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The Effects of Pomegranate Juice on Proinflammatory Cytokines and Physical Function in Patients With Knee Osteoarthritis

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Background: Osteoarthritis (OA) is the most common musculoskeletal disorder causing disability and loss of function, particularly in the elderly. Preclinical and in-vitro studies for mitigation of OA symptoms suggest the protective role of pomegranate juice against OA; this effect is mainly attributed to its polyphenol content.

Objectives: This study aimed to evaluate the effects of pomegranate juice on physical function of and proinflammatory cytokines in patients with knee OA.

Patients and Methods: Thirty-eight patients with knee OA were enrolled in this randomized clinical trial. The participants were randomly divided into two groups. The pomegranate juice group (n = 19) consumed 200 mL of pomegranate juice free of sugar and additives daily for 6 weeks. The control group (n = 19) did not receive any intervention. The levels of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) were measured using serum samples and physical function of patients was evaluated according to the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) at baseline and at the end of intervention. Data were analyzed using SPSS statistical software version 17.

Results: Significant decreases in difficulty of physical function were observed after intervention in the pomegranate juice group (P = 0.01). There was no significant difference in serum levels of TNF-α and IL-1β before and after the intervention in the two study groups.

Conclusions: Taken together, pomegranate juice did not affect serum cytokines levels in patients with OA. Its role in improving physical function in these patients most likely involves other mechanisms.

Keywords: Osteoarthritis; Pomegranate; Tumor Necrosis Factor-Alpha; Interleukin-1beta

1. Background

Osteoarthritis (OA) is the most common musculoskeletal disorder that leads to disability and loss of function, particularly in the elderly (1, 2). The prevalence of knee OA is 7.9% worldwide and 15.3% in the urban areas of Iran (3). Prevalence of OA is high among women and the elderly. The most common risk factors for OA are age, sex, race, obesity, joint trauma, and genetics (4). The major pathological characteristics of OA (progressive loss of articular and subchondral bone) are associated with excessive production of proinflammatory molecules such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) (5). Proinflammatory cytokines contribute to the progression of OA by up-regulating metalloproteinase gene expression, stimulating reactive oxygen species (ROS) production, altering chondrocyte metabolism, and possibly by increasing osteoclastic bone resorption (6). These cytokines are also involved in the degradation of connective tissue by stimulating collagenase (7).

Currently, no treatment exists for this disease. The available treatments including pharmacological and non-pharmacological therapies focus on reducing symptoms (pain and inflammation), maintaining joint mobility, and limiting the loss of function. Long-term use of available pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs) is associated with serious adverse effects including promoting cardiovascular diseases and gastrointestinal disorders (1, 5, 8).

In recent years, there has been an increasing interest in alternative and safe strategies for mitigation of symptoms. Preclinical and in vitro studies have suggested that the protective role of pomegranate was attributable to dietary polyphenols.

Pomegranate fruit is native to Asia, especially Iran, but it is popular worldwide owing to its benefits in many diseases such as cancer, diabetes, and cardiovascular diseases (9-11). Edible parts of pomegranate comprise

80% juice and 20% seed. There are two types of polyphenolic compounds, 1) anthocyanins such as delphinidin, cyanidin, and pelargonidin and 2) hydrolysable tannins such as punicalin, pedunculagine, punicalagin, and gallagic and ellagic acid esters of glucose. Both these types of polyphenolic components are found in abundance in pomegranate and are attributable to pomegranate's antioxidant properties (5). Pomegranate is a good source of vitamin C, providing between 10% and 20% of the recommended daily requirement per cup. It is rich in anthocyanins, which possess antioxidant and anti-inflammatory capabilities, and are responsible for the brilliant color of pomegranate juice (PJ) (1, 10). Related studies suggested that cyclooxygenase2 (COX2) and lipoxygenase activity and production of prostaglandin E2 (PGE-2) were inhibited and the synthesis of type2 collagen was activated in human chondrocytes by prodelphinidines (12, 13).

