Turk J Phys Med Rehab 2024;70(3):335-343
DOI: 10.5606/tftrd.2024.13186
Available online at www.turkishjournalpmr.com

Original Article

Clinical effect of acupuncture on knee osteoarthritis and its effect on p38 MAPK signaling pathway

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ABSTRACT

Objectives: This study aims to investigate the curative efficacy of acupuncture on knee osteoarthritis (KOA) and its improvement on related scores and blood indexes.

Patients and methods: Between January 2019 and January 2020, a total of 108 patients (48 males, 60 females; mean age: 61.0±6.8 years; range 43 to 79 years) with KOA were randomly divided into control group (n=54) and patient group (n=54). Both groups received standard treatment, including adequate rest and exercise and oral celecoxib capsules. The patient group performed acupuncture operations on the Inner knee eye (EX-LE4), outer knee eye (EX-LE5), Yanglingquan (GB34), and Zusanli (ST36). In the control group, three non-acupuncture points were determined for sham acupuncture. The level of Michel Lequesne index of severity for osteoarthritis (ISOA) score, Visual Analog Scale (VAS), Lysholm Knee Score Scale (LKSS), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interleukin-1beta (IL-1β), IL-6, transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2) and p38 mitogen-activated protein kinase (p38 MAPK) were compared before and after treatment.

Results: The reduction of inflammatory markers in the patient group was greater than that in the control group after treatment. The levels of cytokines such as TGF-β, IGF-1, and FGF-2 were significantly increased after treatment, and the levels in the patient group were higher than those in the control group during the same period. In addition, p38 MAPK messenger ribonucleic acid (mRNA) was significantly downregulated after treatment, and the level in the patient group was lower than that in the control group during the same period.

Conclusion: Acupuncture combined with standard treatment can effectively promote the relief of symptoms and the improvement of knee joint function and effectively inhibit the expression of p38 MAPK signaling pathway.

Keywords: Acupuncture; inflammation; knee osteoarthritis; p38 MAPK signaling pathway.

Knee osteoarthritis (KOA) is an usual degenerative disease of knee joint in middle-aged and elderly individuals.[1] At the time of onset, the symptoms are mild only with joint pain and obvious discomfort. When the symptoms become more severe, the patient can develop into disability, which would not only greatly affect the mental health and quality of life of patients, but also greatly increase the economic burden of patients. Currently, the coming of the aging society would greatly increase the incidence of KOA, and the impact on medical treatment and society cannot be ignored.[2] The clinical treatment of KOA is mainly conservative, including active exercise recovery and the use of non-steroidal anti-inflammatory drugs (NSAIDs). However, the long-term use of exercise therapy is a challenge for most patients, and the gastrointestinal reaction and kidney damage of NSAIDs also limit the long-term use of these drugs.[3] In addition, total knee replacement is also a surgical treatment for KOA patients, if necessary. However, given the high risk of surgery and the additional cost of treatment, finding new treatments remains an extremely important priority for this study.

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Received: June 02, 2023 **Accepted:** November 24, 2023 **Published online:** July 17, 2024

Cite this article as: Wei Y, Liu L, Ge H. Clinical effect of acupuncture on knee osteoarthritis and its effect on p38 MAPK signaling pathway. Turk J Phys Med Rehab 2024;70(3):335-343. doi: 10.5606/tftrd.2024.13186.

