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ORIGINAL ARTICLE

# The synergistic effects of ω-3 fatty acids and nano-curcumin supplementation on tumor necrosis factor (TNF)-α gene expression and serum level in migraine patients

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**Abstract** Migraine is a destabilizing neuroinflammatory disorder characterized by recurrent headache attacks. Evidences show tumor necrosis factor (TNF)-α play a role in neuroimmunity pathogenesis of migraine. TNF-α increase prostanoid production, hyperexcitability of neurons, and nociceptor activation resulted in neuroinflammation and neurogenic pain. ω-3 fatty acids and curcumin exert neuroprotective and anti-inflammatory effects via several mechanisms including suppression of TNF-α gene expression and its serum levels. The aim of this study is an evaluation of synergistic effects of ω-3 fatty acids and nano-curcumin on TNF-α gene expression and serum levels in migraine patients. The present study performed as a clinical trial over a 2 month period included 74 episodic migraine patients in 4 groups and received ω-3 fatty acids, nano-curcumin, and combination of them or placebo. At the start and the end of the study, the gene expression of TNF-α and TNF-α serum levels was measured by real-time PCR and ELISA method, respectively. Our results showed that the combination of ω-3 fatty acids and nano-curcumin downregulated TNF-α messenger RNA (mRNA) significantly in a synergistic manner ($P < 0.05$). As relative to gene expression, a significant greater reduction in serum levels of TNF-α were observed in the combination group, but no significant differences in other groups. Supplementation with ω-3 fatty acids or nano-curcumin alone did not show significant reduction either in mRNA or serum levels of TNF-α. In addition, a much greater reduction in attack frequency was found in the combination group

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($P < 0.001$). These findings indicated that ω-3 fatty acids and curcumin supplementation can be considered as a new promising approach in migraine management.

**Keywords** Migraine · Curcumin · ω-3 fatty acids · TNF-α · Gene expression

## Introduction

Migraine is a chronic neurovascular disorder characterized by recurrent headache attacks accompanied by nausea, vomiting, phonophobia, and photophobia (Haut et al. 2006). Migraine is ranked as one of the top 20 most debilitating disorders affecting approximately 11% of the population in the worldwide (Stovner et al. 2007; Yu et al. 2012). The etiology of migraine is not clear, but it seems that genetic and environmental factors play important roles (Mulder et al. 2003). It is now indicating that neurogenic inflammation and changes in neuroimmune balance can contribute to migraine pathogenesis (Malhotra 2016). In neuroinflammatory condition, the increased neuronal activity leads to the release of inflammatory mediators from perivascular neuronal terminals such as tumor necrosis factor (TNF)-α (Fidan et al. 2006). TNF-α is an important proinflammatory cytokine that plays a pivotal role in the pathophysiology of migraine through several mechanisms; TNF-α can promote calcitonin gene-related peptide (CGRP) transcription and stimulate hyperalgesia via enhancing prostanoid production, increasing sensitization and activation of meningeal nociceptors or neuronal hyperexcitability, and contributing to persistent headache (Liu et al. 2014; Longoni and Ferrarese 2006). The observations have been found that certain TNF gene polymorphisms increase genetic susceptibility to migraine disease (Chen et al. 2015). Additionally, studies have demonstrated changes in plasma, serum, or urine levels of TNF-α in migraine patients during attacks and attack free intervals (Covelli et al. 1991a; Mueller et al. 2001; Perini et al. 2005). Also, increased serum levels of TNF-α decreased to a normal concentration after treatment (Covelli et al. 1991b).

