**ORIGINAL ARTICLE**
Iran J Allergy Asthma Immunol
In press.

# Curcumin-mediated Modulation of T-bet and CD8$^+$ T Cells: A Potential Anti-inflammatory Mechanism in Knee Osteoarthritis

**Mohsen Ghoryani[1], Soroush Gorgani[2], Mahdi Atabaki[3], Elmira Noori[4], Zhaleh Shariati-Sarabi[5], and Mojgan Mohammadi[6]**

*[1] Department of Laboratory Sciences, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran*
*[2] Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*[3] Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
*[4] Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
*[5] Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
*[6] Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran*

***Received: 31 July 2025; Received in revised form: 18 September 2025; Accepted: 11 October 2025***

## ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis, characterized by pathological changes in joint components. Increasing evidence suggests that helper T ($T_H$) lymphocytes play a pivotal role in the inflammatory processes associated with OA. Curcumin, the primary polyphenolic compound found in *Curcuma longa*, exhibits potent antioxidant and anti-inflammatory properties. This study aimed to evaluate the effects of curcumin on the gene expression of key transcription factors of $T_H1$ and $T_H2$ cells and to explore their associations with clinical and immunological parameters in patients with knee OA.

This mechanistic sub-study presents a secondary molecular analysis of RNA biospecimens from a previously completed double-blind, placebo-controlled clinical trial involving 30 patients with knee OA. Participants were randomly assigned to receive either 80 mg/day of nano-micelle curcumin or a placebo for 3 months. Expression levels of T-box transcription factor 21 (T-bet) and GATA binding protein 3 (GATA3), the key transcription factors of $T_H1$ and $T_H2$ cells, respectively, were quantified using SYBR Green-based real-time PCR. Their associations with changes in visual analogue scale (VAS) score, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and percentages of CD4$^+$ and CD8$^+$ T cells were analyzed.

Curcumin administration significantly reduced T-bet gene expression compared to baseline and showed a positive correlation with the frequency of CD8$^+$ T cells, while GATA3 expression remained unchanged.

These findings may provide a novel molecular perspective on curcumin's potential to influence CD8$^+$ T cell dynamics by modulating $T_H1$-associated transcriptional programs.

**Keywords:** CD4-positive T-lymphocytes; CD8-positive T-lymphocyte; Curcumin; GATA3 transcription factor; Knee; Osteoarthritis, T-bet protein

**Corresponding Author:** Mojgan Mohammadi, PhD; Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: (+98 51) 3800 2374, Fax: (+98 51) 3882 8560, Email: MohammadiMZH@mums.ac.ir

## INTRODUCTION

Osteoarthritis (OA) is the most prevalent form of arthritis and a type of joint degeneration characterized

Copyright©, Iran J Allergy Asthma Immunol. All rights reserved.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

by pathological changes in components of the joint.[1,2] The primary mechanism underlying this condition is the degeneration of hyaline cartilage, which is accompanied by subchondral sclerosis, the formation of osteophytes, stretching of the joint capsule, synovial inflammation, and weakness of the surrounding muscles. The knee joint is the most commonly affected joint in cases of OA.[2,3] Age, obesity, trauma, genetic predisposition, decreased levels of sex hormones, and immune system status are recognized as common risk factors for developing OA.[4,5]

Accumulating evidence identifies T helper ($T_H$) cell-mediated immunity as a critical driver of the inflammatory cascade in OA. A predominant $T_H1$-polarized response is well documented, characterized by significant infiltration of $T_H1$ cells into the synovial compartment and elevated systemic and local levels of their signature cytokines, including interferon-γ (IFN-γ) and interleukin (IL)-2.[2,6–8] Conversely, the $T_H2$ subset does not show significant quantitative changes in the peripheral blood or synovial tissues of OA patients, suggesting a limited direct role in disease immunopathogenesis.[6,9] Nonetheless, $T_H2$ cells may indirectly contribute to joint damage by facilitating B lymphocyte activation and the subsequent production of autoantibodies targeting cartilage structural components, such as collagen and osteopontin.[1,7,8]

The most common pharmacological treatments for managing pain and symptoms are non-steroidal anti-inflammatory drugs (NSAIDs), which can be administered orally or applied topically. The use of NSAIDs is associated with a variety of side effects. The most significant of these include gastrointestinal complications, such as mucosal damage and gastrointestinal bleeding; renal complications, which may involve acute kidney injury, electrolyte imbalances, and an increased risk of renal tumors; and cardiovascular complications, which encompass a heightened risk of heart attack and stroke.[2,10,11]

The potential of plant-derived compounds as therapeutic agents for OA has attracted considerable scientific interest in recent years.[12–14] Curcumin is the primary polyphenolic compound found in the plant *Curcuma longa*, commonly known as turmeric. This compound exhibits antioxidant and anti-inflammatory properties and has been utilized in the treatment of various conditions, including inflammatory skin and joint diseases, diabetes, and febrile illnesses.[15,16] Curcumin prevents the onset and exacerbation of inflammation by inhibiting key factors involved in inflammatory pathways, including lipoxygenase, cyclooxygenase, phospholipase, collagenase, elastase, and hyaluronidase. Additionally, curcumin reduces the production of inflammatory cytokines such as IL-1β and IL-8. It is considered a potential inhibitor of the secretion of inflammatory mediators from chondrocytes.[17,18]