Acupuncture has a long history. It has been practiced in China for more than 2,500 years and has been used to treat various types of chronic pain. Clinical studies have shown that acupuncture has a better long-term effect on KOA than operative treatment and has few side effects on the body.[4,5] In recent years, many studies at home and abroad have reported the exact efficacy of acupuncture in relieving joint pain and promoting joint function recovery in patients with KOA.[6-8] A prospective, randomized-controlled clinical trial showed that acupuncture treatment was similar with physical therapy in pain relief and functional improvement in KOA, and did not differ significantly.[9] An updated meta-analysis showed that acupuncture therapy was superior to the pharmacotherapy group in alleviating pain in KOA in the short term and in the long term.[6] The 2019 American College of Rheumatology (ACR) updated evidence-based guidelines for the comprehensive treatment of Osteoarthritis recommend the use of acupuncture for osteoarthritis patients.[10]

Previous studies have shown that the early and progressive stages of KOA are often accompanied by inflammation and are associated with cartilage degeneration and defect.[11] Some inflammatory factors, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), can be detected in early KOA, indicating that inflammatory factors are associated with the occurrence and development of KOA. Moreover, since cartilage lesions can easily lead to osteoarthritis, changes in the levels of some growth factors related to cartilage formation, such as transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), also affect the occurrence and development of KOA.[12,13] The p38 mitogen-activated protein kinase (MAPK) signaling pathway has been shown to be associated with inflammation.[14] Wei et al.[15] reported that inhibition of the p38 MAPK signaling pathway was associated with delayed progression of KOA. Wang et al.[16] indicated that electroacupuncture could significantly inhibit the activation of p38 MAPK and improve the inflammatory response of 2, 4-dinitrofluorobenzene induced contact dermatitis in rats. In addition, the p38 MAPK signaling pathway plays a key role in cartilage injury and apoptosis.[17,18] Based on the aforementioned study, patients with KOA were treated with acupuncture and sham acupuncture combined with standard treatment. By evaluating the therapeutic effect, the differences in cytokines, inflammatory factors and p38 MAPK signaling pathway before and after treatment were compared to provide more valuable data reference for clinical practice.

PATIENTS AND METHODS

This study was conducted at Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Department of Needle Knife between January 2019 and January 2020. A total of 108 patients (48 males, 60 females; mean age: 61.0±6.8 years; range 43 to 79 years) patients with KOA were included in the study. The diagnosis of KOA was based on the clinical standards of the ACR.[19] Inclusion criteria were as follows: *(i)* knee pain occurring within the last one month; *(ii)* X-ray showing osteophyte formation; *(iii)* the joint fluid test meeting the osteoarthritis standard; *(iv)* morning stiffness for ≤30 min; *(v)* bone fractures; *(vi)* unilateral KOA; and *(vii)* Kellgren-Lawrence (KL) Grades 2-3. Exclusion criteria were as follows: *(i)* other rheumatic diseases, such as gouty arthritis, or rheumatoid arthritis; *(ii)* previous history of knee surgery; *(iii)* mental disorders; *(iv)* coagulation disorders, immune dysfunction; *(v)* malignant tumors; and *(vi)* serious cardiovascular and cerebrovascular diseases.

Randomization

The patients were randomly grouped into control group (n=54) and patient group (n=54) based on a list of computer-generated random numbers, which were placed in opaque sealed envelopes. The control group received standard treatment plus sham acupuncture treatment, and the patient group received standard treatment plus acupuncture treatment.

Interventions

Standard treatment refers to teaching and counseling KOA patients and reminding them to keep their joints warm in daily life. Individuals with obesity are advised to exercise relevant functions properly after losing weight, minimize weight-bearing activities and strenuous exercise, with the help of walking sticks when necessary, and wear professional knee pads to protect the knee joint. At the same time, each patient was given celecoxib capsules (Jiangsu Hengrui Pharmaceuticals, China) orally, 200 mg/day, once a day, with warm water after meals. The standard treatment period was four weeks.

Acupuncture treatment was carried out according

to the acupoints determined after diagnosis and treatment of traditional Chinese medicine (TCM) syndrome differentiation. Acupoints: Inner knee eye (EX-LE4), outer knee eye (EX-LE5), Yanglingquan (GB34), Zusanli (ST36). The patient was seated with the knees bent and the knee joint at 90°. After routine disinfection of the acupoints, sterile needle was used to acupuncture the acupoints. The depth of the needles was about 0.5 to 1.0 cun (cun is a unit of length developed in ancient China, 1 cun ≈ the width of the patient's own thumb knuckle). After Deqi (Deqi is a sensation that refers to the skin tension around the needle felt by the physicians during the acupuncture process, as well as numbness and sore swelling at the acupoint), a small range of lifting and thrusting twist was performed, and the needles were removed 30 min later. Acupuncture was performed once a day in the morning and six times a week for four weeks.