It is well known that some nutrients have neuroprotective, anti-inflammatory, and suppressive effects on TNF-α gene expression and secretion (Bazan et al. 2011; Shehzad et al. 2013). A large body of studies have been reported that ω-3 fatty acids and curcumin (diferuloylmethane), a famous flavonoid separated from the ground rhizome of the Curcumalonga, are able to inhibit nuclear factor-kappa B (NF-kB) signaling pathway and its target genes which play a pivotal role in inflammatory response and mediated proinflammatory molecule production including TNF-α, IL-1β, and IL-6 as well as iNOS and COX-2 (Bauer et al. 2014; Goel et al. 2008; Shehzad and Lee 2010). It has been found that ω-3 fatty acid supplementation significantly decreases headache attacks in migraine patients (Pradalier et al. 2001). Both of ω-3 fatty acids and curcumin also can reduce neurogenic pain trough downregulation of proinflammatory mediators (TNF-α and COX-2) (Ji et al. 2011; Shishodia 2013). These compounds also can synergistically enhance the effects of some drugs which are common in migraine treatment (non-steroidal anti-inflammatory drugs (NSAIDs), Valproate Sodium) (Mittal et al. 2009; Tajmirriahi et al. 2012).

Additionally, recent in vitro and in vivo studies in neuroinflammatory conditions have suggested a synergistic effect between ω-3 fatty acids and curcumin (Mirza et al. 2013; Saw et al. 2010). In this context, we hypothesized that ω-3 fatty acids and curcumin may potentially reinforce each other's effects in suppression of neuroinflammation response. Thus, the present study was designed to evaluate synergistic effects of ω-3 fatty acids and nano-curcumin on TNF-α inhibition of gene expression and serum levels in migraine patients.

## Method and materials

### Study design and patients

The present study was conducted as a randomized double blind placebo controlled clinical trial. Eighty patients with episodic migraine (80% female and 20% male, aged 20–50) enrolled in this study. The patients had no any other disease (renal, liver, and cardiovascular disease, cancer, diabetes, or inflammatory disorders based on patient's reports). Six participated patients (five female and one male) were excluded from the study because of changes in their drugs. Exclusion criteria included pregnancy, allergic or adverse reaction to ω-3 fatty acids or nano-curcumin supplementation, and changes in treatment trend or drugs.

At the beginning of the study, participants were informed about the aim and possible benefit or risk of the study. A written informed consent approved by the Ethics Committee of the Tehran University of Medical Sciences (TUMS) was obtained from all participants as well as medical history (included attack frequency in the last month) based on patients report and they were free to withdraw the trial at any time of study. Also, the patients were divided into four groups based on stratified randomization method for sex, gender, and body mass index.

The present RCT was approved by the ethics committee of TUMS as ID IR.TUMS.REC.1394.462 and registered in ClinicalTrials.gov as ID: NCT02532023.

The International Headache Society (IHS) was used as a standard criteria for diagnosis of migraine type (Ward 2012) characterized by neurologists. All patients had episodic migraine characterized by fewer than 15 days per month with >three migraine attacks per month (≥one attack per week) and received three cyclic antidepressants (amitriptyline or

nortriptyline which is a major metabolite of amitriptyline with the same mechanisms) with β-blocker (propranolol). The mechanisms of action of these drugs are different from anti-inflammatory properties of omega-3 and nano-curcumin and act trough inhibition of uptake of neurotransmitters (serotonin or norepinephrine) and beta-adrenergic blocking, respectively (Garza and Swanson 2006). It is necessary to mention that according to defined protocol by physicians, all of patients who have >three attack per month received 20–40 mg propranolol and 25–50 mg amitriptyline or nortriptyline as a prophylaxis treatment based on body weight of patents and if any of patients change their drug or dose, they will be excluded from the study.

### Supplementation

As mentioned above, the patients were divided into four groups. The groups of study included (I) the group treated with ω-3 fatty acids and nano-curcumin supplementation ($n = 17$), (II) the group treated with ω-3 fatty acid supplementation and nano-curcumin placebo ($n = 19$), (III) the group treated with nano-curcumin supplementation and ω-3 fatty acid placebo ($n = 19$), and (IV) the control group that treated with ω-3 fatty acid placebo and nano-curcumin placebo ($n = 19$).

