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Clinical Trial Study

The Effects of Nano-curcumin Supplementation on Leptin and Adiponectin in Migraine Patients: A Double-blind Clinical Trial Study from Gene Expression to Clinical Symptoms

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Abstract: ***Background*:** Migraine is a disabling neurogenic disorder characterized by recurrent headache attacks. Adipokines act as inflammatory and pain mediators that contribute to migraine pathogenesis. Leptin and adiponectin levels change in migraine patients and are associated with headache attacks. Curcumin can exert modulatory and analgesic effects on adipokines through several mechanisms, from gene expression to suppressing pain. The aim of the present study was to evaluate the effects of nano-curcumin supplementation on leptin and adiponectin gene expression, their serum levels and migraine symptoms in patients with migraine.

***Methods*:** Forty-four episodic migraine patients enrolled in this trial were divided into two groups as nano-curcumin (80 mg/day) and placebo group, over a two-month period. At the beginning and the end of the study, the mRNA expression of leptin and adiponectin from isolated PBMCs and their serum levels were measured using real-time PCR and ELISA method, respectively. The headache frequencies, severity and duration of pain were also recorded.

***Results*:** The results of the present research showed that nano-curcumin can up-regulate adiponectin mRNA and increase its serum level significantly ($P < 0.05$). In the case of leptin, a reduction in gene expression and concentration was found in the nano-curcumin group but it was not statistically significant ($P > 0.05$). Nano-curcumin also significantly reduced the frequency, severity and duration of headaches ($P < 0.05$).

***Conclusion*:** These findings indicate that nano-curcumin supplement can be considered as a promising approach to migraine management and clinical symptoms improvement.

***Clinical Trial Registration*:** IRCT20160626028637N2.

Keywords: Migraine, leptin, adiponectin, headache, curcumin, gene expression.

1. INTRODUCTION

Migraine is a disabling neurogenic disorder characterized by recurrent headache episodes often associated with photophobia, phonophobia, nausea, or vomiting [1]. The prevalence of migraine has been reported to be 2.6% to 21.7% and proximally 12% in the general population [2]. Migraine has a genetic component and is mainly derived from genetic interference with environmental factors [3]. Based on previous evidence, obesity plays an important role in migraine pathogenesis, leading to an increase in headache attacks [4]. The adipokines secreted from adipose tissue act as inflammatory mediators of migraine [5]. Clinical studies show that pain intensity and response to treatment in episodic migraine are associated with changes in adipokines concentration, such as adiponectin and leptin. In addition to adipose tissue, leptin and adiponectin receptors are expressed in the cerebral cortex, brain endothelium, bone marrow, and hypothalamus, and are involved in the regulation of nutrition and various headache disorders, including migraines [6, 7]. Leptin levels are significantly higher in patients with migraines [8]. Leptin has a known role in the process of pain and inflammation and through several mechanisms, it is involved in the pathogenesis of migraines,

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such as induction of nociceptive receptors activity, production of pain mediators including interleukin (IL)-6 and tumor necrosis factor (TNF)-α [9], interference with cellular signaling pathways, such as nuclear factor kappa B (NF-κB), endothelial nitric oxide synthase (eNOS) and phosphorylated extracellular signal-regulated kinases 1 and 2 (PERK1/2) [10]. It seems that adiponectin levels also elevate in migraine patients [11]. At normal levels, adiponectin has anti-inflammatory effect and inhibits inflammatory cytokines (such as IL-6, IL-8, and IL-1), but at lower than normal levels, adiponectin activates NF-κB and causes an inflammatory condition that contributes to migraine pathogenesis [12].

Evidence suggests that some compounds have modulatory effects on adipokine and leptin levels as well as inflammation and pain intensity, including curcumin [13]. Curcumin, the yellow active ingredient of turmeric, is a member of the Zingiberaceae family isolated from the Curcuma longa rhizome [14]. Numerous studies have demonstrated that curcumin carries out regulatory properties on adiponectin and leptin mRNA expression through multiple mechanisms; curcumin can suppress NF-κB signaling in adipocytes and significantly inhibit the secretion and expression of leptin gene and other inflammatory cytokines. It also reduces peroxisome proliferator-activated receptors (PPARs) activity and oxidative stress by reducing leptin receptor phosphorylation and inhibiting leptin gene expression and signaling. Curcumin also regulates leptin and adiponectin secretion by acting on the adenosine monophosphate (AMP)-kinase pathway [13]. Curcumin also affects adiponectin through epigenetic phenomena as it regulates the expression of adiponectin by reducing methylation of adiponectin mRNA [15].

