EPA = Eicosapentaenoic Acid
- hs-CRP = High Sensitivity C-reactive Protein
- IL = Interleukin
- iNOS = Inducible Nitric Oxide Synthase
- NF-κB = Nuclear Factor-kappa B
- TNF = Tumor Necrosis Factor

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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The present study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) as ID: IR.TUMS.REC.1394.462 and identified in ClinicalTrials.gov as ID: NCT02532023. Written informed consent was obtained from all patients before participating in the study. Those who participated in this study are kindly acknowledged.

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