Sham acupuncture treatment refers to the selection of non-acupoints close to acupoints, shallow injection of sterile needles into the skin. The sham acupuncture group neither needed to obtain "Deqi" in the process of acupuncture, nor did they need to twist or lift the needles. The locations of acupoints and non-acupoints are shown in Table 1.

Assessment and clinical indicators

1) Knee function evaluation

Michel Lequesne index of severity for osteoarthritis (ISOA) score,[20] Visual Analog Scale (VAS),[21] and Lysholm Knee Scoring Scale (LKSS)[22] were used to evaluate the changes of knee function in the two groups before and four-week after treatment, respectively.

ISOA: The score is evaluated for pain and discomfort, walking ability and involvement degree of daily life. The normal score is 0, and the heaviest score is 8. The total score of 1 to 4 is mild, 5 to 7 is moderate, 8 to 10 is severe, 11 to 13 is very severe, and ≥14 is extremely severe.

VAS: Pain is rated on a scale of 0 to 10, with higher scores indicating more severe pain.

LKSS: The scale contains eight different items: pain, instability, lock-in, swelling, lameness, stair climbing, squatting, and use of support. The maximum score is 100, with higher scores indicating less disability.

2) Laboratory examination

Fasting venous blood of patients was collected before treatment and four weeks after treatment, and various indexes of patients were detected by automatic biochemical analyzer. Serum was isolated from whole blood after centrifugation, and the following indexes were detected: C-reactive protein (CRP), IL-1β (IL-1β), IL-6, TGF-β, IGF-1, and FGF-2. The erythrocyte sedimentation rate (ESR) was detected by an automatic ESR analyzer. Elevated levels of the pro-inflammatory cytokines, IL-1β and IL-6, indicate inflammation in the body and an inflammatory response is activated. The CRP is an acute phase protein synthesized by liver cells when the body is subjected to inflammatory stimuli such as microbial invasion or tissue damage. In addition, patients with acute inflammation tended

TABLE 1
Locations of acupoints and non-acupoints

Description	International code	Locations
		Acupoints
Inner knee eye	EX-LE4	Located in the depression outside the patella and patellar ligament when the knee is bent 90 degrees.
Outer knee eye	EX-LE5	Located in the depression inside the patella and patellar ligament when the knee is bent 90 degrees.
Yanglingquan	GB34	On the outside of the calf, in the depression of the anterior and inferior fibula head.
Zusanli	ST36	It is exactly 3 cun below EX-LE4.
		Non-acupoints
Point 1	-	Replace EX-LE4. Located on the non-acupoint 1 cun below EX-LE4.
Point 2	-	Replace GB34. Located on the non-acupoint 0.5 cun behind GB34.
Point 3	-	Replace ST36. Located on the non-acupoint 3 cun behind ST36.

Cun is a unit of length. The cun described in Chinese medicine is a method of determining the location of acupoints based on the patient's own fingers. Due to individual differences, 1 cun is equivalent to the width of a patient's thumb knuckle.

to have an increased ESR, which could be determined when the patient's ESR was not less than 20 mm/h) The expression of p38 MAPK messenger ribonucleic acid (mRNA) was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA in serum was extracted for reverse transcription, and the complementary deoxyribonucleic acid (cDNA) obtained by reverse transcription was amplified for PCR reaction. The reaction conditions were as follows: pre-denaturation at 95° for 1 min, followed by 40 cycles of denaturation at 95° for 30 sec, annealing at 60° for 15 sec, and extension at 72° for 20 sec. The β-actin acts as the internal parameter.