Considering the involvement of $T_H1$-mediated inflammation in OA and the reported immunomodulatory effects of curcumin, it was hypothesized that curcumin supplementation could modify $T_H1/T_H2$ transcriptional profiles, thereby influencing systemic inflammatory responses. Therefore, the present study was designed to investigate the effects of curcumin on the expression of *T-box transcription factor 21* (*TBX21* or *T-bet*) and *GATA binding protein 3* (*GATA3*)-the master transcription factors of $T_H1$ and $T_H2$ cells, respectively-as well as their relationship with clinical and immunological parameters in patients with knee OA.

## MATERIALS AND METHODS

This study is a mechanistic sub-study and a secondary molecular analysis of biospecimens (RNAs) from our previously completed double-blind, placebo-controlled clinical trial.[19] In that trial, 30 patients diagnosed with knee OA were randomly assigned to two groups, with 15 individuals in each group, based on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire and the Kellgren-Lawrence (K-L) criteria. The first group received a daily dose of 80 mg of nano-micelle curcumin, branded as SinaCurcumin (produced by Exir Nano Sina Corporation, Tehran, Iran), for a duration of three months. The second group received a placebo daily for the same period. Moreover, a dosage of 50 mg of sodium diclofenac was administered to all patients as part of a conventional pain management therapy. For details about the CONSORT flow diagram and clinical trial registration, please refer to our previous study.[19] The present study was approved by the ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.IRH.REC.1402.043).

### RNA Extraction and cDNA Preparation

The RNA samples extracted from peripheral blood mononuclear cells (PBMCs) of all patients were obtained from our biobank. Total RNA was isolated

IN PRESS

using the Total RNA Extraction Kit (Favorgen, Taiwan) following the manufacturer's protocol. The purity and concentration of the extracted RNA were assessed spectrophotometrically using a NanoDrop™ (Thermo Fisher Scientific, USA). Samples with an A260/280 ratio between 1.8 and 2.0 were considered to have acceptable purity and were used for cDNA synthesis. RNA integrity was further confirmed by visualizing distinct 28S and 18S rRNA bands on a 1% agarose gel electrophoresis.

**SYBR Green Real-Time PCR**

Forward and reverse primers for *T-bet* (primary transcription factor of $T_H1$ lymphocytes) and *GATA3* (primary transcription factor of $T_H2$ lymphocytes) were developed in-house as target genes, with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) serving as the housekeeping gene. This was accomplished using Beacon Designer 7.9 (Premier Biosoft International, USA) (Primer sequences are provided in Supplementary Table 1); next, primer sequences were subjected to a specificity check using the Primer-BLAST tool on the NCBI website. Real-time PCR was performed on cDNA samples using a LightCycler 96 instrument (Roche, Germany) and Master Mix Green Without ROX (Ampliqon, Denmark). The SYBR Green real-time PCR conditions were initial denaturation at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 20 seconds. All reactions were performed in duplicate for each sample, and mean Ct values were used for subsequent $2^{-\Delta\Delta CT}$ analysis. Replicates showing a Ct difference greater than 0.3 were repeated to ensure accuracy and reproducibility. Expression levels of transcription factors are presented as relative expression compared to measurements taken prior to the intervention, using intra-patient normalization.

**Statistical Analysis**

After collecting and processing the initial data, the data were entered into IBM SPSS Statistics 27 (IBM Corp, USA) for analysis. The paired samples *t* test and the Wilcoxon signed-rank test were used to compare data collected before the administration of curcumin and placebo with data obtained three months later. Comparative analyses of immunological and clinical parameters between the curcumin and placebo groups were conducted using the Mann–Whitney *U* test and the independent samples *t* test. Pearson's correlation analysis was conducted to assess the relationships between immunological and clinical parameters. A significance threshold of $p<0.05$ was set for all tests.

## RESULTS

**Demographic Data for Patients with Knee OA**

A total of 30 female patients with knee OA were included in this mechanistic sub-study, with 15 receiving curcumin and 15 receiving placebo. The mean age of participants was $49.13 \pm 1.50$ years in the curcumin group and $48.26 \pm 1.32$ years in the placebo group. As reported in our previous clinical trial,[19] there were no significant differences between the two groups regarding body mass index (BMI) or disease duration.

**Gene Expression Analysis**

The expression level of the transcription factor *T-bet* gene in the group of patients who received curcumin showed a significant within-group decrease of 47% three months after curcumin consumption compared to the levels before consumption. In the placebo group, the expression level of the *T-bet* gene exhibited a non-significant decrease three months after placebo administration (Table 1, Figure 1). In the group of patients who received curcumin, the expression level of the transcription factor gene *GATA3* showed a non-significant decrease three months after the initiation of treatment compared to baseline levels. Conversely, the placebo group exhibited a non-significant increase in the expression level of this gene three months after placebo administration (Table 1, Figure 1).

