Reports of Biochemistry & Molecular Biology
Vol.9, No.1, Apr 2020

*Original article*

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# The Effect of Nano-Curcumin Supplementation on Pentraxin 3 Gene Expression and Serum Level in Migraine Patients

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## Abstract

***Background:*** This study was designed to investigate the effect of nano-curcumin supplementation on pentraxin 3 (PTX3) gene expression and serum level in migraine patients.
***Methods:*** The present study, performed as a clinical trial, included 38 episodic migraine patients in two groups that received either nano-curcumin or placebo over a two-month period. At the start and the end of the study, PTX3 gene expression and serum levels were measured.
***Results:*** After two months of treatment, PTX3 gene expression and serum levels were both significantly less in the nano-curcumin than in the placebo group (P= 0.01 and P< 0.001, respectively). No significant gene expression differences were found between the two groups.
***Conclusions:*** Curcumin may have a potential inhibitory effect on PTX3 gene expression and serum levels in migraine disease and can be considered as an efficient therapy in migraine management.

**Keywords:** Clinical trial, Curcumin, Inflammation, Migraine, Pentraxin 3.

## Introduction

Migraine is a chronic neurovascular disorder affecting approximately 18% of women and 6% of men (1). Migraine is ranked as the third-most prevalent disorder and seventh-highest specific cause of disability worldwide (2). The exact cause of migraine is still not completely understood, but it seems that genetic and environmental factors play important roles (3). It has been suggested that neuro-inflammation plays a pivotal role in migraine pathogenesis (4). Neuro-inflammatory condition derived from glial cell activation includes microglia, astrocytes, and other pro-inflammatory mediators (5). Pro-inflammatory cytokines have a crucial role in pain, inflammation, and migraine progression through cell-to-cell interaction and increasing the vascular permeability (6, 7). Inflammatory cytokines e.g. TNF-α, and IL-6 are pain mediators in neuro-inflammation and vascular disorders (8). Moreover, a strong correlation between migraine and increased serum CRP levels has been demonstrated (9). Previous reports have established the association between various inflammatory markers and migraine pathophysiology (8).

Pentraxins, a superfamily of acute phase proteins, are a novel and important part of innate immunity (10). Previous reports have shown the association between pentraxin 3 (PTX3) and various neurological disorders including Parkinson's disease and stroke (10). Ceylan et. al. found greater PTX3 serum levels in migraine

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Received: 1 Nov, 2019; Accepted: 29 Nov, 2019

attack patients than in interictal and control patients. Moreover, they showed that patients with newly-diagnosed and relatively short-lasting migraine attacks exhibit greater PTX3 serum levels than patients with longer disease and attack durations (11).

It is well known that some nutrients have anti-inflammatory and neuroprotective effects (12). Previous reports suggested that curcumin, an active polyphenol of turmeric, plays a crucial role in suppressing inflammation-mediated pro-inflammatory molecular synthesis (13). Moreover, curcumin relieves neurogenic pain by down-regulating inflammatory mediator expression (14). The aim of this study was to evaluate the effects of nano-curcumin supplementation on PTX3 gene expression and serum protein level in migraine patients.

## Materials and methods

### *Study design*

The present study was performed as a randomized, double-blind, placebo-controlled clinical trial. With 5% significance level, a power of 90%, and a 20% probability of missing patients during the study, the sample size of was determined for each group. Forty patients with episodic migraines were enrolled. Two patients withdrew from the study because of altered medication. The study was performed 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 of the aim and possible benefits and risks of the study. The study was approved by the Ethics Committee of TUMS (ID: IR.TUMS.REC.1394.462) and identified in Clinical Trials. gov as ID: NCT02532023. All the patients were considered to have episodic migraines as per the International Headache Society (IHS) criteria used as the standard by neurologists. All participants received three cyclic antidepressants plus a β-blocker. No patients had any other diseases such as diabetes, renal or heart disease, thyroid disorder, cancer, or 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. The capsules containing nano-curcumin or placebo were coded by a third person. Group A received 80 mg of nano-curcumin and group B received a paraffin oil placebo for 2 months. All the subjects received a single capsule daily and all the capsules were similar in appearance.

### *PBMC separation and serum collection*

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by the standard Ficoll-Hypaque (Hamburg, Germany) method. Patient blood was centrifuged at 1,500 rcf for 10 min. The serum was collected, transferred to microtubes, and stored at -80 °C. Serum analyzed for PTX3 protein by ELISA according to the manufacturer's protocol (Mediagnost, Germany).