It is necessary to mention that, because the bioavailability of curcumin is very low, we used a nanocurcumin (nanoparticles of curcumin) supplement in this study, which has a high absorption (27-fold higher) and is safe [16].

Based on previous evidence, the drugs used for migraine treatment (such as amitriptyline and beta-blockers) affect adipokines levels, indicating the important role of adipokines in the treatment of migraines [17, 18]. Curcumin significantly reduces the number of pain attacks, severity, and duration of pain, as well as pro-inflammatory factors in migraine patients [19-21], but the mechanism of action of curcumin on adipokines in migraine is not clear. In this regard, the present study hypothesizes that nanocurcumin can exert modulatory effects on adipokines involved in migraine pathogenesis resulting in an improvement of clinical signs, headache attacks, and pain severity in these patients. Thus, the present study aimed to investigate the effects of nanocurcumin supplementation on leptin and adiponectin gene expression and serum levels, as well as provide a clinical manifestation of disease in migraine patients.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

The present investigation was conducted as a randomized double-blind placebo-controlled clinical trial (RCT) study. Forty-four episodic migraine patients participated in this study, including 42 females and 2 males, from April to September 2021 at the faculty of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran (Fig. **1**). Inclusion criteria of the present study included age 20-50, body mass index (BMI) between 25-35, definitive diagnosis of episodic migraine by the neurologist based on International Headache Society (IHS) criteria (defined as ≥ 1 attack per week or fewer than 15 days headache per month), no presence of any disorders, such as cancer, thyroid disorders, diabetes, liver disease, renal disorder, cardiovascular disease or inflammatory condition, as well as no specific diet, supplements consumption or special activity at least in last 3 months.

Exclusion criteria included allergic reaction to curcumin, pregnancy during study, any changes in regular treatment drugs, any inflammatory diseases or headache attacks that lead to long-term use (more than 2 weeks) of analgesics, and less than 90% of the supplement consumption at the end of the study.

In general, 2 participants withdrew from the research due to unwillingness to cooperate. The current clinical trial study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) as ID: IR.TUMS.MEDICINE.REC.1399.190 and registered in Iranian Registry of Clinical Trials (IRCT) as ID: IRCT20160626028637N2.

At the start of the clinical trial, a written informed consent, which was approved by the TUMS ethics committee, was obtained from all patients. The details of the trial, including goals, benefits, and potential risks of the study, were explained to the participants. The patients were also free to leave the study at any time during the trial. The anthropometric and demographic data, the medical history, usage of drugs, the frequency of headache attacks (per week), and the headache duration (hours) based on patients' reports were recorded at the beginning and the end of the study as well as the severity of pain using Visual Analogue Scale (VAS) questionnaire.

To determine compliance, participants were contacted per two weeks to find out about supplement consumption or possible side effects. Less than 90% consumption of the total supplements or placebo at the end of the trial was considered as non-compliant.

The sample sizes calculation was performed based on the leptin levels, which calculates the highest number of sample size in comparison to other dependent variables of the study. Considering the $\alpha = 0.05$ and power= 80% ($1 - \beta = 1$), the sample size of 20 participants in each group was calculated. Considering the 10% probability of missing, 22 patients were considered in each group [22].

We used stratified randomization to control BMI and gender variables. Then, the participants were divided equally into 2 groups by Permuted Block Randomization method. In each group, 21 women and 1 man were entered. The groups of the study included: 1) the intervention group (taking the nano-curcumin supplement), and 2) the control group (taking the placebo). The patients in group 1 received 80 mg nano-curcumin (two capsules of 40 mg/day) and the

Table 1. Sequencing and information of primers.