The participants in group I received 2500 mg ω-3 fatty acids (two capsules, each one 1250 mg/day fish oil) and 80 mg nano-curcumin (one capsule/day), the group II patients were supplemented with 2500 mg ω-3 fatty acids (two capsules, each one 1250 mg/day fish oil) and nano-curcumin placebo (paraffin oil, one capsule/day), the patients in group III received 80 mg nano-curcumin (one capsule/day) and ω-3 fatty acid placebo (paraffin oil, 2 capsules/day), and the patients in the control group (group IV) were supplemented with ω-3 fatty acid placebo (paraffin oil, 2 capsules/day) and nano-curcumin placebo (paraffin oil, 1capsules/day). Each capsule of ω-3 fatty acids contains 600 mg EPA and 300 mg DHA. The ω-3 fatty acids, nano-curcumin, and placebo capsules were coded by a third person. The ω-3 fatty acids and nano-curcumin capsules were the same in their placebo in shape, size, and color. The duration of intervention was 2 months. None of the patients in four groups of the study reported any side effects including allergic reaction, diarrhea, nausea, and vomiting to ω-3 fatty acids and nano-curcumin compound.

### Peripheral blood mononuclear cell separation and serum collection

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by the standard Ficoll-Hypaque (Hamburg, Germany) method. The serum of patients was collected and transferred to microtubes after 10 min centrifugation (3000 RPM) and were stored at −80 °C to further measurements of TNF-α concentration via ELISA method (Mediagnost, Germany).

### RNA extraction and cDNA synthesis

The cytoplasmic RNAs were extracted and purified using RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA) based on kit protocol. Then, the quantity and purity of RNAs were assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and a ratio of 260/280 nm between 1.9–2.1 considered as pure extracted RNAs. QuantiTect Reverse Transcription Kit (Qiagen, Germany) was used in order to single strand complementary DNA (cDNA) synthesis and afterwards stored at −20 for the determination of gene expression in the next step.

### Real-time polymerase chain reaction

The primers of TNF-α and β-actin genes as housekeeping were designed by Primer Express 3 software (Applied Biosystems). The information and sequencing of these primers are illustrated in Table 1. Real-time polymerase chain reaction (PCR) for gene expression was performed by the StepOne system (Applied Biosystems, Foster City, CA, USA) and SYBR Green detection method as described previously (Mottaghi et al. 2013). The fold change of TNF-α gene expression was calculated by Ct ($2^{-\Delta\Delta Ct}$) equation.

### Statistical analysis

The statistical analysis of data was performed using SPSS 22.0 for windows. The Kolmogorov–Smirnov distribution test was used in order to assess data normality. For normal data, Paired *t* test and one-way ANOVA were used for comparison of variables within and between groups, respectively. Moreover, two related sample *t* tests (Wilcoxon) and Kruskal–Wallis tests that were used for data did not have a normal distribution after the log transformation.

**Table 1** Sequencing and primer information

| Gene name | Sequence | Length | Tm | CG% |
|---|---|---|---|---|
| TNF-α | Forward: 5′-TCCTTCAGACACCCTCAACC-3′ | 20 | 58.94 | 55.00 |
| | Reverse: 5′- GGTTGCCAGCACTTCACTG-3′ | 19 | 59.34 | 57.89 |
| β-actin | Forward: 5′- TGGCACCCAGCACAATGAAG-3′ | 20 | 61.18 | 55.00 |
| | Reverse: 5′- AGTCATAGTCCGCCTAGAAGC-3′ | 21 | 59.04 | 52.38 |

Additionally, ANCOVA test was used to control confounding factors. Data are expressed as the mean ± SE. The test level for statistical significance of differences in groups of study was defined as $P \leq 0.05$.

## Results

### Basic patient information

The clinical properties of migraine patients in all of groups of study have been illustrated in Table 2. As it has been shown, there were no statistically significant differences in age, sex, body mass index, height, weight, and waist circumstance in all of the patients in the four groups of study.