### *RNA extraction and cDNA synthesis*

RNA was extracted from the PBMCs and purified by the RNeasy Plus Mini Kit (Qiagen, Valencia, Calif., USA) based on the manufacturer's protocol. The purity and quantity of the extracted RNA were analyzed on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA) cDNA was synthesized from the extracted RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) and stored at -20 °C.

### *Real-time polymerase chain reaction*

The PTX3 and β-actin gene primers (Table 1) were designed by Primer Express 3 software (Applied Biosystems). Real-time polymerase chain reaction (PCR) for gene expression was performed on a Step One system (Applied Biosystems, Foster City, CA, USA) using the SYBR Green detection method as described previously (15). The fold change of PTX3 gene expression was calculated by the Ct ($2^{-\Delta\Delta Ct}$) equation.

### *Data analysis*

Statistics were analyzed using SPSS 22.0 software. The Kolmogorov–Smirnov distribution test was used to assess data normality. For normal data, the paired t test and the independent samples t-test were used to compare variables within and between groups, respectively. The Mann-Whitney

U test was used for data lacking a normal distribution after the log transformation. Additionally, the ANCOVA test was used to control confounding factors. Data were expressed as means ± SEs. Statistical significance was defined as $P \leq 0.05$ for all tests.

**Table 1.** Primer sequences

| Gene | Sequence |
|---|---|
| PTX3 | Forward: 5'- ATGGTGAACTGGCGGCTAC-3'<br>Reverse: 5'- GGATGTGACAAGACTCTGCT-3' |
| β-actin | Forward: 5'- TGGCACCCAGCACAATGAAG-3'<br>Reverse: 5'- AGTCATAGTCCGCCTAGAAGC-3' |

## Results

Baseline characteristics of the study subjects are shown in Table 2. No statistically significant differences were found for age, sex, body mass index, height, or weight between the nano-curcumin and placebo groups.

No significant differences in PTX3 gene expression were found after treatment between the nano-curcumin and placebo groups PTX3 (P= 0.24). However, within group analysis showed significant reduction in PTX3 gene expression followed by nano-curcumin supplementation (P= 0.01).

No significant difference in the fold change of PTX3 gene expression was found between the two groups (P= 0.13) (Table 3).

The PTX3 serum levels did not have normal distribution on the basis of statistical tests and did not normalize even after logarithmic transformation; therefore, non-parametric statistical tests were used for analysis. After treatment, PTX3 serum levels were significantly less in the nano-curcumin than in the placebo group (P= 0.01) and were also significantly less after nano-curcumin treatment than before (P< 0.001) (Table 4).

No significant correlations were found between PTX3 gene or protein expression changes and patient or control subject headache frequency, duration, or severity (Table 5).

**Table 2.** Baseline characteristics of study subjects

| | Nano-curcumin (n= 19) | Placebo (n= 19) | P value[1] |
|---|---|---|---|
| Age (Years) | 37.36±1.95 | 36.57±1.87 | 0.95 |
| Sex (Male/Female) | 4/15 | 4/15 | 0.99[2] |
| Weight (kg) | 72.63±3.87 | 75.05±2.43 | 0.77 |
| Height (cm) | 161.47±1.84 | 162.84±1.45 | 0.95 |
| BMI (kg/m$^2$) | 27.59±1.05 | 26.94±0.89 | 0.65 |

All values are expressed as means ± SE or numbers
1 Obtained from Independent T test
2 Obtained from Mann-Whitney U test

**Table 3.** ΔCT and mean of PTX3 gene expression in freshly-obtained PBMCs

| | | Nano-curcumin (n= 19) | Placebo (n= 19) | P value[1] | P value[2] |
|---|---|---|---|---|---|
| PTX3 gene expression in fresh PBMCs | before | 0.41±9.37 | 0.47±9.57 | 0.71 | 0.24 |
| | after | 0.50±10.47 | 0.50±9.94 | 0.57 | |
| | difference | 0.39±1.09 | 0.40±0.37 | 0.29 | |
| | P value[3] | 0.01 | 0.37 | | |
| Fold change of PTX3 gene expression | | 0.20±0.83 | 0.44±1.41 | 0.13[4] | |

1. Obtained from Independent T test
2. Obtained from ANCOVA, adjusted for baseline values
3. Obtained from paired T test
4. Obtained from Mann-Whitney U test