Gene Name	Sequence	Length	Tm
Adiponectin	Forward: 5′-AACATGCCCATTCGCTTTACC-3′	21	59
	Reverse: 5′-TAGGCAAAGTAGTACAGCCCA-3′	21	59
Leptin	Forward: 5′-TGCCTTCCAGAAACGTGATCC-3′	21	61
	Reverse: 5′-CTCTGTGGAGTAGCCTGAAGC-3′	21	63
GAPDH	Forward: 5′-ACAACTTTGGTATCGTGGAAGG-3′	22	62
	Reverse: 5′-GCCATCACGCCACAGTTTC-3′	19	61

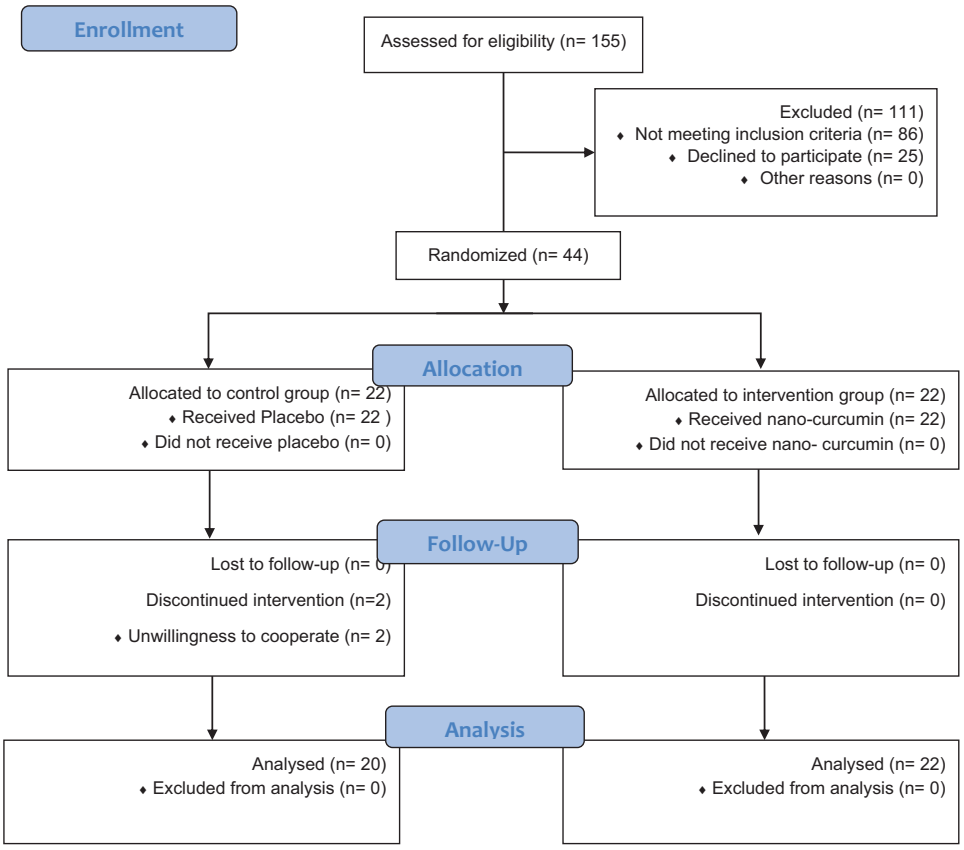

Fig. (1). Consort flow diagram of the study.

control group received nano-curcumin placebo (containing paraffin oil, 2 capsules/day).

The nano-curcumin and the placebo capsules were in the form of red soft gels and were manufactured by Cina Curcumin Pharmaceutical Company. Each 40 mg nano-curcumin capsule contained approximately 100% curcumin along with negligible amounts of other compounds, such as curcuminoid, emulsifier (polysorbate), gelatin, and glycerin. Nano-curcumin increases the absorption of curcumin up to 40-fold in rats and 27-fold in humans and is safe up to a dose of 210 mg, and each 40 mg of nano-curcumin is approximately equivalent to 1 g curcumin [16, 23]. Patients in the nano-curcumin group were asked to take 2 of 40 mg nano-curcumin capsules daily after meals (after lunch and dinner) to prevent possible digestive problems. In fact, each patient in the intervention group received a total of 80 mg of nano-curcumin equivalent to 2 gr curcumin per day.