Recent evidence suggests that pomegranate extract has anti-inflammatory effects and protects chondrocytes against IL-1-induced cartilage damage in vitro (13). Hadipour Jahromy et al. demonstrated that feeding PJ to OA model mice improved histopathological damage of knee joints (14). These studies suggested that pomegranate could be effective in mitigation of OA symptoms. To the best of our knowledge, no clinical trial on the effect of pomegranate juice in patients with knee OA has been published.

2. Objectives

This study aimed to evaluate the effects of PJ on physical function of and proinflammatory cytokines in patients with knee OA.

3. Patients and Methods

3.1. Participants

Participants of this randomized clinical trial were patients with knee OA according to the American College of Rheumatology (ACR) criteria (based on pain history, clinical examination, radiological and laboratory findings) and who had no allergy to pomegranate or contraindication to PJ. The age range of the participants was 30 - 80 years. The exclusion criteria included conditions such as rheumatoid arthritis, diabetes mellitus, cardiovascular, liver, and renal diseases, cancer, and pregnancy. Patients who consumed commercially prepared antioxidant supplements and/or were treated with oral or injected corticosteroids agents within 4 weeks or 6 months prior to the first visit were also excluded. Sample size was estimated based on a statistical power of 95%, on the basis of a study conducted by Shadmanfard et al. (15).

3.2. Study Design

Thirty-eight patients with knee OA (34 females and 4 males) were enrolled in this study. The patients were randomly divided into two groups: The intervention group (n = 19) consumed 200 mL sugar- and additive-free PJ daily for 6 weeks. Takdaneh Agro-industrial company produced the PJ used for the study. The control group did not receive any intervention. We asked patients in the supplement group to continue their usual lifestyles during the study.

3.3. Measurements

Demographic characteristics were determined by interview; weight and height were measured according to standard protocols with participants in light clothes and without shoes, respectively. Body mass index (BMI) was calculated by: weight (kg)/height^2 (m^2).

Fasting blood samples (10 mL) were taken and subjected to centrifugation and serum separation for biochemical analyses. The levels of TNF-α and IL-1β were measured using the serum samples by an immunoassay method by Boster Immunoleader Human TNF-α ELISA kit (cat No: EK0525) and Boster Immunoleader Human IL-1β kit (cat No: EK0392), respectively. To evaluate physical function of patients, a 17-item questionnaire about the degree of difficulty for certain activities of daily living based on the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) was used at baseline and end of study (16).

3.4. Statistical Analysis

Data were analyzed using SPSS statistical software version 17 by independent-samples t-test and paired-samples t-test for parametric variables and Man-Whitney or Wilcoxon signed rank test for nonparametric variables and Chi-square for nonquantitative variables. P values less than 0.05 were considered statistically significant. This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and registered in the Iranian Registry of Clinical Trials (IRCT number: IRCT2014031517017N1).

4. Results

Demographic characteristics of the participants at baseline are shown in Table 1. There were no significant differences with regard to demographic characteristics and variables at baseline between the groups. About 90% of the participants were female. The mean BMI in PJ and control groups was 32.4 ± 4.5 and 29.3 ± 5.5, respectively, which was not statistically significant (P = 0.07). Twelve patients in the PJ group and 10 patients in the control group were obese (BMI ≥ 30) (P > 0.05).

Patients treated with PJ reported significant decrease in the score of difficulty of physical function compared with controls. Although the mean TNF-α levels decreased in the PJ supplement group after treatment, a significant difference in TNF-α levels was not observed between the two groups (P > 0.05). The IL-1β levels also did not differ significantly between the two groups (Table 2).