**Table 4.** PTX3 serum levels

| | | Nano-curcumin (n=19) | Placebo (n=19) | P value[1] | P value[2] |
|---|---|---|---|---|---|
| PTX3 (ng/L) | before | 0.23±5.50 | 0.19±5.48 | 0.39 | |
| | after | 0.27±4.77 | 0.16±5.36 | 0.007 | 0.01 |
| | difference | 0.14±0.73- | 0.13±12/0- | 0.003 | |
| | P value[3] | 0.001< | 0.28 | | |

1. Obtained from Mann-Whitney U test
2. Obtained from ANCOVA, adjusted for baseline values
3. Obtained from paired T test

**Table 5.** Correlation of PTX3 gene expression and serum level changes with subject clinical symptoms

| | | Nano-curcumin (n=19) | | | Placebo (n=19) | | |
|---|---|---|---|---|---|---|---|
| | | Number of attacks | Duration of attacks | Severity of attacks | Number of attacks | Duration of attacks | Severity of attacks |
| PTX3 gene expression | r | 0.01 | -0.06 | -0.33 | -0.22 | 0.23 | 0.02 |
| | P value[1] | 0.94 | 0.79 | 0.16 | 0.29 | 0.33 | 0.91 |
| PTX3 serum levels (ng/L) | r | -0.09 | 0.14 | 0.23 | 0.07 | -0.12 | -0.30 |
| | P -value | 0.72 | 0.57 | 0.34 | 0.76 | 0.61 | 0.21 |

1. Obtained from Spearman correlation test

## Discussion

In the current study, 38 patients with episodic migraine enrolled in a randomized clinical trial with 2-month nano-curcumin supplementation to determine the effect of nano-curcumin supplementation on PTX3 gene expression and serum levels. The findings indicated that nano-curcumin supplementation significantly reduced PTX3 gene expression after intervention, but did not show any significant changes in the expression of genes in the control group. Also, PTX3 serum levels were significantly less after nano-curcumin intervention than before, and also less than those of the placebo groupd after intervention.

Recent study results have suggested that neurogenic inflammation is common in the pathogenesis of many neurological diseases including Alzheimer's, Parkinson's, multiple sclerosis, and especially migraine (16). Neurogenic inflammation plays a key role in the immune pathogenesis, manifestation, and progression of migraine disorder (17), and contributes to the sensitization and activation of perivascular meningeal afferents during attacks (18). In neuroinflammation status, the activated glial cells and neurons produce proinflammatory cytokines and other mediators including TNF-α, IL-1β, IL-6, CRP, and PTX3 from the vessels around the neuronal terminals (16). Migraine has a special pattern of inflammatory markers including TNF-α, IL-1β, IL-6, CRP, and PTX3, which increase nitric oxide synthase activity, producing oxidative stress and nitrous oxides. These cytokines play a role in the progression of disease as well as endothelial disorders in migraine. TNF-α, a potential pain mediator in neurovascular inflammatory conditions, is involved in the initiation and progression of a migraine attack (19). It also upregulates transcription of CGRP, a potent vasodilator, and PTX3 (20).

To date, no study on the effect of nano-curcumin on PTX3 gene expression or serum levels has been reported, but several studies have examined the effects of nano-curcumin on other inflammatory cytokines and the factors and signals involved in PTX3 transcription. PTX3 transcription involves several factors, including AP1 and NF-κB transcription factors. NF-κB increases PTX3 transcription in response to the inflammatory cytokines TNF-α and IL-1β. The TNF-α, IL-1β, and Toll-like receptor (TLR) inflammatory cytokines are the main motifs for PTX3 gene expression (21).

Curcumin, a lipophilic substance that easily passes through the blood-brain barrier, is able to

reduce the inflammation of the nervous system through various mechanisms of cell signaling to genes, and has neuro-protective effects (22). In this regard, Abdolahi et al reported that 80 mg of curcumin for eight weeks significantly decreased TNF-α gene expression and serum levels in migraine patients (23). TNF-α is a major inducer of PTX3 expression, and nano-curcumin can play an important role in reducing the expression and secretion of PTX3 in migraine sufferers by reducing TNF-α expression. IL-1β is another PTX3 gene-stimulating cytokine. In 2014, Singh and Vinayak observed that after induction of pain in the cerebrospinal fluid of rats, curcumin downregulated expression of the inflammatory cytokines IL-1β and IL-6 (24).