The placebo capsules were quite similar to nano-curcumin supplements in shape, size, and color. The nano-curcumin or placebo capsules were coded by a third person. The period of intervention was 2 months in this research.

2.2. Peripheral Blood Mononuclear Cell and Serum Isolation

Peripheral Blood Mononuclear Cells (PBMCs) were isolated according to standard Ficoll-Hypaque (Hamburg, Germany) density gradient centrifugation from the hepa-

rinized peripheral blood sample. Then, patients' serum was collected after 10 minutes of centrifugation at 3000 RPM and stored at -80 °C for subsequent measurements of serum leptin and adiponectin levels using the ELISA method (Mediagnost, Germany).

2.3. RNA Extraction, cDNA Synthesis, and Real-time Polymerase Chain Reaction

The cytoplasmic RNA extraction and purification were conducted using Hybrid-R™ Blood RNA (GeneAll®, Korea), based on the protocol of the kit. The purity and quantity of the extracted RNA were determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA) with a ratio of 260/280 nm. The measurements between 1.9-2.1 were considered pure RNA. In continuation, the single strand complementary (cDNA) was synthesized by Easy™ cDNA Synthesis Kit (Parstour, Iran), which was stored at -20 °C to examine the leptin and adiponectin gene expression.

NCBI Primer-Blast was used to design the primers of leptin, adiponectin, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping genes. The sequencing and properties of primers are presented in Table **1**. The StepOne system (Applied Biosystems, Foster City, Calif., USA) and the SYBR Green detection method were used for real-time polymerase chain reaction (PCR) for leptin and adiponectin gene expression. The fold change of leptin and adiponectin gene expression was calculated using the Ct ($2^{-\Delta\Delta Ct}$) formula [24].

2.4. Clinical Signs of Pain Recording

In the present study, the headache attacks frequency (the number of attacks per week) and the headache duration (average hours of headache) based on patient self-reports were recorded before and after the study. The severity of headache was recorded using the VAS questionnaire at the start and the end of the 2-month intervention. VAS is a tool that patients rate their pain on a scale of 0 to 10. In this regard, pain intensity is divided into five categories as 0= No pain, 1-3= Mild pain, 4-6= Moderate pain, 7-9= Severe pain, and 10= Worst pain [25].

3. STATISTICAL ANALYSIS

Data are expressed as mean ± Standard Error (SEM). The collected data were transferred to SPSS software version 22 for analysis. The normality of data was assessed by the Kolmogorov-Smirnov distribution test. For normal data, the Paired t-test and independent t-test were used for comparison of the results within and between groups, respectively. For data not normally distributed, the Wilcoxon test and Mann–Whitney U test were used for within and between-group comparisons, respectively. A Chi-squared test was used to compare qualitative factors between the groups. ANCOVA test was used to remove the effect of confounding variables. In this study, P value ≤ 0.05 was accepted as a statistically significant difference. It should be noted that the intention to treat method was used to replace missing data and outliers. Outlier data were detected by ± 3SD Calculation.

4. RESULTS

4.1. Participant Information

The clinical and anthropometric data of patients between the nano-curcumin group and control groups are shown in Table **2**. There were no statistically significant differences found in age (P=0.59), gender (P=0.75), the onset of migraine age (P= 0.54), weight (P=0.25), height (P=0.55), BMI (P=0.40), or waist circumference (P=0.58) between the groups. Also, the clinical pain features of the patients showed no significant differences among groups (headache frequencies P=0.43, duration of headaches P=0.70, and pain severity P=0.87) (Table **2**). In addition, based on the Chi-squared test, no significant differences were observed in medication used (P=0.98), job (P=0.73), or education (P=0.08) between the groups. Also, no side effects were seen in any of the study groups.

Table 2. Baseline characteristics and pain manifestation of patients in nano-curcumin and control groups.