### TNF-α gene expression in freshly isolated PBMCs

The results of the present study show that the combination of ω-3 fatty acids and nano-curcumin significantly can reduce the expression of TNF-α messenger RNA (mRNA) but not in the other groups including ω-3 fatty acids alone, nano-curcumin alone or control group after 2 months supplementation. As shown (Table 3), ω-3 fatty acid + nano-curcumin-supplemented patients showed 1.82 ± 0.50 ($P$ = 0.002) decreases in TNF-α mRNA versus 0.62 ± 0.37 ($P$ = 0.11) and 0.51 ± 0.58 ($P$ = 0.39) decline in patients in ω-3 fatty acid and nano-curcumin groups respectively that were not significant statistically, which demonstrated a synergistic effect between ω-3 fatty acids and nano-curcumin. The control group who received ω-3 fatty acids and nano-curcumin placebo showed no significant changes in TNF-α mRNA (0.04 ± 0.37, $P$ = 0.90). The levels of TNF-α expression were much lower in the patients that received combination of ω-3 fatty acids and nano-curcumin (0.47 ± 0.08 fold change) compared to patients receiving ω-3 fatty acids or nano-curcumin only or placebo group (1.61 ± 0.46, 1.32 ± 0.36 and 1.91 ± 0.85 respectively). However, the Kruskal–Wallis test showed no statistically significant differences among groups ($P$ = 0.06, Fig. 1). Additionally, the results of TNF-α gene expression also show a statistically significant difference between the combination group and the ω-3 fatty acid-treated group ($P$ = 0.03) but not between the combination group and nano-curcumin ($P$ = 0.08). It seems that in gene expression level, ω-3 fatty acids and nano-curcumin have an additive effect and further studies are needed to confirm synergistic effects.

### TNF-α serum levels

The results of serum level of TNF-α are shown in Table 4. As to the relative gene expression of TNF-α, a significant difference was observed in the serum level of TNF-α in patients who took combination of ω-3 fatty acids and nano-curcumin ($P$ = <0.05) but not in other groups of study. In ω-3 fatty acid + nano-curcumin-supplemented group, patients showed much more reduction (47.56 ± 7.82 ng/dl, $P$ = 0.001) in TNF-α serum concentration but patients who received ω-3 fatty acids alone, nano-curcumin alone, or placebo showed a small reduction which were not statistically significant ($P$ = > 0.05). Also, a statistically significant difference in TNF-α serum levels was observed among groups at the end of the study ($P$ = <0.001). Moreover, ANCOVA test shows significant differences between the omega-3 + nano-curcumin group with ω-3 fatty acid group ($P$ < 0.001) as well as the ω-3 fatty acid + nano-curcumin group with the nano-curcumin group ($P$ < 0.001). These findings may indicate a synergistic relation of omega-3 and nano-curcumin in migraine disorder.

### Evaluation of attack frequency

As shown in Table 5, the frequency of headache attack decreased significantly in the combined ω-3 fatty acid + nano-

**Table 2** Characteristics of patients in groups of study

| Characteristics | | ω-3 fatty acid + nano-curcumin group ($n$ = 17) | ω-3 fatty acid group ($n$ = 19) | Nano-curcumin group ($n$ = 19) | Control group ($n$ = 19) | $P$ value[a] |
|---|---|---|---|---|---|---|
| Age (years) | | 35.82 ± 1.99 | 36.15 ± 1.99 | 37.36 ± 1.95 | 36.57 ± 1.87 | 0.95 |
| Sex | Male | 3 | 4 | 4 | 4 | 0.99[b] |
| | Female | 14 | 15 | 15 | 15 | |
| Weight | | 68.41 ± 2.72 | 69.26 ± 2.89 | 72.63 ± 3.87 | 75.05 ± 2.43 | 0.77 |
| Height | | 162.41 ± 2.16 | 162.68 ± 2.02 | 161.47 ± 1.84 | 162.84 ± 1.45 | 0.95 |
| BMI | | 26.02 ± 0.98 | 26.16 ± 0.98 | 27.59 ± 1.05 | 26.94 ± 0.89 | 0.65 |
| WC | | 80.52 ± 1.84 | 80.00 ± 1.65 | 83.89 ± 2.23 | 81.94 ± 1.74 | 0.37 |