TLRs are predominantly expressed in glial cells and play a key role in the pathogenesis of inflammatory neurological diseases. Also, TLRs, especially TLR4, play an important role in PTX3 gene expression (25). Curcumin reduces the activity of microglia by inhibiting TLR4/MYD88 signalling and subsequently decreases PTX3 gene expression (26, 27).

Curcumin also significantly decreases NF-κB expression and NF-κB-α phosphorylation. NF-κB, which plays a key role in regulating inflammation, decreases TLR4 and downstream signalling, decreasing the activity of astrocytes and reducing inflammation (28). Curcumin also reduces PTX3 transcription by disabling AP1 and NF-κB transcription factors (29). The main signalling pathways effective in expressing the PTX3 gene are NF-κB/IKBa and MAPK. Of the MAPK members (JNK/ERK 1,2/P38MAPK), only JNK and ERK 1,2 play roles in PTX3 gene expression. Curcumin, by inhibiting JNK and ERK1,2 phosphorylation, inhibits their activity. Inhibiting JNK and ERK1,2 phosphorylation reduces PTX3 gene expression. The signalling pathway of P38MAPK does not directly play a role in PTX3 gene expression (30).

As previously noted, in this study also, supplementation with nano-curcumin significantly reduced PTX3 gene expression and serum levels after intervention. It is important to mention that curcumin bioavailability is poor and high doses are needed to exhibit efficacy in clinical trials. For this reason, in this study, we used nano-curcumin instead of curcumin. Curcumin nanoparticles safely increase curcumin absorption over that of standard curcumin by about 27-fold (31, 32).

In contrast to a previous report in which ω-3 fatty acids and nano-curcumin reduced attack frequency in migraine patients (23), in our study nano-curcumin had no effect on the frequency, duration, or severity of attacks in migraine patients. However, this study is the first one to examine PTX3 and more clinical trials are needed to determine an effective dose of active nutrients on PTX3 gene expression and serum levels.

In conclusion, regarding the findings of this study, we conclude that curcumin may have a potential inhibitory effect on PTX3 gene expression and serum levels in migraine disease, and can be considered as an efficient therapy in migraine management.

## Acknowledgment

The study was approved by the Ethics Committee of TUMS (ID: IR.TUMS.REC.1394.462) and identified in Clinical Trials. gov as ID: NCT02532023.

## References

1. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed M, Stewart WF. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology. 2007;68(5):343-9.

2. Steiner, T. J., Stovner, L. J., & Birbeck, G. L. (2013). Migraine: the seventh disabler. The journal of headache and pain, 14(1), 1.

3. Mulder EJ, Van Baal C, Gaist D, Kallela M, Kaprio J, Svensson DA, et al. Genetic and environmental influences on migraine: a twin study across six countries. Twin Res. 2003;6(5):422-31.

4. Malhotra R. Understanding migraine: Potential role of neurogenic inflammation. Ann Indian Acad Neurol. 2016;19(2):175-82.

5. Honarvar NM, Saedisomeolia A, Abdolahi M, Shayeganrad A, Sangsari GT, Rad BH, et al. Molecular anti-inflammatory mechanisms of retinoids and carotenoids in Alzheimer's disease: A review of current evidence. J Mol Neurosci. 2017;61(3):289-304.