Over the study period, the change in *T-bet* expression (post–pre) did not differ significantly between the curcumin and placebo groups (Table 1, Figure 1), whereas the change in *GATA3* expression was significantly greater in the curcumin group than in the placebo group (Table 1, Figure 1).

IN PRESS


Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

**Table 1. Gene expression levels of *T-bet* and *GATA3*.**

| Gene | Group | Relative Expression, Mean ± SEM | Test | *p* |
|---|---|---|---|---|
| **A. Within-group gene expression data** | | | | |
| | | **Before vs 3 months** | **Within-group test** | |
| *T-bet* | Curcumin | 1.00 ± 0.20 vs 0.53 ± 0.08 | Wilcoxon signed-rank test | 0.046 |
| | Placebo | 1.00 ± 0.24 vs 0.57 ± 0.10 | Wilcoxon signed-rank test | 0.182 |
| *GATA3* | Curcumin | 1.00 ± 0.17 vs 0.73 ± 0.15 | Paired samples test | 0.302 |
| | Placebo | 1.00 ± 0.23 vs 1.62 ± 0.17 | Paired samples test | 0.135 |
| **B. Between-group gene expression data** | | | | |
| | | **Changes from baseline to 3 months** | **Between-group test** | |
| *T-bet* | Curcumin | −0.42 ± 0.12 | Mann–Whitney *U* test | 0.806 |
| | Placebo | −0.42 ± 0.11 | | |
| *GATA3* | Curcumin | −0.26 ± 0.14 | Independent samples *t* test | <0.001 |
| | Placebo | 0.62 ± 0.15 | | |

GATA3: GATA binding protein 3; SEM: standard error of the mean; T-bet: T-box transcription factor (TBX21).

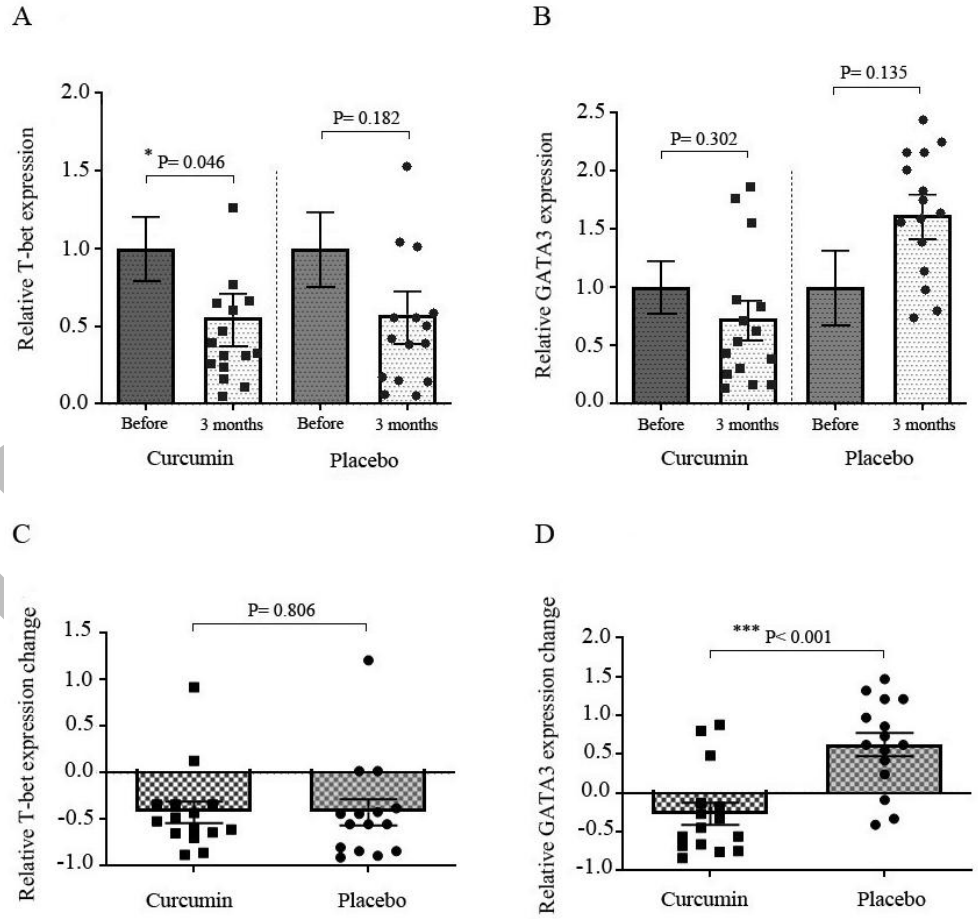


**Figure 1. Gene expression levels of *T-bet* and *GATA3*. Data are presented as mean ± SEM relative expression compared to before administration. A. Relative *T-bet* expression; B. Relative *GATA3* expression; C. Relative *T-bet* expression change; D. Relative *GATA3* expression change. GATA3 indicates GATA binding protein 3; SEM: standard error of the mean; T-bet, T-box transcription factor (TBX21).**

**Clinical and Immunological Factors Change Analysis**

Baseline and 3-month values of the visual analogue scale (VAS) score, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and the percentages of $CD4^+$ and $CD8^+$ T cells have been reported previously.[19] In the present analysis, we assessed the corresponding changes (post–pre) over the study period to facilitate the evaluation of potential correlations with changes in the expression of the target genes *T-bet* and *GATA3*.