3) Criterion of curative effect

Clinical control: Knee pain disappeared or almost disappeared, and the affected joint moved freely. Markedly effective: significant relief of knee pain with occasional mild pain or limited mobility. Effective: Knee joint pain was relieved, but there was still pain and limited activity. Ineffective: No improvement in pain or mobility restriction. Total effective rate (%) = (number of clinical control cases + number of markedly effective cases + number of effective cases)/ total number of cases × 100%.

Statistical analysis

Study power and sample size calculation were performed using the G*Power version 3.1.3 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Considering 95% confidence interval (CI) and 80% power of the study, the minimum sample size was determined to be 108 cases, 54 cases in the study group and 54 cases in the control group according to a 1:1 ratio.

Statistical analysis was performed using the SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The normality of the quantitative data was assessed using the Kolmogorov-Smirnov test. The paired t-test was used for comparison of differences within groups, and independent sample t-test was used for comparison of differences between groups. The Pearson correlation analysis was performed to evaluate the correlation between p38 MAPK and various indicators before and after treatment. A p value of <0.05 was considered statistically significant.

RESULTS

Comparison of baseline data

Baseline data of patients are shown in Table 2. There was no significant difference in age, sex ratio, body mass index (BMI), duration of disease, and location of injury between the two groups (p>0.05).

Comparison of knee function scores, laboratory biochemical indexes, and p38 MAPK protein levels before and after treatment

As shown in Table 3, before treatment, there was no significant difference in the Michel Lequesne scores between the two groups. After treatment, the scores of both groups were significantly lower than those before treatment (p<0.001). After treatment, the Michel Lequesne score in the patient group was lower than that in the control group (p<0.01). The VAS scores showed no significant difference between the two groups before treatment. The VAS scores

TABLE 2
Baseline data of participants

	Control group (n=54)		Patient group (n=54)		
Items	n	Mean±SD	n	Mean±SD	p
Age (year)		61.1±6.6		61.1±7.1	0.978
Sex					0.439
Male	22		26		
Female	32		28		
Body mass index (kg/m^2)		23.23±2.01		23.13±1.98	0.997
Disease duration (year)		3.94±1.51		4.27±1.55	0.773
Location of injury					0.441
Left	24		25		
Right	30		29		

SD: Standard deviation.

TABLE 3
Comparison of various indexes between the two groups

	Control group			Patient group			
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment		
Items	Mean±SD	Mean±SD	p^1	Mean±SD	Mean±SD	p^2	p^3
Michel Lequesne (score)	8.54±1.62	4.00±1.44	<0.001	8.46±1.42	3.15±1.37	<0.001	0.002
VAS (score)	4.11±1.19	2.93±1.08	<0.001	4.39±1.09	1.33±1.03	<0.001	<0.001
LKSS (score)	49.98±5.55	72.91±7.40	<0.001	50.83±5.55	86.72±8.59	<0.001	<0.001
ESR (mm/h)	21.91±10.22	11.18±2.55	<0.001	21.66±10.30	9.72±3.59	<0.001	0.017
CRP (mg/L)	8.26±3.01	2.07±0.30	<0.001	8.20±3.15	1.90±1.01	<0.001	<0.001
IL-1β (ng/mL)	87.26±10.59	64.91±9.24	<0.001	85.18±10.02	45.40±6.74	<0.001	<0.001
IL-6 (pg/mL)	233.75±38.53	163.67±20.91	<0.001	225.68±43.14	102.29±16.14	<0.001	<0.001
TGF-β (μg/L)	19.07±9.20	26.27±7.00	<0.001	19.59±9.92	31.04±5.46	<0.001	<0.001
IGF-1 (μg/L)	79.94±9.67	89.87±7.40	<0.001	79.89±11.08	95.16±8.12	<0.001	0.001
FGF-2 (μg/L)	21.70±2.84	26.42±4.57	<0.001	22.88±2.93	31.85±3.41	<0.001	<0.001
p38 MAPK	1.02±0.22	1.00±0.19	<0.001	0.82±0.20	0.32±0.03	<0.001	<0.001