6. Soveyd N, Abdolahi M, Bitarafan S, Tafakhori A, Sarraf P, Togha M, et al. Molecular mechanisms of omega-3 fatty acids in the migraine headache. Iran J Neurol. 2017;16(4):210-217.
7. Hamed SA. The vascular risk associations with migraine: relation to migraine susceptibility and progression. Atherosclerosis. 2009;205(1):15-22.
8. Longoni M, Ferrarese C. Inflammation and excitotoxicity: role in migraine pathogenesis. Neurol Sci. 2006;27 suppl 2:s107-10.
9. Lippi G, Mattiuzzi C, Cervellin G. C-reactive protein and migraine. Facts or speculations?. Clin Chem Lab Med. 2014;52(9):1265-72.
10. Ryu WS, Kim CK, Kim BJ, Kim C, Lee SH, Yoon BW. Pentraxin 3: a novel and independent prognostic marker in ischemic stroke. Atherosclerosis. 2012;220(2):581-6.
11. Ceylan M, Bayraktutan OF, Becel S, Atis Ö, Yalcin A, Kotan D. Serum levels of pentraxin-3 and other inflammatory biomarkers in migraine: association with migraine characteristics. Cephalalgia. 2016;36(6):518-25.
12. Wärnberg J, Gomez-Martinez S, Romeo J, Díaz LE, Marcos A. Nutrition, inflammation, and cognitive function. Ann N Y Acad Sci. 2009;1153:164-75.
13. Shehzad A, Wahid F, Lee YS. Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. Arch Pharm (Weinheim). 2010;343(9):489-99.
14. Shishodia S. Molecular mechanisms of curcumin action: gene expression. Biofactors. 2013;39(1):37-55.
15. Mottaghi A, Salehi E, Keshvarz A, Sezavar H, Saboor-Yaraghi A-A. The influence of vitamin A supplementation on Foxp3 and TGF-β gene expression in atherosclerotic patients. J Nutrigenet Nutrigenomics. 2012;5(6):314-26.
16. Tietjen GE. Migraine as a systemic vasculopathy. Cephalalgia. 2009;29(9):989-96.
17. Gerring ZF, Powell JE, Montgomery GW, Nyholt DR. Genome-wide analysis of blood gene expression in migraine implicates immune-inflammatory pathways. Cephalalgia. 2018;38(2):292-303.
18. Levy D. Migraine pain and nociceptor activation—where do we stand?. Headache.2010;50(5):909-16.
19. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. Annu Rev Physiol. 2013;75:365-91.
20. Liu R, Ma M, Cui M, Dong Z, Wang X, Zhang W, et al. Effects of tumor necrosis factor-β (TNF-β) 252A> G polymorphism on the development of migraine: a meta-analysis. PLoS One. 2014;9(6):e100189.
21. Casula M, Montecucco F, Bonaventura A, Liberale L, Vecchié A, Dallegri F, et al. Update on the role of Pentraxin 3 in atherosclerosis and cardiovascular diseases. Vascul Pharmacol. 2017;99:1-12.
22. Bisht K, Wagner KH, Bulmer AC. Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto-and DNA-protective dietary compounds. Toxicology. 2010;278(1):88-100.
23. Abdolahi M, Tafakhori A, Togha M, Okhovat AA, Siassi F, Eshraghian MR, et al. 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. Immunogenetics. 2017;69(6):371-378.
24. Singh AK, Vinayak M. Curcumin attenuates CFA induced thermal hyperalgesia by modulation of antioxidant enzymes and down regulation of TNF-α, IL-1β and IL-6. Neurochem Res. 2015;40(3):463-72.
25. Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, et al. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. Eur J Immunol. 2003;33(10):2886-93.
26. Fu Y, Gao R, Cao Y, Guo M, Wei Z, Zhou E, et al. Curcumin attenuates inflammatory responses by suppressing TLR4-mediated NF-κB signaling pathway in lipopolysaccharide-induced mastitis in mice. Int Immunopharmacol. 2014;20(1):54-8.
27. Zhu HT, Bian C, Yuan JC, Chu WH, Xiang X, Chen F, et al. Curcumin attenuates acute inflammatory injury by inhibiting the TLR4/MyD88/NF-kappaB signaling pathway in experimental traumatic brain injury. J Neuroinflammation. 2014;11:59.
28. Yu S, Wang X, He X, Wang Y, Gao S, Ren L, et al. Curcumin exerts anti-inflammatory and antioxidative properties in 1-methyl-4-

phenylpyridinium ion (MPP (+))-stimulated mesencephalic astrocytes by interference with TLR4 and downstream signaling pathway. Cell Stress Chaperones. 2016;21(4):697-705.
29. Divya CS, Pillai MR. Antitumor action of curcumin in human papillomavirus associated cells involves downregulation of viral oncogenes, prevention of NFkB and AP-1 translocation, and modulation of apoptosis. Mol Carcinog. 2006;45(5):320-32.
30. Zhang J, Koussih L, Shan L, Halayko AJ, Chen BK, Gounni AS. TNF up-regulates Pentraxin3 expression in human airway smooth muscle cells via JNK and ERK1/2 MAPK pathways. Allergy Asthma Clin Immunol. 2015;11:37.
31. Kanai M, Imaizumi A, Otsuka Y, Sasaki H, Hashiguchi M, Tsujiko K, et al. Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. Cancer Chemother Pharmacol. 2012;69(1):65-70.
32. Sasaki H, Sunagawa Y, Takahashi K, Imaizumi A, Fukuda H, Hashimoto T, et al. Innovative preparation of curcumin for improved oral bioavailability. Biol Pharm Bull. 2011;34(5):660-5.