Table 1. Baseline Characteristics of 38 Patients With Knee Osteoarthritis in the Pomegranate Juice Supplement Study (n= 19) [a]

Variables	Pomegranate Juice	Controls	P Value
Age, y [b]	56.74 ± 10.23	53.84 ± 11.95	0.42
Gender [c]			1.000
Male	2 (10.52)	2 (10.52)	–
Female	17 (89.47)	17 (89.47)	–
Weight, kg [b]	79.68 ± 13.80	72.47 ± 14.86	0.13
Height, cm [b]	155 ± 6.55	157 ± 7.54	0.42
Physical activity [c]			0.572
Very low	8 (42.10)	9 (47.36)	–
Low	8 (42.10)	9 (47.36)	–
Moderate	3 (15.78)	1 (5.26)	–
High	–	–	–
Education [c]			0.910
Illiterate	6 (31.57)	7 (36.84)	–
High school	8 (42.10)	8 (42.10)	–
University	5 (26.31)	4 (21.05)	–

[a] TNF-α = tumor necrosis factor α; IL-1β = interleukin1β.
[b] Data are expressed as mean ± SD and were tested using by Independent-Samples t-test.
[c] Data are expressed in number (percentage) and were tested using Chi-Square test.

Table 2. Comparison of Variables at Baseline and 6 Weeks after Intervention in 38 Patients With Knee Osteoarthritis in the Pomegranate Juice Supplement Study (n = 19) [a]

Variables	Pomegranate Juice	Controls	P Value
Physical function [b]			
Baseline	27.74 ± 10.56	25.47 ± 14.12	0.57
Final	22.53 ± 11.19	26.68 ± 14.35	0.32
P value	0.01	0.20	–
TNF-α, pg/mL [b]			
Baseline	29.02 ± 10.92	24.59 ± 6.24	0.134
Final	26.09 ± 10.18	29.44 ± 11.82	0.356
P value	0.472	0.131	–
IL1β, pg/mL [c]			
Baseline	12.7 (6.4)	14.350 (9.8)	0.248
Final	10.7 (3.9)	15.15 (9.4)	0.052
P value	0.223	0.133	–

[a] TNF-α = tumor necrosis factor α; IL1β = interleukin1β.
[b] Data are expressed as mean ± SD and tested using Paired Sample t-test or Independent-Samples t-test.
[c] Data are expressed as median (IQR = Interquartile range) and tested using Wilcoxon signed-rank test or Mann-Whitney.

5. Discussion

This study showed that consumption of 200 mL PJ daily for 6 weeks improved physical function in daily activities. To the best of our knowledge, this study is the first clinical trial on the effect of PJ in patients with OA. The effect of consumption of 10 mL pomegranate extract (polyphenol component equivalent to that in 200 mL PJ) for 12 weeks in patients with rheumatoid arthritis (RA) was investigated by Balbir-Gurman et al. (17). The results of their pilot study showed no change in Health Assessment Questionnaire Disability Index (HAQ-DI) in 6 patients with RA. This inconsistency may be owing to differences in study participants and to the small sample size of that study.

Recently, the use of anti-inflammatory or antioxidants supplements and vitamins has been suggested for the

management of joint problems. Schumacher et al. (18) conducted a randomized double blind crossover study, which reported the effects of 8 oz tart cherry juice intake (contains 50 mg gallic acid and 450 mg anthocyanins) for 6 weeks. This intervention was effective in improving physical function score in the cherry juice group. According to the author's opinion, improved physical function was associated with decline in hsCRP in the cherry juice group. Long-term treatment (8 months) with Mariva, a curcumin-phosphatidylcholine complex, improved physical function score of patients with OA in a controlled clinical trial (19). This was accompanied with a decrease in the levels of IL-1β and IL-6 in the treatment group. These findings are consistent with our results and suggest that administration of antioxidants or anti-inflammatory dietary compounds may be effective in alleviating symptoms of OA. Antioxidant and anti-inflammatory activity of PJ, attributable to anthocyanins and hydrolyzable tannins, is predicted to alleviate the symptoms of knee OA. This may have occurred via inhibition of IL-1β-induced proteoglycan breakdown in cartilage explants and expression of matrix metalloproteinases (MMPs) 1, 3, and 13, which was previously reported (13).