Characteristics		Nano-Curcumin Group	Control Group	*P* Value
Age (years)		39.27±2.15	41.00±2.42	0.59 [a]
Gender	Female	21 (95.5%)	21 (95.5%)	0.75 [b]
	Male	1 (4.5%)	1 (4.5%)	
Onset of migraine age (year)		26.14±1.94	28.06±2.53	0.54 [a]
Weight (kg)		77.52±2.37	74.18±1.66	0.25 [a]
Height (cm)		161.82±1.58	160.50±1.53	0.55 [a]
BMI (kg/m^2)		29.66±0.86	29.03±0.75	0.40 [a]
WC (cm)		98.18±1.05	96.91±1.08	0.58 [a]
Severity of pain (VAS scoring 0-10)		7.64±0.31	7.45±0.38	0.87 [c]
Headache frequency (number/week)		3.23±0.37	3.59±0.38	0.43 [c]
Duration of attacks (hours)		17.55±3.21	14.41±2.45	0.70 [c]

Note: BMI: Body Mass Index; WC: Waist Circumference. All values are expressed as means ± SE or numbers.
[a]Independent t test
[b]Chi-squared test
[c]Mann-Whitney U test

4.2. Leptin and Adiponectin Gene Expression in Isolated PBMCs

As shown in Fig. (**2**), the mRNA expression analysis for adiponectin indicated a significant increase in nano-curcumin-treated groups (P<0.05) compared to the control group with a 13.9% effect size. In the case of leptin gene expression, a reduction in change fold was seen in the nano-curcumin group. Although, after ANCOVA analysis, a P value = 0.06 was observed, which was close to significant levels but still not statistically significant between the groups (Fig. **2**).

Table 3. The serum concentration of leptin and adiponectin.

Adipokines		Nano-Curcumin Group	Control Group	*P* Value [b]	Effect Size
Leptin (ng/ml)	Before	16.65±1.41	16.77±1.58	0.95	3.4%
	After	15.50±1.24	16.71±1.17	0.23 [c]	
	Difference	-1.14±0.77	-0.06±0.83	0.34	
	P value [a]	0.15	0.94		
Adiponectin (ng/ml)	Before	21.15±2.46	21.12±2.27	0.99	11.2%
	After	24.02±2.86	20.46±1.80	0.02 [c]	
	Difference	2.87±0.040	-0.66±1.11	0.03	
	P value [a]	0.017	0.56		