The changes in VAS scores, CRP levels, and the percentages of $CD4^+$ and $CD8^+$ T cells from pre- to post-intervention showed a significantly greater reduction in the curcumin group compared to the placebo group, whereas no significant differences were observed in ESR changes between the two groups (Table 2, Figure 2).

**Table 2. Changes in clinical and immunological indicators during the study period**

| Clinical/immunological indicators | Group | Change from baseline to 3 months, Mean ± SEM | Between-group test | *p* |
|---|---|---|---|---|
| VAS | Curcumin | −4.53 ± 0.50 | Mann–Whitney *U* test | <0.001 |
| | Placebo | 0.64 ± 0.34 | | |
| CRP, mg/L | Curcumin | −0.91 ± 0.38 | | <0.001 |
| | Placebo | 0.68 ± 0.22 | | |
| ESR, mm/h | Curcumin | −0.4 ± 0.43 | | 0.381 |
| | Placebo | 0.06 ± 0.47 | | |
| $CD4^+$ T cells, % | Curcumin | −4.46 ± 1.41 | | <0.001 |
| | Placebo | 1.43 ± 0.31 | | |
| $CD8^+$ T cells, % | Curcumin | −4.51 ± 0.81 | | <0.001 |
| | Placebo | 1.86 ± 0.64 | | |

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SEM: standard error of the mean; VAS: visual analogue scale.

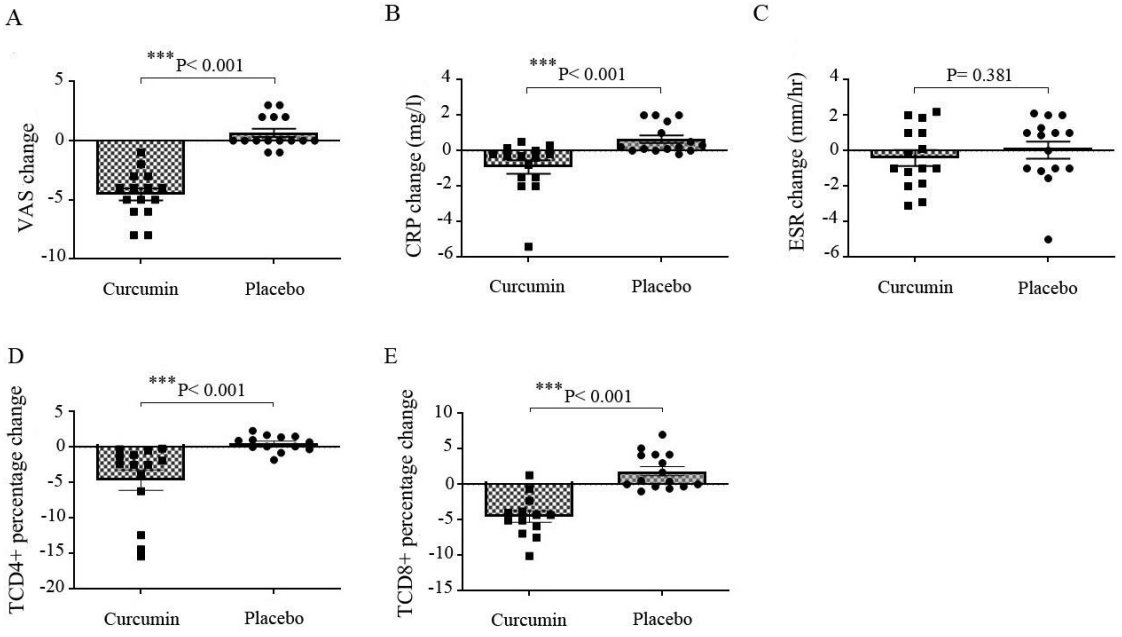


**Figure 2. Changes in clinical and immunological indicators during the study period. Data are presented as mean ± SEM. A. VAS score; B. CRP; C. ESR; D. T $CD4^+$ percentage; E. T $CD8^+$ percentage. CRP indicates C-reactive protein; ESR: erythrocyte sedimentation rate; SEM: standard error of the mean.**


Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

**Correlation Analysis**

The observed changes in clinical and immunological factors were used to evaluate potential associations with alterations in the expression of the transcription factors T-bet and GATA3 throughout the study period. Correlation analysis revealed that only two relationships reached statistical significance: a positive correlation between changes in *T-bet* expression and the frequency of $CD8^+$ T cells, and a negative correlation between changes in *GATA3* expression and ESR in the curcumin-treated group (Figure 3). No other correlations were statistically significant (Table 3).