SD: Standard deviation; VAS: Visual Analog Scale; LKSS: Lysholm Knee Scoring Scale; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; IL-1β: Interleukin-1beta; IL-6: Interleukin-6; TGF-β: TGF-beta; IGF-1: Insulin-like growth factor-1; FGF-2: Fibroblast growth factor-2; p38 MAPK: p38 mitogen-activated protein kinase; p^1 Represents the intra-group comparison of the control group; p^2 represents intra-group comparison of the patient group; p^3 represents an intergroup comparison between the patient group and the control group after treatment.

in both groups were significantly lower than after treatment, and the scores in the patient group were lower than those in the control group (p<0.001). After treatment, the LKSS scores in both groups were significantly higher than before treatment. Notably, the LKSS scores after treatment was higher in the patient group than in the control group (p<0.001). The results of inflammatory indicators showed that after treatment, ESR, CRP, IL-1β, and IL-6 in both groups were significantly downregulated compared to pre-treatment values. After treatment, the levels of these indicators in the patient group were lower than those in the control group (p<0.05). Moreover, the results of growth factor assessment showed that the serum levels of TGF-β, IGF-1, and FGF-2 were significantly upregulated after treatment than before, and the above growth factors were higher in the patient group after treatment than in the control group (p<0.001). Additionally, qRT-PCR showed that the level of p38 MAPK mRNA in the blood of both groups decreased significantly after treatment. Meanwhile, the reduction of p38 MAPK was greater in the patient group after treatment than in the control group (p<0.001).

Comparison of clinical efficacy

The evaluation of clinical efficacy was summarized in Table 4. The overall effective rate of the patient group was 90.74%, which was significantly higher than that of the control group (72.22%) (p<0.001), indicating that the efficacy of the study group was better than that of the control group.

TABLE 4
Comparison of clinical efficacy between the two groups

	Control group (n=54)		Patient group (n=54)		
Satisficing	n	%	n	%	p
Clinical control	7	12.96	12	22.22	
Markedly effective	25	46.30	27	50.00	
Effective	7	12.96	10	18.52	<0.001
Ineffective	15	27.78	5	9.26	
Total effective rate	39	72.22	49	90.74	

TABLE 5
Correlation analysis between p38 MAPK and various indicators

	Pre-treatment		Post-treatment	
Characteristics	r	*p*	r	*p*
Michel Lequesne score	0.493	<0.001	0.477	<0.001
LKSS (score)	−0.381	0.005	−0.309	0.023
VAS (score)	0.318	0.019	0.274	0.045
ESR (mm/h)	0.401	0.003	0.516	<0.001
CRP (mg/L)	0.683	<0.001	0.644	<0.001
IL-1β (ng/mL)	0.515	<0.001	0.541	<0.001
IL-6 (pg/mL)	0.726	<0.001	0.741	<0.001
TGF-β (μg/L)	−0.532	<0.001	−0.378	0.005
IGF-1 (μg/L)	−0.307	0.024	−0.307	0.024
FGF-2 (μg/L)	−0.417	0.002	−0.326	0.016

p38 MAPK: p38 mitogen-activated protein kinase; LKSS: Lysholm Knee Scoring Scale; VAS: Visual Analog Scale; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TGF-β: TGF-beta1; IGF-1: Insulin-like growth factor-1; FGF-2: Fibroblast growth factor-2.