No significant differences were observed in serum TNF-α and IL-1β levels between the two groups. The hallmark of OA is imbalance between anabolic synthesis or repair of matrix components by growth factors and catabolic breakdown of matrix by inflammatory cytokines, MMPs, aggrecanases, PGE-2, and proteases (1). Excessive production of proinflammatory molecules, such as IL-1β and TNF-α, activates a cascade of intra-articular signal transduction mechanisms. These cytokines stimulate chondrocytes to degrade the surrounding cartilage matrix and secrete PGE-2 and destructive enzymes, which leads to exacerbation of clinical symptoms (20). Concentration of TNF-α in the synovial fluid significantly correlates to the WOMAC score in the study conducted by Orita et al. (21).

Administration of PJ in an experimental study of OA was investigated by Hadipour-Jahromy et al. (14). They observed that pomegranate juice prevented the negative effects of monoiodoacetate (MIA) in mice in a dose-dependent manner. Histopathological changes in knee osteoarthritic joints indicated that at the highest dose of PJ, chondrocyte organization was preserved by approximatly 50% and the number of cells was increased around 65% with less damage to proteoglycan over the treatment. No inflammatory cells or cell proliferation in the synovial fluid were observed in either of the PJ groups (high-dose or low-dose). These changes showed chondro-protective and anti-inflammatory effects of PJ in a mouse model of MIA-induced osteoarthritis. Shukla et al. investigated the effect of pomegranate extract (PE) on inflammatory cytokines in arthritic joints in an RA model (22). The results indicated no statistically significant difference in levels of TNF-α and IL-1β in the arthritic joints of PE-fed mice compared to that in non-fed mice. This finding was consistent with our results. Although a 12 week consumption study of PJ in patients with diabetes did not affect plasma TNF-α concentration (23), findings from an in vitro study demonstrated that punicalagin treatment had inhibitory effect on lipopolysaccharide-induced release of IL-1β and TNF-α in RAW264.7 cells, by suppressing the activation of toll-like receptor 4 (TLR4)-mediated mitogen activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) signaling pathways (24). More clinical trials are needed to explain this conflict of results in in-vitro and in-vivo studies.

To our knowledge, this study is the first randomized clinical trial to evaluate the effects of PJ in patients with OA. This is the strength of the current study. Moreover, we used a control group comprising patients with OA to compare the results with the intervention group. Study limitations include inability to use a placebo and lack of evaluation of the concentration of the cytokines in the synovial fluid of the knee, which would be the purported site of action for the signals. Therefore, it is recommended that these cytokines be evaluated in the joints in future studies.

In conclusion, intake of PJ improved physical function in daily activities but did not affect serum cytokine levels in patients with OA. This effect may be owing to other mechanisms that were not investigated in this trial. Further studies with a longer intervention period and a larger sample size in a double blind placebo-controlled setting are needed to evaluate the protective role of PJ against OA.

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Authors' Contributions

Study concept and design: Nasrin Ghoochani, Majid Karandish, Karim Mowla, Mohammad Hossein Haghighizadeh, Mohsen Khorami, Mohammad Taha Jalali. Acquisition of data: Nasrin Ghoochani, Majid Karandish, Karim Mowla, Mohsen Khorami. Analysis and interpretation of data: Nasrin Ghoochani, Majid Karandish, Mohammad Hossein Haghighizadeh. Drafting of the manuscript: Nasrin Ghoochani, Majid Karandish, Karim Mowla, Mohammad Hossein Haghighizadeh, Mohsen Khorami, Mohammad Taha Jalali. Critical revision of the manuscript for important intellectual content: Nasrin Ghoochani, Majid Karandish, Karim Mowla, Mohammad Hossein Haghighizadeh, Mohsen Khorami, Mohammad Taha Jalali. Statistical analysis: Nasrin Ghoochani, Majid Karandish, Karim Mowla, Mohsen Khorami. Administrative, technical, and material support: Nasrin Ghoochani, Majid Karandish. Study supervision: Nasrin Ghoochani.

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