**Table 3. Associations between changes in clinical and immunological indicators and *T-bet* and *GATA3* expression levels in the curcumin group during the study period.**

| Clinical/Immunological parameter | Relative *T-bet* expression changes | | Relative *GATA3* expression changes | |
|---|---|---|---|---|
| | ***r*** | ***p*** | ***r*** | ***p*** |
| VAS | 0.116 | 0.679 | −0.145 | 0.606 |
| CRP, mg/L | −0.374 | 0.170 | 0.086 | 0.761 |
| ESR, mm/h | −0.297 | 0.282 | −0.583 | 0.023 |
| $CD4^+$ T cells, % | 0.154 | 0.599 | −0.217 | 0.456 |
| $CD8^+$ T cells, % | 0.664 | 0.013 | −0.995 | 0.745 |

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; GATA3: GATA binding protein 3; T-bet: T-box transcription factor (TBX21); VAS: visual analogue scale.

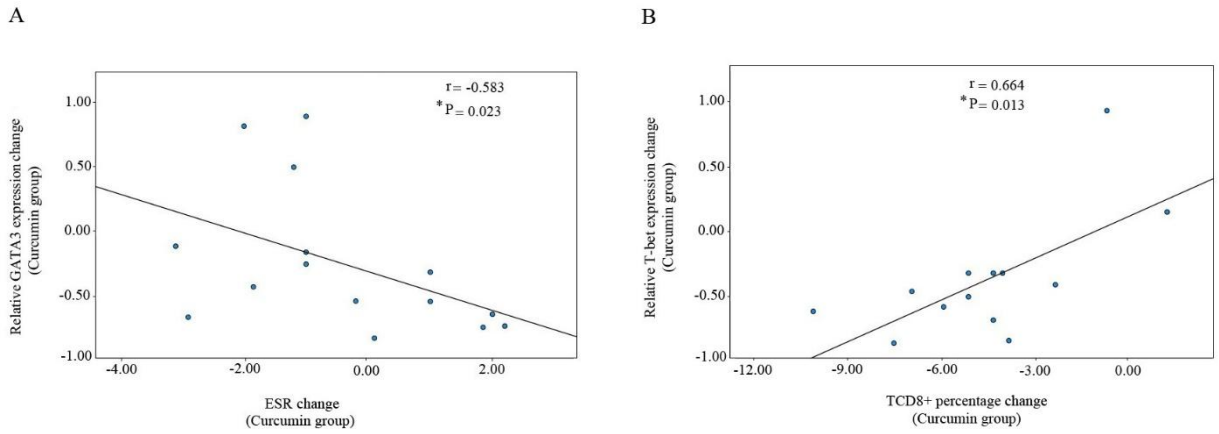


**Figure 3. Correlations between changes in *T-bet* and *GATA3* relative expression levels and immunological indicators in the curcumin group during the study period. ESR indicates erythrocyte sedimentation rate; GATA3, GATA binding protein 3; T-bet, T-box transcription factor (TBX21).**

## DISCUSSION

According to the studies conducted, the effect of curcumin on the expression of transcription factor genes specific to $T_H1$ and $T_H2$ lymphocytes in patients with OA has not yet been evaluated. The results of our study indicated that the consumption of curcumin resulted in a significant reduction in the expression of the transcription factor genes *T-bet* in patients with knee OA.

**The Effect of Curcumin on the Expression Levels of the *T-bet* Gene, the Main Transcription Factor of $T_H1$ Lymphocytes**

$T_H1$ lymphocytes are a subgroup of T lymphocytes distinguished by their primary transcription factor, T-

bet. These cells play a critical role in the pathogenesis of autoimmune diseases by secreting IFN-γ.[20–22] As a key pro-inflammatory cytokine, IFN-γ significantly influences immune responses by enhancing the expression of Toll-like receptors (TLRs) on innate immune cells, promoting the shift of antibody isotypes toward immunoglobulin G (IgG), stimulating the expression of major histocompatibility complex (MHC) molecules, facilitating antigen presentation, and activating macrophages.[23,24] The number of $T_H1$ lymphocytes and their cytokine levels, including IL-2 and IFN-γ, are elevated in patients with OA. Furthermore, the accumulation of $T_H1$ lymphocytes in the synovial fluid and synovial membrane of individuals with osteoarthritis has been documented.[6,7,25]

In our study, the administration of curcumin for three months to patients with knee OA resulted in a significant decrease in the expression level of the *T-bet* gene. As no studies have been published to date regarding the effect of curcumin on T-bet expression in patients with OA, we will utilize other related published studies for a comparative analysis of the findings. Kanakasabaia et al[26] investigated the effects of curcumin administration in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis (MS). Their findings revealed that curcumin administration increased *T-bet* expression in the spleen and lymph nodes, while simultaneously decreasing the expression of this transcription factor in the spinal cord of the mice.[26] In a study by Castro et al,[27] the administration of curcumin to type 1 diabetic mice resulted in a reduction of *T-bet* gene expression in the spleen cells of these mice. Furthermore, Khosropour et al[28] reported a decrease in *T-bet* gene expression in both the spleen and spinal cord of mice in the MS model (EAE) following curcumin administration. These findings indicate that curcumin may modulate $T_H1$ differentiation by downregulating *T-bet* expression.