Correlation analysis between p38 MAPK and each indicator

The correlation analysis results of p38 MAPK level and each index in the patient group before and after treatment are shown in Table 5. The results showed that the levels of ISOA score, VAS score, LKSS score, ESR, CRP, IL-1β, IL-6, TGF-β, IGF-1, and FGF-2 in the patient group were correlated with the levels of p38 MAPK before and after treatment. Among them, the LKSS score, TGF-β, IGF-1, and FGF-2 were significantly negatively correlated with p38 MAPK, while the ISOA score, VAS score, ESR, CRP, IL-1β, and IL-6 were significantly positively correlated with p38 MAPK. Among these markers, inflammatory markers such as ESR, CRP, IL-1β, and IL-6 showed a strong correlation with p38 MAPK.

DISCUSSION

Acupuncture, as one of the important treatment methods of TCM, has a certain curative effect on the treatment of KOA.[23] Acupoints selected for the treatment of KOA are mainly those around the knee joint, which is determined according to the anatomical structure of the knee joint.[24] In the present study, we selected four acupoints, namely, inner knee eye (EX-LE4), outer knee eye (EX-LE5), Yanglingquan (GB34), and Zusanli (ST36). The function of the aforementioned acupoints is to promote blood circulation, and they are often used to treat leg pain, joint pain, peripheral soft tissue inflammation and other skeletal muscle diseases caused by various reasons.

In the current study, there was no significant difference in baseline data between the two groups of patients, indicating that the two groups of patients have the research value of comparative study. The results in this study showed that the overall curative effect of acupuncture combined with standard treatment on KOA was significantly higher than that of sham acupuncture combined with standard treatment (90.74% *vs.* 72.22%). After four weeks of treatment, acupuncture combined with standard treatment can significantly reduce Michel Lequesne scores and VAS scores, and improve LKSS score. In addition, compared to the sham acupuncture combined with standard treatment, the levels of ESR, CRP, IL-1β, and IL-6 in the acupuncture group were significantly reduced after treatment, while the levels of TGF-β, IGF-1, and FGF-2 were significantly increased. Meanwhile, through the detection of MAPK signaling pathway, acupuncture combined with standard treatment could significantly reduce the relative expression of p38 MAPK mRNA. The results suggest that acupuncture combined with standard treatment can yield significant positive effects on the improvement of knee pain and function.

The occurrence and development of KOA is closely related to the inflammatory response. The acute KOA is mainly characterized by joint swelling, pain, and functional limitation, which is related

to the accumulation of oxidative stress products and the activation of inflammation induced by meniscus and synovial injury during KOA. Synovial inflammation can induce a cascade reaction through the release of inflammatory factors such as IL-1β and TNF-α, promote the progression of synovial inflammation, and further aggravate the injury of KOA. Shi et al.[25] found that acupuncture could significantly reduce the levels of pro-inflammatory cytokines (TNF-α, IL-1β) and cartilage degradation biomarkers (MMP-3, MMP-13), and significantly increase the levels of anti-inflammatory cytokines IL-13. In this study, inflammation indexes in both groups were significantly lower after treatment than before, indicating that inflammation in patients with KOA was suppressed after treatment. However, it can be obviously observed that the inflammation index of acupuncture combined with standard treatment group is lower than that of sham acupuncture combined with standard treatment group.

The IL-1β is one of the strongest inflammatory cytokines in the body, and its content is very low under physiological conditions. However, it would be greatly increased, when diseases such as gout and arthritis occur, which can directly participate in cartilage degradation, accelerate synovial hyperplasia, and induce chondrocyte growth arrest.[26] Cartilage injury is a key feature of KOA. In the current clinical practice, there is still a lack of effective treatment to reverse cartilage injury, and it can only delay the injury process as much as possible. The TGF-β is an important factor in chondrogenesis and cartilage damage repair, and the continuous reduction of its level is not conducive to the recovery of KOA.[27] Similarly, IGF-1 and FGF-2 are growth-promoting endocrine hormones that can protect cartilage and delay cartilage degeneration in joints, but if they are low expressed in serum of KOA patients, they may be unfavorable to disease control.[28,29] It could be seen that acupuncture combined with standard treatment in this study can alleviate the clinical symptoms of patients and reduce the inflammatory reaction of joints, thus promoting the recovery of joints.