All values are expressed as means ± SE or numbers

*BMI* body mass index, *WC* waist circumference

[a] ANOVA

[b] Kruskal–Wallis test

**Table 3** ΔCT and mean of TNF-α gene expression in freshly obtained PBMCs

| | | ω-3 fatty acid + nano-curcumin group ($n$ = 17) | ω-3 fatty acid group ($n$ = 19) | Nano-curcumin group ($n$ = 19) | Control group ($n$ = 19) | $P$ value[b] |
|---|---|---|---|---|---|---|
| TNF-α gene expression in fresh PBMCs | Before | 11.97 ± 0.88 | 11.61 ± 0.82 | 11.92 ± 0.63 | 12.04 ± 0.61 | 0.97 |
| | After | 13.79 ± 0.86 | 12.23 ± 0.70 | 12.43 ± 0.71 | 12.08 ± 0.70 | 0.03[c] |
| | Difference | 1.82 ± 0.50 | 0.62 ± 0.37 | 0.51 ± 0.58 | 0.04 ± 0.37 | 0.06 |
| | $P$ value[a] | 0.002 | 0.11 | 0.39 | 0.90 | |
| Fold change of TNF-α gene expression | | 0.47 ± 0.08 | 1.61 ± 0.46 | 1.32 ± 0.36 | 1.91 ± 0.85 | 0.06[d] |

Data are reported as means ± SE. ΔCT = Ct of target gene – Ct of β-actin

[a] Paired sample $t$ test

[b] One-way ANOVA

[c] ANCOVA

[d] Kruskal–Wallis test

curcumin group, the ω-3 fatty acid group, and the nano-curcumin group but not in the control group. At the end of the study, the patients treated with ω-3 fatty acids + nano-curcumin showed a 2.09 ± 0.34/week reduction in the number of attacks ($P < 0.001$) which was approximately twofold more reduction compared to ω-3 fatty acid or nano-curcumin-supplemented groups (1.10 ± 0.33 and 0.98 ± 0.33 reduction, respectively) indicated a synergistic effects of these compounds. These differences were statistically significant among groups ($P = 0.01$). Also ANCOVA test showed a significant difference between the ω-3 fatty acid + nano-curcumin group and the ω-3 fatty acid group ($P = 0.01$) as well as among the omega-3+ nano-curcumin group and nano-curcumin group ($P < 0.001$). These results can suggest a synergistic relation between omega-3 and nano-curcumin in management of migraine attacks.

## Discussion

In the current study, 74 patients with episodic migraine enrolled in a 2-month ω-3 fatty acids, nano-curcumin, the combination of them and placebo supplementation clinical trial to determine the synergistic effect of ω-3 fatty acids and nano-curcumin supplementation on gene expression and serum levels of TNF-α.

During recent decades, evidences have suggested that neurogenic inflammation plays a key role in the immunopathogenesis, manifestation, and progression of migraine disorder (Gerring et al. 2017) and contributes to the sensitization and activation of perivascular meningeal afferents during attacks (Levy 2010). In neuroinflammation status, the activated glia (microglia and astrocytes) produces proinflammatory cytokines and other mediators which disrupted the integrity of blood brain barrier (BBB). The TNF-α and IL-1β released from glia regulate development and neuronal circuits function, including nociceptive pathways (Deverman and Patterson 2009). TNF-α, a potential pain mediators in neurovascular inflammatory condition, is involved in the initiation and progression of a migraine attack (Pietrobon and Moskowitz 2013). In addition, TNF-α lead to CNS sensitization trough reducing inhibitory currents, increasing excitatory and the induction of COX-2 that contributes to inflammatory hyperalgesia development (Cunha et al. 1992). Franceschin et al. found that the mRNA expression of TNF-α increased following migraine the induction in animal models (Franceschini et al. 2013). Also, the elevated TNF-α serum levels in human and children, even in outside of attacks, confirm the pathogenic role of TNF-α in migraine (Boćkowski et al. 2010; Tanure et al. 2010).