Note: Data are reported as means ±SE

a Paired t test

b Independent t test

c ANCOVA

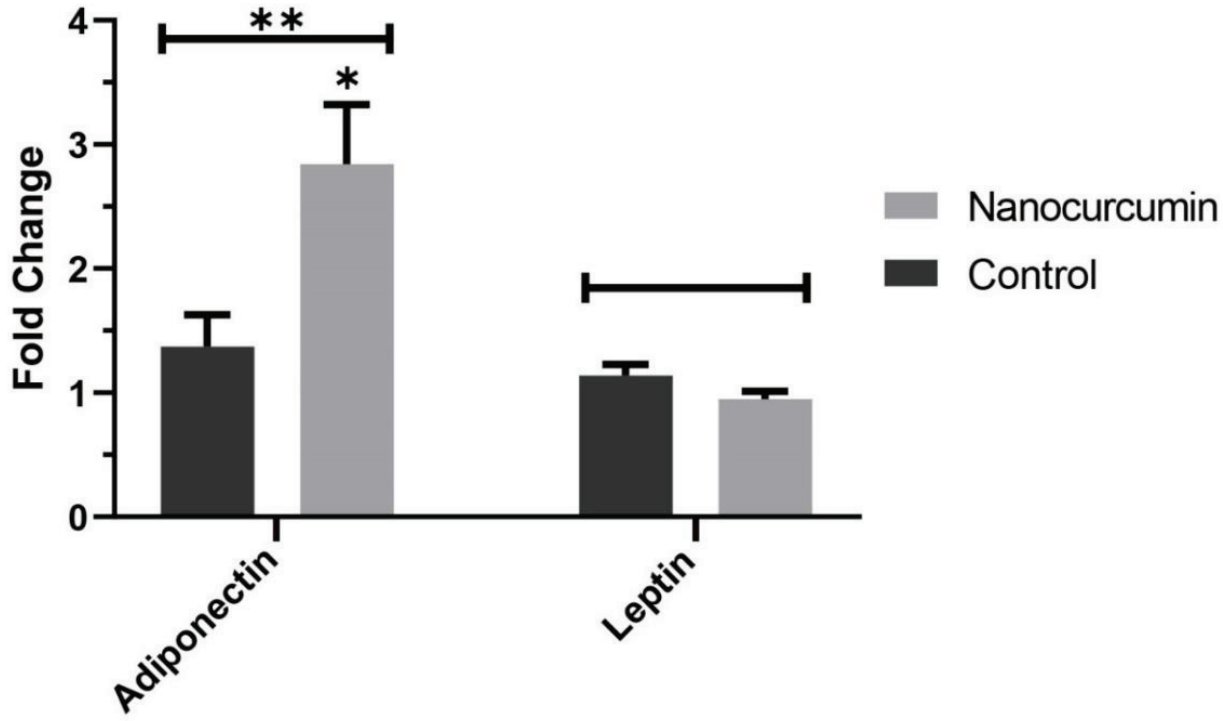

Fig. (2). The fold change of adiponectin and leptin gene expression in PBMC extracted mRNA. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

4.3. Leptin and Adiponectin Serum Levels

According to the results of serum levels of adiponectin and leptin shown in Table **3**, a significant increase in the serum levels of adiponectin was observed in patients who received nano-curcumin supplements. Also, this difference was significant between the groups (P=0.02). In the case of leptin, no significant reduction was found either within or between groups in both nano-curcumin supplemented and control groups, even after adjustment based on baseline serum levels (P> 0.05) (Table **3**).

4.4. Clinical Features of Headache

Analysis of the data of the present study shows that treatment with nano-curcumin for 2 months can reduce headache frequencies significantly between the groups (P<0.001), with a size effect of 37%. However, these effects were not observed in the placebo group.

In addition, a significant difference was found in the headache duration in the nano-curcumin-treated group (P<0.001) but not in the control groups. Also, this reduction was statistically significant between the groups (P <0.001,

size effect= 34%). As presented in Table **4**, based on VAS measurement (scoring from 0 to 10), the nano-curcumin therapy exerted significant reductions in the severity of pain (P <0.001, size effect 37%) (Table **4**) (Fig. **3**).

Table 4. Clinical properties of headache.

Symptom			Nano-Curcumin Group	Control Group	P Value [b]	Effect Size
Attack frequencies (number/ week)	Before		3.23±0.37	3.59±0.38	0.43	47%
	After		1.82±0.19	3.95±0.31	<0.001[c]	
	Difference		-1.40±0.37	0.36±0.22	<0.001	
	P value [a]		0.002	0.12		
Duration of headache (hour)	Before		17.55±3.21	14.41±2.45	0.70	34%
	After		4.42±0.87	12.34±2.02	<0.001[c]	
	Difference		-13.12±3.08	-2.06±1.85	0.004	
	P value [a]		<0.001	0.42		
Severity of pain (Scoring 0-10)	Before	No Pain	0 (0%)	0 (0%)	0.87	37%
		Mild Pain	0 (0%)	1 (4.5%)		
		Moderate Pain	8 (36.4%)	6 (27.3%)		
		Sever Pain	12 (54.5%)	13 (59.1%)		
		Worst Pain	2 (9.1%)	2 (9.1%)		
	After	No Pain	1 (4.5%)	0 (0%)	<0.001[c]	
		Mild Pain	6 (27.3%)	1 (4.5%)		
		Moderate Pain	13 (59.1%)	7 (31.8%)		
		Sever Pain	2 (9.1%)	13 (59.1%)		
		Worst Pain	0 (0%)	1 (4.5%)		
	P value [a]	-	<0.001	0.48	-	-

Note: Data are reported as means ±SE.
a Will Coxon t test
b Mann U Wittney Test
c ANCOVA