**The Effect of Curcumin on the Expression Levels of the *GATA3* Gene, the Main Transcription Factor of $T_H2$ Lymphocytes**

$T_H2$ lymphocytes are a distinct subset of T cells characterized by the expression of the master transcription factor GATA3.[20–22] IL-4 is a key cytokine produced by $T_H2$ lymphocytes, playing a crucial role in regulating humoral immune responses.[9,23,29] In the context of autoimmune diseases, $T_H2$ lymphocytes are generally considered anti-inflammatory cells due to their capacity to suppress cellular immune responses.[22] The quantity of $T_H2$ lymphocytes in the peripheral blood, synovial membrane, and synovial fluid of patients with OA does not exhibit a significant difference when compared to healthy individuals. Furthermore, evidence suggests that $T_H2$ lymphocytes are unlikely to play a major role in the immunopathogenesis of OA.[6,7,9]

The results of our study indicate that administering curcumin for three months to patients with knee OA led to a non-significant reduction in the expression level of the *GATA3* gene. Considering that there is currently no available information regarding the effect of curcumin on the expression of the *GATA3* gene in patients with OA, we will compare our findings with those from other related published studies. The study conducted by Castro et al[27] demonstrated a decrease in the expression of the *GATA3* gene in the spleen cells of type 1 diabetes model mice following curcumin administration. Similarly, Chong et al[30] reported that curcumin administration reduced the expression of the *GATA3* gene in lung tissue cells of asthmatic mice. Furthermore, research by Khosropour et al[28] revealed that administering curcumin to multiple sclerosis model mice (EAE) resulted in an increase in the expression of the *GATA3* gene in both the spinal cord and spleen tissues of the mice.

**Correlation Analysis of T-bet and GATA3 Expression with Clinical and Immunological Parameters**

In curcumin-treated patients, an inverse correlation between changes in *GATA3* expression and ESR was observed throughout the study period, suggesting a potential mechanistic link between $T_H2$ polarization and systemic inflammatory status. GATA3, the master transcription factor that drives $T_H2$ differentiation, regulates the production of anti-inflammatory cytokines, including IL-4.[20,22,23] Enhanced expression of GATA3 may therefore shift the immune response toward a more immunoregulatory phenotype, contributing to the attenuation of systemic inflammation, as evidenced by reduced ESR levels. These findings suggest that curcumin may exert immunomodulatory effects by promoting a $T_H2$-biased immune response, which is associated with anti-inflammatory properties.

The observed downregulation of T-bet, the master transcription factor driving $T_H1$ lineage commitment, following curcumin administration can be mechanistically explained within an integrated

IN PRESS

immunological framework. This reduction positively correlated with decreased frequencies of $CD8^+$ T cells, suggesting a coordinated modulation of $T_H1$-mediated cellular responses. T-bet is a key regulator of the $T_H1$ cytokine network, including IL-2 and IFN-γ, which are essential for the activation, proliferation, and cytotoxic differentiation of $CD8^+$ T cells.[20] Therefore, curcumin-induced suppression of T-bet may attenuate this supportive cytokine environment, thereby limiting the pool of effector $CD8^+$ T cells. These findings align with the well-established immunomodulatory properties of curcumin, suggesting that its anti-inflammatory effects may be mediated, at least in part, through the modulation of $T_H1$-driven immunity and its associated cellular effectors. Overall, this study may provide a novel molecular perspective on the potential of curcumin to regulate $CD8^+$ T cell responses by targeting upstream $T_H1$ transcriptional programs.

The absence of a significant correlation between $T_H1/T_H2$ transcriptional markers (T-bet/GATA3) and clinical or immunological indicators underscores the multifactorial nature of systemic inflammation. While curcumin modulates adaptive immune polarization, systemic markers like CRP are primarily driven by innate immune activation—for example, interleukin-6 (IL-6) signaling from macrophages—and the hepatic acute-phase response,[18,27] which may operate independently of T-helper cell transcriptional dynamics. This dissociation highlights the potential for compartmentalized immunomodulation, wherein T-cell programming is altered without immediate reflection in systemic inflammatory markers.

**Other Points of Discussion**

The patients in the present study had been taking common medications, including diclofenac (an NSAID) and methyl salicylate ointment, to prevent the exacerbation of knee pain during the period of curcumin or placebo administration. Given that various studies have highlighted the effects of medications, including NSAIDs, on cellular processes such as the expression of inflammatory genes,[31–33] it is important to note that the expression levels of transcription factor genes in our studied patients may have also been influenced by their regular medication regimen.

Gene expression regulation within the cells is a complex process that occurs during transcription and post-transcription.[34] RNA-binding proteins (RBPs) and microRNAs are examples of molecules that play significant roles in this regulation.[35–38] Furthermore, the quantity of RNA present in a cell often does not correlate with the level of the corresponding protein, primarily due to post-transcriptional processes that influence protein levels.[34] Therefore, in this study, the effects of curcumin on the expression levels of the transcription factors T-bet and GATA3 may be affected by the previously mentioned regulatory mechanisms.