The results of the present study showed that the combination of ω-3 fatty acids and nano-curcumin is able to significantly reduce TNF-α gene expression and TNF-α serum

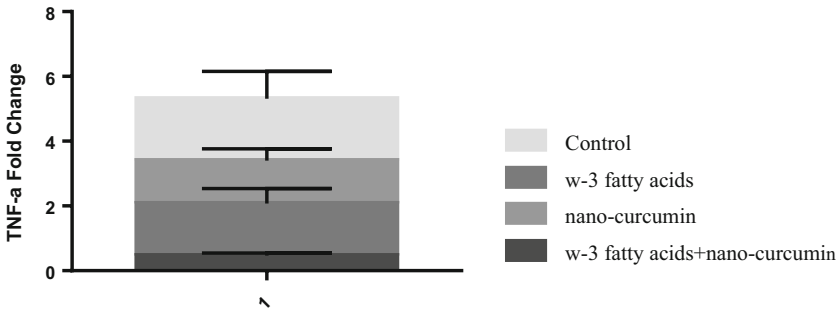


**Fig. 1** The fold change of TNF-α gene expression in PBMC extracted mRNA. A much greater reduction has been observed in the combination group. However, based on the Kruskal–Wallis test, these differences in TNF-α fold change among groups are not statistically significant

**Table 4** Serum level of TNF-α

| | | ω-3 fatty acid + nano-curcumin group ($n$ = 17) | ω-3 fatty acid group ($n$ = 19) | Nano-curcumin group ($n$ = 19) | Control group ($n$ = 19) | $P$ value[b] |
|---|---|---|---|---|---|---|
| TNF-α serum levels | Before | 99.65 ± 11.21 | 101.03 ± 7.19 | 103.90 ± 13.29 | 99.22 ± 8.48 | 0.80 |
| | After | 52.08 ± 7.06 | 86.90 ± 5.11 | 92.56 ± 8.70 | 89.99 ± 9.35 | <0.001[c] |
| | Difference | −47.56 ± 7.82 | −14.13 ± 8.33 | −11.33 ± 8.75 | −9.22 ± 8.15 | 0.05 |
| | $P$ value[a] | 0.001 | 0.14 | 0.19 | 0.21 | |

Data are reported as means ± SE

[a] Two related sample tests (Wilcoxon)

[b] Kruskal–Wallis test

[c] ANCOVA

levels ($P < 0.05$) while our results showed no statistically significant decreases in TNF-α gene expression in fresh PBMCs obtained from participants who were treated with ω-3 fatty acids alone, nano-curcumin alone, or control group. These findings indicated a potent synergistic relation between ω-3 fatty acids and nano-curcumin which is parallel with TNF-α serum level results (much more significant reduction in the combination group ($P = 0.001$) versus a no significant small reduction in other groups).

Recently, in cellular and animal studies, the synergistic effects of poly unsaturated fatty acids and curcumin have been noted, but there are a few studies in this area. Saw et al. demonstrated that the combination of curcumin with both of EPA and DHA (long poly unsaturated fatty acids) synergistically decreased COX-2 and iNOS gene expression as well as nitric oxide (NO) production in macrophage cells and increased antioxidant effects of heme oxygenase-1 (Saw et al. 2010). Similar to these reports, Jia et al. showed curcumin plus fish oil, which is rich in ω-3 poly unsaturated fatty acids, significantly inhibited gene expression of inflammatory mediator, including NF-kB much more than fish oil or curcumin in rats (Jia et al. 2011). In another in vivo study in a neurodegenerative model, curcumin-DHA combined therapy could regulate microglia activity and improve retina degeneration (Mirza et al. 2013). These results are parallel with our results but the present study was the first clinical trial, which examines the synergistic effects of ω-3 fatty acids and nano-curcumin. However, the synergistic effects of these compounds have been evaluated in human studies as the effects of curcumin and long-chain omega-3 polyunsaturated fatty acids in diabetes type 2 prevention in processing (Thota et al. 2016).

It is necessary to mention that bioavailability of curcumin is very low and very high doses are needed to exhibit beneficial clinical impacts. In this regard, in our study, we used nano-curcumin instead of curcumin, which is nanoparticle of curcumin and safely increases curcumin absorption about 27-fold (Kanai et al. 2012; Sasaki et al. 2011).