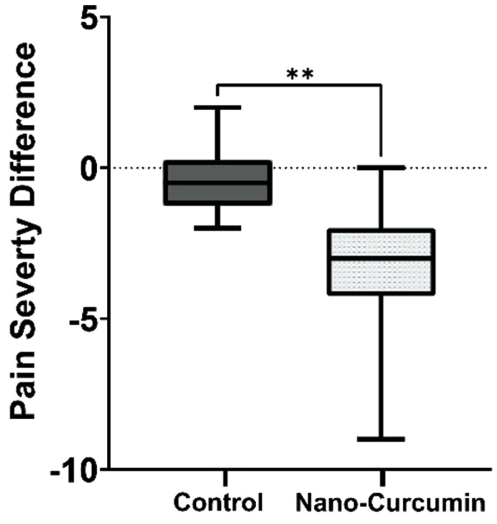

Fig. (3). The severity of pain in migraine based on VAS measurements. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

5. DISCUSSION

In the present double-blind clinical trial study, 44 patients with episodic migraine participated and underwent a supplementation program with nano-curcumin or placebo for 2 months for evaluation of modulatory effects of nano-curcumin on leptin and adiponectin gene expression, serum levels, and clinical signs of disease in migraine.

Adipokines, which are mainly secreted from adipose tissue (such as leptin, adiponectin, resistin, visfatin, *etc*.), play important roles in a variety of physiological functions, including regulation of endothelial function, weight, immune response, inflammation, and insulin resistance [26]. Recent studies have shown that some of these adipokines are involved in migraine pathogenesis. They have abundant receptors in the central nervous system that are considered potential biomarkers for migraine disorder [27]. Based on previous studies in patients with migraine, levels of adiponectin, leptin, pro-inflammatory mediators, and cytokines increased significantly [11]. Additionally, there is a significant correlation between adiponectin levels and other in-

flammatory factors involved in migraine pathogenesis, pain, and clinical symptoms in migraine patients [28, 29]. These facts indicate that leptin or adiponectin modulation can be promising in the treatment of migraine.

To our knowledge, the present study is the first clinical trial that focused on the modulatory effects of curcumin on adipokines at gene levels as the clinical sign of migraine disorder.

The results of this study show that nano-curcumin increased mRNA expression as well as serum levels of adiponectin significantly in comparison to the placebo group (P<0.05). Parallel to our finding, metabolic trial studies have demonstrated curcumin to increase adiponectin levels and improve adiponectin to leptin ratio [30, 31]. Also, a meta-analysis by Clarck *et al*. found curcumin to significantly improve adiponectin concentrations [32]. Conversely, an experimental study by Ali *et al*. showed curcumin supplementation to reduce adiponectin levels in chronic kidney disease model [33]. However, the observations indicate that adiponectin levels increase in migraine patients [34]. It seems that adiponectin has dual influences on inflammation; different adiponectin oligomers probably explain this paradox. At normal levels, it has an anti-inflammatory effect and inhibits inflammatory cytokines (such as IL-6, IL-8 and IL-1), but at lower than normal levels, adiponectin activates NF-κB, resulting in an inflammatory condition [35]. The current hypothesis is that chronic inflammation associated with visceral obesity inhibits adiponectin production, but in migraine, even in non-obese patients, it appears to increase as positive feedback to an increase in inflammation [11]. Adiponectin signaling occurs through several mechanisms, including suppressed nuclear factor NF-κB, AMP-kinase, and the inflammatory cytokines of TNF-α and IL-6, which are known to play a role in migraine pathogenesis and attacks [12]. Although the functional mechanism of adiponectin in migraine is not known and there are conflicting data in this field, our study showed that curcumin can increase the level and expression of its gene and improve clinical symptoms.

In the case of leptin, the nano-curcumin supplementation reduced leptin mRNA or concentration, but it was not statistically significant (P> 0.05). Contrary to the results of this study, Atkin et al., in their meta-analysis, showed that curcumin significantly reduces serum leptin [36]. Many clinical and cellular studies show the reducing effects of curcumin on leptin [30, 37-39], which occur through several mechanisms, including the block of NF-κB signaling pathway in adipocytes, reduction of phosphorylation of leptin receptor, suppressing of AMP-kinase and p38 Mitogen-activated protein kinase (MAPK) signaling, and affecting gene expression of leptin [40, 41]. Overall, these conflicting results may be due to study conditions, the nature of the disease or duration, the sample size and supplementation dosage. However, further studies are needed on the effects of curcumin on leptin levels in migraine.