The differentiation of T lymphocytes into $T_H1$ and $T_H2$ subgroups is influenced not only by the primary transcription factors T-bet and GATA3 but also by other transcription factors, including the RUNX family and STAT.[39,40] Therefore, by simultaneously examining these transcription factors at the protein level using techniques such as flow cytometry, a more accurate interpretation of the effects of curcumin on these transcription factors—and subsequently on lymphocyte differentiation—can be achieved.

The sample size, dosage, and duration of curcumin administration are additional factors that may have influenced the study outcomes. Future studies involving larger cohorts, varied dosing regimens, and extended intervention periods are warranted to better elucidate the dose- and time-dependent effects of curcumin on immune-related gene expression.

In conclusion, to the best of our knowledge, our study was the first attempt to evaluate the effects of curcumin on the expression of transcription factor genes specific to $T_H1$ and $T_H2$ cells in patients with knee OA. A three-month administration of curcumin at a daily dose of 80 mg in patients with knee OA resulted in a significant downregulation of *T-bet* expression, which was positively correlated with $CD8^+$ T cell frequency. This finding indicates that curcumin may modulate cytotoxic T cell responses by regulating $T_H1$-associated transcriptional activity. Given the side effects associated with conventional therapy in OA, further studies on the immunomodulatory effects of curcumin could enhance our understanding of the mechanisms of action of this herbal compound, potentially paving the way for new approaches in the management and treatment of OA.

## STATEMENT OF ETHICS

The study was approved by the ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.IRH.REC.1402.043).

IN PRESS

## FUNDING

The study was funded by Mashhad University of Medical Sciences, Mashhad, Iran (the Vice Chancellor for Research; Grant No. 4011775).

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

Not applicable.

## DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author.

## AI ASSISTANCE DISCLOSURE

The authors acknowledge the use of Wordvice AI (wordvice.ai) for language editing and clarity enhancement. This tool was employed exclusively for linguistic refinement; no AI system was involved in data analysis, interpretation, or the generation of scientific content. All intellectual contributions remain solely those of the authors.

## REFERENCES

1. Courties A, Kouki I, Soliman N, Mathieu S, Sellam J. Osteoarthritis year in review 2024: Epidemiology and therapy. Osteoarthritis Cartilage. 2024;32(11):1397–404.
2. Jang S, Lee K, Ju JH. Recent Updates of Diagnosis, Pathophysiology, and Treatment on Osteoarthritis of the Knee. Int J Mol Sci. 2021;22(5):2619.
3. Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, et al. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020;11(8):854.
4. Demehri S, Kasaeian A, Roemer FW, Guermazi A. Osteoarthritis year in review 2022: imaging. Osteoarthritis Cartilage. 2023;31(8):1003–11.
5. Dong Y, Yan Y, Zhou J, Zhou Q, Wei H. Evidence on risk factors for knee osteoarthritis in middle-older aged :a systematic review and meta analysis. J Orthop Surg Res. 2023;18(1):634.
6. Li YS, Luo W, Zhu SA, Lei GH. T Cells in Osteoarthritis: Alterations and Beyond. Front Immunol. 2017;8:356.
7. Wen Z, Qiu L, Ye Z, Tan X, Xu X, Lu M, et al. The role of Th/Treg immune cells in osteoarthritis. Front Immunol. 2024;15:1393418.
8. Zou Y, Liu C, Wang Z, Li G, Xiao J. Neural and immune roles in osteoarthritis pain: Mechanisms and intervention strategies. Journal of Orthopaedic Translation. 2024;48:123–32.
9. Chow YY ,Chin KY. The Role of Inflammation in the Pathogenesis of Osteoarthritis. Mediators Inflamm. 2020;2020:8293921.
10. Katz JN, Arant KR, Loeser RF. Diagnosis and Treatment of Hip and Knee Osteoarthritis: A Review. Jama. 2021;325(6):568–78.
11. Richard MJ, Driban JB, McAlindon TE. Pharmaceutical treatment of osteoarthritis. Osteoarthritis Cartilage. 2023;31(4):458–66.
12. Fang S, Zhang B, Xiang W, Zheng L, Wang X, Li S, et al. Natural products in osteoarthritis treatment: bridging basic research to clinical applications. Chin Med. 2024;19(1):25.
13. Lee YT, Yunus MHM, Ugusman A, Yazid MD. Natural Compounds Affecting Inflammatory Pathways of Osteoarthritis. Antioxidants (Basel). 2022;11(9):1852.
14. Poursamimi J, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohajeri SA ,Ghoryani M, Mohammadi M. Immunoregulatory Effects of Krocina™, a Herbal Medicine Made of Crocin, on Osteoarthritis Patients: A Successful Clinical Trial in Iran. Iran J Allergy Asthma Immunol. 2020;19(3):253–63.
15. Hewlings SJ, Kalman DS. Curcumin: A Review of Its Effects on Human Health. Foods. 2017;6(10):92.
16. Liu S, Liu J, He L, Liu L, Cheng B, Zhou F, et al. A Comprehensive Review on the Benefits and Problems of Curcumin with Respect to Human Health. Molecules. 2022;27(14):4400.
17. Dehzad MJ, Ghalandari H ,Nouri M, Askarpour M. Antioxidant and anti-inflammatory effects of curcumin/turmeric supplementation in adults: A GRADE-assessed systematic review and dose-response meta-analysis of randomized controlled trials. Cytokine. 2023;164:156144.
18. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, et al. Anti-Inflammatory Effects of Curcumin in the Inflammatory Diseases: Status, Limitations and Countermeasures. Drug Des Devel Ther. 2021;15:4503–25.
19. Atabaki M, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohammadi M .Significant immunomodulatory properties of curcumin in patients with osteoarthritis; a successful