As mentioned previously, in this study, supplementation with ω-3 fatty acids or nano-curcumin alone did not show significant reduction in either mRNA of TNF-α or its serum levels. A large body of in vitro and animal studies have indicated that both of ω-3 fatty acids and curcumin can suppress the gene expression of proinflammatory cytokines such as TNF-α in experimental models and declined the TNF-α immunoreactivity (de Alcântara et al. 2017; Xu et al. 2010) but it seems that in human trials, higher doses of these nutrients are needed to observe inhibitory effects on TNF-α levels. In this context, Ramirez-Ramirez et al. showed that supplementation therapy with 4 g/day of fish oil for 12 months significantly reduced TNF-α serum concentration in multiple sclerosis

**Table 5** Evaluation of attack frequency

| | | ω-3 fatty acid + nano-curcumin group ($n$ = 17) | ω-3 fatty acid group ($n$ = 19) | nano-curcumin group ($n$ = 19) | Control group ($n$ = 19) | $P$ value[c] |
|---|---|---|---|---|---|---|
| Headache attacks[a] | Before | 2.72 ± 0.40 | 2.77 ± 0.26 | 2.81 ± 0.31 | 2.76 ± 0.24 | 0.99 |
| | After | 0.62 ± 0.08 | 1.67 ± 0.42 | 1.82 ± 0.27 | 2.22 ± 0.38 | 0.009 |
| | Difference | −2.09 ± 0.34 | −1.10 ± 0.33 | −0.98 ± 0.33 | −53 ± 0.32 | 0.01 |
| | $P$ value[b] | <0.001 | 0.004 | 0.009 | 0.11 | |

Data are reported as means ± SE

[a] Number of headache attacks per week

[b] Paired sample $t$ test

[c] One-way ANOVA

(Ramirez-Ramirez et al. 2013) which is twofold more than omega-3 supplementation in our study. Also 1 g/day curcumin has no significant effects on TNF-α serum levels (Ganjali et al. 2014) which is the same to our results. However, these findings show the synergistic relation of ω-3 fatty acids and curcumin, i.e., lower doses of them lead to larger effect in suppression of TNF-α expression.

Additionally, our results show that ω-3 fatty acids and nano-curcumin are able to reduce attack frequency in migraine patients. In this context, Harel et al. and Pradalie et al. reported that omega-3 fatty acid supplementation in migraine patients can decrease headache frequency (Harel et al. 2002; Pradalier et al. 2001). Based on observation, omega-3 fatty acid and curcumin can reduce neurogenic pain (Ji et al. 2011; Shishodia 2013). Moreover, we found much greater reduction in attack frequency in the combination group ($P < 0.001$).

Also, based on the results of present study, comparison of ω-3 fatty acids + nano-curmin with ω-3 fatty acids alone- or nano-curcumin alone-treated groups showed a significant difference between frequency of migraine attacks and serum levels of TNF-α ($P < 0.05$). In context of TNF-α gene expression, a significant difference was observed between the combination group and the ω-3 fatty acid-supplemented group but not between the combination group and nano-curcumin supplementation. These findings suggested a potent synergistic relation between ω-3 fatty acids and nano-curcumin in management of migraine symptom. However, this study is the first one in this regard and more clinical trials are needed to determine an effective dose of active nutrients and combination theraphy in nutrigenomic level.

Thus, regarding the evidences and the results of the current study, it can conclude that combination of ω-3 fatty acids and curcumin may have a potential inhibitory effect on TNF-α gene expression, serum levels, and pain attacks in migraine disease and can be considered as an efficient combined therapy in migraine management.

## Conclusion

To our knowledge, this is the first clinical trial of the effects of ω-3 fatty acid and nano-curcumin supplementation on TNF-α gene expression and serum level in migraine patients. Many studies have shown that both ω-3 fatty acids and curcumin have anti-inflammatory, neuroprotective, and suppressive effects on TNF-α expression and levels. Several studies have pointed to the fact that ω-3 fatty acids and curcumin synergistically can reinforce each other's impact. We demonstrated that the combination of ω-3 fatty acids and nano-curcumin synergistically is able to induce an anti-neuroinflammatory response through the downregulation of TNF-α gene expression and gene production, i.e., TNF-α serum concentration as well as headache frequency more than each of ω-3 fatty acids or no curcumin alone. These findings show that combination therapy of effective nutrients may be targeted as new approaches in migraine prevention and treatment. However, further studies are needed to understand the mechanisms of these effects.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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