In addition, the findings of the present study demonstrated that nano-curcumin treatment can significantly decrease headache attack frequency, the duration of headache, and headache severity in patients with migraine with a size effect of 47%, 34%, and 37%, respectively. In this context, Abdolahi *et al*. showed nano-curcumin to reduce headache frequency as well as pain severity in migraine patients while it did not have a significant effect on the duration of headache [42]. However, the clinical trial studies in the field of headache and curcumin are limited, and most evidence in this field has focused on cellular and animal studies intending to discover the relevant mechanisms [4, 43]. Curcumin declines neurogenic pain through several mechanisms, including downregulating pro-inflammatory cytokines (such as TNF-α, IL-6, and cyclooxygenase-2) as well as calcitonin gene-related peptide (CGRP) and substance P, which are pain mediators and have a known role in the pathogenesis of migraine [4, 44]. Curcumin also suppresses nociceptor activity, such as transient receptor potential vanilloid 1 (TRPV1) [45], stress oxidative and NF-κB transcription factor [46]. It seems that curcumin probably acts on multiple levels, from gene expression and cell signaling pathways to nociceptors at the sensory neurons. However, in the case of curcumin's effects on headache and adipokines, the human studies are very limited, and the mechanisms proposed are largely based on cellular and animal studies, so extending these mechanisms to humans may be challengeable.

In general, considering the evidence of regulatory effects of curcumin on adiponectin and leptin, analgesic and anti-inflammatory effects of curcumin as well as the laboratory and clinical findings of the present study, it can be concluded that curcumin can be promising in the treatment of migraine disease or clinical improvement as an adjuvant therapy with no potential side effects.

CONCLUSION

Based on our knowledge, the present clinical trial is the first study on the effects of nano-curcumin supplementation on adiponectin and leptin gene expression and their product (adiponectin and leptin serum levels) or clinical sign of headache in migraine patients. The changes in adiponectin and leptin concentrations in migraine patients and their relationship with inflammation and pain signaling demonstrated that these adipokines play a pivotal role in the pathogenesis of migraine. In this study, we found that nano-curcumin can significantly up-regulate adiponectin gene expression and serum concentrations and reduce headache attacks frequencies, the severity of headache, and duration of headaches. Therefore, targeting curcumin can be a promising approach to migraine treatment. However, further comprehensive trials are necessary to confirm these findings and clarify the mechanisms involved in this field.

LIST OF ABBREVIATIONS

AMP	=	Adenosine Monophosphate
BMI	=	Body mass index
CGRP	=	Calcitonin Gene-Related Peptide
eNOS	=	Endothelial Nitric Oxide Synthase
IL	=	Interleukin

MAPK = Mitogen-Activated Protein Kinase

NF-κB = Nuclear Factor kappa B

PERK = Phosphorylated Extracellular Signal-Regulated Kinases

PPAR = Peroxisome Proliferator-Activated Receptor

TNF = Tumor Necrosis Factor

TRPV1 = Transient Receptor Potential Vanilloid 1

VAS = Visual Analogue Scale

AUTHORS' CONTRIBUTIONS

MS contributed to the conception or design of the work. EJ and SS contributed to the interpretation of data for the work. MA contributed to drafting of the manuscript. MSY and EA contributed to the analysis of the data. The study was conducted under the supervision of MJ. The final version of the manuscript has been finally approved by all the authors for publication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present clinical trial was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) as ID: IR.TUMS.MEDICINE.REC.1399.190 and registered in the Iranian Registry of Clinical Trials (IRCT) as ID: IRCT20160626028637N2.

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. The research involving human participants was performed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

At the start of the study, a written informed consent, approved by the TUMS Ethics Committee, was obtained from all participants.

STANDARDS OF REPORTING

CONSORT guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from Mohsen Sedighiyan, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of Mohsen Sedighiyan (m.sedighiyan86@gmail.com).

FUNDING

None.

CONFLICT OF INTEREST

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

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