In recent years, the pathogenesis and targeted therapy of KOA have gradually become a focus of research, mainly focusing on proteomics, gene expression, signaling pathway and other aspects, among which the signaling pathway is closely related to the occurrence and development of KOA. The NF-κB pathway, Wnt pathway, Notch pathway, and MAPKs pathway have been shown to be involved in the regulation of KOA.[30] The MAPKs are a group of serine-threonine protein kinases that can be activated by cytokines, growth factors and neurotransmitters.[31] As one of the important intracellular signal transduction systems, MAPKs are responsible for regulating and maintaining inflammatory response, cell growth and differentiation, and the body adaptation to environmental stress.[32] The p38 MAPK signaling pathway is a crucial part of the MAPKs family. Both inflammatory factors and stress responses can promote the phosphorylation of p38 MAPK, thereby activating p38 MAPK to participate in the occurrence and regulation of inflammatory pain.[33] In the current study, through the detection of p38 MAPK mRNA in the blood of patients, the expression of p38 MAPK mRNA in patients was decreased after treatment, which was more reduced in the patient group than in control group during the same period. These results suggest that acupuncture stimulation at acupoints may have a certain inhibitory effect on local tissue inflammation and reduce the production of inflammatory factors, thus reducing the phosphorylation level of p38 MAPK. The correlation analysis also revealed that, before and after treatment, the level of p38 MAPK in the patient group was correlated with ISOA score, VAS score, LKSS score, ESR, CRP, IL-1β, IL-6, TGF-β, IGF-1, and FGF-2 levels to varying degrees. The IL-1β and IL-6 showed a significant positive correlation with p38 MAPK levels, respectively. This result may be explained by the involvement of the p38 MAPK signaling pathway in inflammation regulation. As Zheng et al.[34] reported in a study on lung injury, serum IL-1β expression was reduced in acute lung injury rats with the inhibition of the p38 MAPK signaling pathway. Other studies have shown that p38 MAPK can promote the expression of a variety of pro-inflammatory factors, and inflammatory factors can in turn activate the p38 MAPK signaling pathway, making the inflammatory response gradually stronger through the positive feedback pathway.[35] Therefore, based on the current results, we can only judge that p38 MAPK is involved in inflammation regulation and is associated with IL-1β and IL-6, but the regulatory mechanisms of p38 MAPK, IL-1β and IL-6 need to be further studied.

This study has some limitations. *(i)* This study is a single-center study with a small sample size, and selection bias may inevitably occur in the process of sample inclusion. Therefore, the results of this study need to be verified in a large multi-center

sample. *(ii)* Current studies suggest that the p38 MAPK signaling pathway may be involved in the regulation of inflammation in KOA, but the specific regulatory mechanisms are unknown because animal studies have not been conducted. *(iii)* This study only evaluated the short-term efficacy, but did not evaluate the long-term efficacy and safety, which needs to be further improved to provide a new basis for the acupuncture treatment of KOA.

In conclusion, our study results show that, based on the standard treatment, acupuncture has a significant effect on the treatment of KOA, which may be achieved by inhibiting the related proteins of p38 MAPK signaling pathway, thereby reducing inflammatory response, and promoting cartilage repair. This is of great value in alleviating the clinical symptoms of KOA and controlling the progression of the disease. We believe that this study provides important clinical data supporting the feasibility of acupuncture in the clinical therapy of KOA.

Ethics Committee Approval: The study protocol wasapproved by the Affiliated Hospital of Integrated TraditionalChinese and Western Medicine, Nanjing University of ChineseMedicine Ethics Committee (no: 2020031). The study was conducted in accordance with the principles ofthe Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Designed the research study: Y.W.; Performed the research: Y.W., L.Y.L., H.Q.G.; Analyzed the data and wrote the manuscript: Y.W. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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