IN PRESS

clinical trial in Iran. Int Immunopharmacol. 2020;85:106607.
20. Evans CM, Jenner RG. Transcription factor interplay in T helper cell differentiation. Brief Funct Genomics. 2013;12(6):499–511.
21. Marks KE, Rao DA. T peripheral helper cells in autoimmune diseases. Immunol Rev. 2022;307(1):191–202.
22. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine. 2015;74(1):5–17.
23. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. Int J Mol Sci. 2019;20(23):6008.
24. Kopitar-Jerala N. The Role of Interferons in Inflammation and Inflammasome Activation. Front Immunol. 2017;8:873.
25. Nees TA, Rosshirt N, Zhang JA, Platzer H, Sorbi R, Tripel E, et al. T Helper Cell Infiltration in Osteoarthritis-Related Knee Pain and Disability. J Clin Med. 2020;9(8):2423.
26. Kanakasabai S, Casalini E, Walline CC, Mo C, Chearwae W, Bright JJ. Differential regulation of CD4(+) T helper cell responses by curcumin in experimental autoimmune encephalomyelitis. J Nutr Biochem. 2012;23(11):1498–507.
27. Castro CN, Barcala Tabarrozzi AE, Winnewisser J, Gimeno ML, Antunica Noguerol M, Liberman AC ,et al. Curcumin ameliorates autoimmune diabetes. Evidence in accelerated murine models of type 1 diabetes. Clin Exp Immunol. 2014;177(1):149–60.
28. Khosropour S, Shahvarooghi E, Rezaeizadeh H, Esmaeelzadeh M. Curcumin and Its Semisynthetic Derivative F-Curcumin Ameliorate the Expression of Cytokines in Autoimmune Encephalomyelitis Mouse Models of Multiple Sclerosis. Iran J Allergy Asthma Immunol. 2023;22(6):575–87.
29. Dong C, Fu T, Ji J, Li Z, Gu Z. The role of interleukin-4 in rheumatic diseases. Clin Exp Pharmacol Physiol. 2018;45(8):747–54.
30. Chong L, Zhang W, Nie Y, Yu G, Liu L, Lin L, et al. Protective effect of curcumin on acute airway inflammation of allergic asthma in mice through Notch1-GATA3 signaling pathway. Inflammation. 2014;37(5):1476-85.
31. Antunes DM, Rodrigues M, Guimarães DM, Duarte CME, Miguita L, Corrêa L, et al. Nonsteroidal Anti-inflammatory Drugs Modulate Gene Expression of Inflammatory Mediators in Oral Squamous Cell Carcinoma. Anticancer Res. 2019;39(5):2385–94.
32. Cho JY .Immunomodulatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) at the clinically available doses. Arch Pharm Res. 2007;30(1):64–74.
33. Pemmari A, Tuure L, Hämäläinen M, Leppänen T, Moilanen T, Moilanen E. Effects of ibuprofen on gene expression in chondrocytes from patients with osteoarthritis as determined by RNA-Seq. RMD Open. 2021;7(3):e001846.
34. Singh KP, Miaskowski C, Dhruva AA, Flowers E, Kober KM. Mechanisms and Measurement of Changes in Gene Expression. Biol Res Nurs. 2018;20(4):369–82.
35. Catalanotto C, Cogoni C, Zardo G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. Int J Mol Sci. 2016;17(10):1712.
36. Corley M, Burns MC, Yeo GW. How RNA-Binding Proteins Interact with RNA: Molecules and Mechanisms. Mol Cell. 2020;78(1):9-29.
37. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne). 2018;9:402.
38. Turner M, Díaz-Muñoz MD. RNA-binding proteins control gene expression and cell fate in the immune system. Nat Immunol. 2018;19(2):120–9.
39. Hosokawa H, Rothenberg EV. How transcription factors drive choice of the T cell fate. Nat Rev Immunol. 2021;21(3):162–76.
40. Luckheeram RV, Zhou R, Verma AD, Xia B. $CD4^+$T cells: differentiation and functions. Clin Dev Immunol. 2012;2012:925135.

IN PRESS