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Effectiveness and Safety of Acupuncture for Treatment of Knee Osteoarthritis: A Systematic Review

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Abstract

Background: Acupuncture is increasingly recognized for its efficacy in managing knee osteoarthritis (KOA), offering significant pain relief and functional improvements.

Aim: This systematic review was conducted with aim to summarize the studies on reporting the effectiveness and safety of acupuncture in KOA patients.

Method: This quasi-experimental study was conducted on 60 diabetic patients in Fars Province, Iran in 2023-2024. Participants were randomly assigned to the control and intervention groups. The intervention group received dignity therapy in three sessions over 10 days. Dignity and Fasting blood sugar levels were assessed before, immediately after, and one month following the intervention by Patient Dignity Inventory (PDI) and fasting blood sugar (FBS) check list. Data were analyzed using SPSS software (version 15) and descriptive statistics, independent tests and one single repeated measures ANOVA. $p<0.05$ was considered statistically significant.

Results: The studies consistently demonstrated that various forms of acupuncture therapy significantly reduce pain (p=0.001-0.008) and enhance functional outcomes (p=0.001-0.027) among KOA patients. Pain relief was evaluated using the visual analogue scale, while functional improvements were assessed through WOMAC scores. Secondary outcome, including quality of life measured by SF-12 and AqoL-SF36 scales, also showed significant improvement (p=0.001-0.057).

Implications for Practice: Healthcare providers are encouraged to consider patient preferences and treatment goals when recommending acupuncture, ensuring personalized care that addresses both symptomatic relief and functional recovery. Further research and clinical integration of acupuncture are pivotal in advancing treatment options for osteoarthritis, promoting improved outcomes and patient well-being.

Keywords: Acupuncture, Knee osteoarthritis, Systematic review

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Introduction

Knee osteoarthritis (KOA) is a generative disease in adults (1, 2). The incidence of KOA in recent years has shown an increasing trend and as one of the main sources of disabling physique, thereby increasing the number of dependencies in daily activities (3). Globally, osteoarthritis affects approximately 10% of men and 13% of women over the age of 60 years. The prevalence of osteoarthritis increases with age, ranging from 30% to 40% in those over the age of 65 years. Prevalence rates are higher in Asians compared to Europeans and North Americans. Progressive degeneration occurs in the cartilage with the main symptoms, including pain, functional limitations and joint swelling and physical disability (4-7).Pain and limited physical mobility will greatly affect the quality of life including physical, psychological, social relations and environment (8, 9). Until now, there is no radical medicine and treatment for this disease, especially those aimed at relieving pain, restoring joint function and reducing the risk of physical disability (10, 11). The latest guidelines from the American College of Rheumatology recommend that management of KOA include regular physical exercising, self-managing, weight loss and using assistive devices (canes and knee pads) as well as pharmacological therapy, which includes topical Nonsteroidal anti-inflammatory drugs (NSAIDs) and intra-articular glucocorticoid injections (12, 13). This management has not shown effective in short term and it takes a long time to implement better self-management (11). Pharmacological treatment may provide effective short-term effects, but long-term use of analgesics will cause joint damage, joint degeneration and addiction as well as side effects on other organs such as kidney, cardiovascular and significant gastrointestinal responses. Therefore, complementary and alternative therapy is of interest and can be considered by the public as a safe and effective treatment in both the short and long term (14, 15).

Traditional Chinese medicine is one of the intervention methods in nursing that is considered safe and effective and many people choose to treat various diseases. Acupuncture is a Traditional Chinese Medicine (TCM) which is effective for reducing pain (16). Acupuncture may be effective and safe to KOA patients, given that the effects are much milder than conventional therapy (17). Patients will not experience problems in exercising and losing weight, so acupuncture therapy is highly recommended as one of the KOA nursing methods (18, 19). Several studies reported the acupuncture's general benefit in improving symptoms such as joint pain, swelling, functional changes and stiffness in KOA patients (20, 21).

According to theoretical biochemistry, acupuncture therapy can trigger various endogenous substances release that can reduce pain (22, 23). It is important to simultaneously evaluate the effectiveness and safety of acupuncture therapy. Treatments that are highly effective but carry significant risks or impacts may not be appropriate, especially for patients with KOA, who require long-term treatment and care. Conversely, treatments that are safe but have low effectiveness can be considered for other therapies that have higher effectiveness. In recent years many randomized controlled trial studies have been published to evaluate the effectiveness and safety of acupuncture (24, 25). Several studies have been conducted, but evidence on the efficacy and safety of acupuncture is still mixed due to variations in patient characteristics, duration of treatment, acupuncture techniques, and study designs. Safety is also important to note, although based on several studies, no serious effects were found in patients with acupuncture therapy. Given the increasing public interest in traditional medicine, one of which is acupuncture in the treatment of KOA, a comprehensive and systematic review of the existing literature is needed. Therefore, this systematic review was performed with aim to summarize the studies on reporting the effectiveness and safety of acupuncture in KOA patients.

Methods

This systematic review was conducted according to guidance introduced by the Cochrane Collaboration Search Strategy and Preferred Reporting Items for Systematic Reviews and Meta Analyzes (PRISMA) statement. Search for literature was conducted by two independent researchers in four international-qualified electronic databases (PubMed, ScienceDirect, Cochrane Library and EMBASE) and four Chinese electronic databases (China National Knowledge Infrastructure, Wanfag, Chinese Biomedical Literature and Chongqing VIP) from January 2018 until December 2022. We included the last 5 years of research to get up-to-date results by paying attention to topic relevance, original articles, published in reputable journals in English. The literature search was conducted by

the following keywords: ("Acupuncture" OR "Acupuncture Therapy" OR "Electroacupuncture" OR "Manual Acupuncture" OR "Warm Acupuncture" OR "Auricular Acupuncture" OR "Acupoints") AND ("Osteoarthritis" OR "Osteoarthritis of the Knee" OR "KOA" OR "Knee Osteoarthritis" OR "Knee Pain") AND ("Randomized Controlled Trial" OR "RCT"). A search of related articles was also carried out to ensure a comprehensive literature search.

In this study, we selected the articles with randomized controlled trials (RCTs) or quasi-RCTs, in which acupuncture therapy was one of the treatments for KOA. The selected participants were patients who were clinically diagnosed with KOA without limitation of age, gender and race. Intervention in the treatment group with various acupuncture therapies (acupuncture, warm acupuncture, auricular acupuncture electroacupuncture) and acupuncture therapy combined with other therapies. Intervention in the control group included comfort therapy (placebo, sham acupuncture or control group without therapy) and other therapies (pharmacological therapy and non-pharmacological therapy, etc).

At least one of the specified outcomes is considered to be reported: effective rate, cure rate, University of Western Ontario and McMaster University Arthritis Index score (WOMAC), Visual Analogue Scale (VAS), side effects and other indicators. Exclusion criteria were duplicate published studies, non-original research articles, comments, conference abstracts, and research whose data cannot be downloaded, and acupuncture intervention in the control group and non-major intervention in the treatment group.

Two researchers entered search results into the "Mendeley" software based on search strategy. After omitting copy, the researchers independently checked the title and abstract of the article in order to remove the irrelevant studies; as well as reading the full text of the studies relevant to inclusion criteria and extracting data was carried out based on the first author, year of publication, country, number of samples, intervention, control measure, outcomes, and overall conclusion. Data extraction was carried out by two researchers and through discussing and consulting to the first author. We endeavor to contact the authors for any addition of missed information, as necessary. Two researchers independently assessed the quality of the included studies using the JBI critical appraisal tool. There are 13 study assessment domains with RCT designs using the critical appraisal tool from JBI (26). Each domain is evaluated as " Yes", "No", "Unclear", "Not Applicable". The results of the assessment are categorized as follows: <4 poor quality, 4-6 moderate quality, 7-9 good quality, and >10 excellent quality. Two authors analyzed studies that met the criteria for analysis and the data obtained were re-examined by the first author. Included studies were analyzed based on the presence of approximately one result specified to be reported: effective rate, cure rate, side effects and other parameters.

Ethical Consideration

The study was approved by the research ethics committee of Buleleng School of Health Sciences (ethical code: 703/EC-KEPK-SB/VI/2024). Ethical considerations in conducting a systematic review on acupuncture therapy for knee osteoarthritis transparency and accuracy in reporting findings was avoiding selective reporting and subjecting the review to rigorous peer review for validation of methodology and ethical integrity. These ethical guidelines ensure the systematic review contributes to healthcare knowledge on acupuncture therapy for knee osteoarthritis.

Results

Literature search and selection

A literature search was conducted on 4 international electronic databases and 4 Chinese electronic databases and found 573 studies. A total of 218 duplicate articles were found and removed. Literature search was conducted in 4 international electronic databases and 4 Chinese electronic databases and 573 studies were found. A total of 218 duplicate articles were found and removed. Therefore, 335 literatures were screened and 316 articles were excluded with details of 282 articles did not have relevant topics, 11 did not have relevant populations, 7 were not research articles, 5 protocol studies, and 11 review studies. In this way, 19 articles were selected in the second stage of screening that 12 were excluded because they were non-RCTs and did not have control or treatment groups. The final result obtained 7 articles that were included in the literature review (Figure 1).

Participants from the 7 studies included in this review with an age range of 40–75 years from hospital patients across China (27-29). All respondents in the included studies were 829 patients with KOA. In each study, respondents were selected based on strict inclusion criteria to obtain homogeneous respondents. BMI, medical history, duration of suffering, and pain intensity were used as the main criteria for determining respondents. Five studies used sham acupuncture or fake acupuncture in the control group (30, 31). One research used acupuncture manuals to compare the effectiveness of therapy to electroacupuncture (32) and used Usual Medical Care from respondents to compare therapeutic acupuncture and electroacupuncture plus usually medical care.

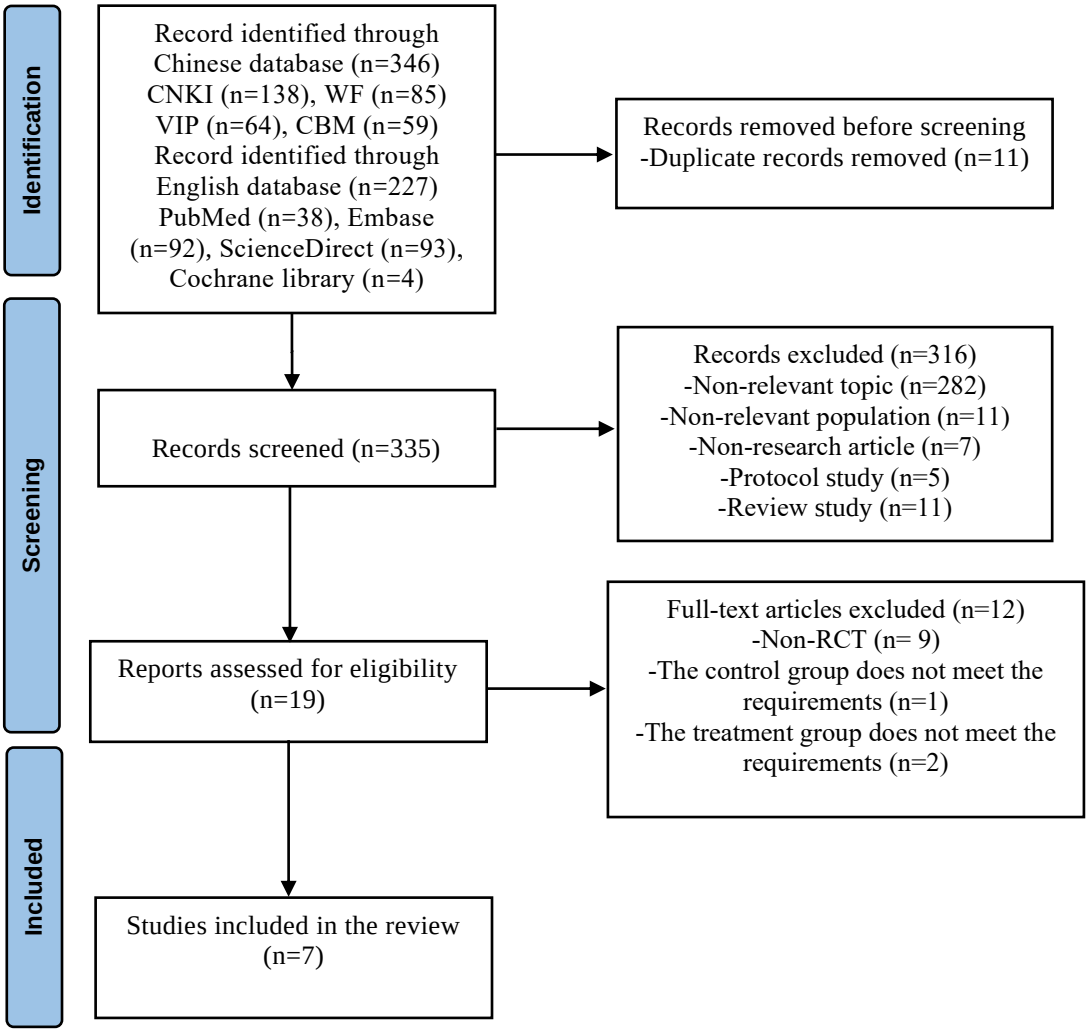

Figure 1. Flowchart of the systematic review selection process

Studies that was included according to the inclusion criteria was shown to assess the achievement of parameters on KOA. In this study, KOA assessment parameters obtained included: WOMAC Arthritis Index scores, Visual Analogue Scale (VAS), Quality of Life (SF-12 and AqoL-SF36), Time Up and Go Test (TUGT), Knee Injury and Osteoarthritis Outcome Score (KOOS), Body Composition, Knee ROM, Quadriceps Muscle Stiffness (QMS), Mental Component Summary Score (MCS), Numeric Pain Rating Scale (NPRS), Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), One Leg Standing (OLS), Physical Component Summary Score (PCS), Present Pain Intensity (PPI), and Conditioned Pain Modulation (CPM).

The results of the review of all included studies showed that 6 types of acupuncture methods were tested on patients, including: Traditional Acupuncture, Electroacupuncture, Laser Acupuncture, Manual Acupuncture, Warm Acupuncture, Needling Acupuncture and Sham Acupuncture therapy were used in the control group. Electroacupuncture in practice is used to obtain significant short-

term effects. This acupuncture therapy is usually able to show its effectiveness with an intensity of 30 minutes of therapy at 5 sessions per week for 2 weeks (Table 1).

Table 1. Studies on the effectiveness of acupuncture treatment for knee osteoarthritis

No	Author (Year)	Country	Intervention		Outcome		Conclusions
			Treatment	Control	Primary	Secondary	
1	Lin et al., 2018 [27]	China	AT	SA	Effective rate, WOMAC	VAS, QoL (SF-12)	Acupuncture intervention given intensively three times a week for eight weeks significantly reduced pain and improved function in patients with knee osteoarthritis. This therapy is safe with minor side effects such as pain at the puncture site and hematoma in some patients.
2	Lv et al., 2019 [32]	China	Strong EA, Weak EA,	Sham EA	Effective rate, WOMAC, CPM, VAS	NPRS, ES, PPI	EA administered for at least 2 weeks have a clinically important effect on improving CPM function in KOA patients. Strong EAs are better than weak or sham EAs in reducing pain intensity and inhibiting chronic pain.
3	Zhang et al., 2019 [38]	China	AP+UC, EA+UC	UC	Effective rate, VAS, WOMAC	QoL (AqoL-SF36)	AP or EA combined with usual medical care is more effective than usual medical care alone for KOA treatment
4	Wang et al., 2020 [30]	China	EA	MA	WOMAC	VAS, QoL (SF-12)	EA and MA interventions are very effective and safe in KOA.
5	Wu et al., 2020 [29]	China	LA+EA	Sham LA + EA	Effective rate, VAS	WOMAC, KOOS, Body Composition, Knee ROM, QMS, OLS, 30s chair stand	Combination of LA and EA is more effective than EA alone in reducing knee pain and stiffness and increasing lower leg muscle strength, which can improve balance and quality of life.
6	Lam et al., 2021[28]	China	NA	SA	VAS	WOMAC, TUGT, SCT, PCS, MCS	Superficial needle acupuncture significantly reduces knee pain and improves KOA symptoms.
7	Chang et al., 2022 [34]	China	MA, EA, WA	SA	Effective rate, WOMAC	VAS, AIMS2-SF, BAI, BDI, Credibility/Expatency	EA therapy is more effective and provides more effect to reduce pain in KOA compared to MA and WA.

AT: acupuncture therapy; SA: sham acupuncture; WA: warm acupuncture; EA: electroacupuncture; FA: fire needle acupuncture; NA: no acupuncture; HA: hyaluronic acid; MT: medicine therapy; PT: physical therapy; HM: herbal medicine; WOMAC: Western Ontario and Mcmaster Universities Arthritis Index; VAS: Visual Analog Scale; BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory

Risk of bias

The Cochrane Risk of Bias Assessment for seven studies of acupuncture for knee osteoarthritis (OA) revealed variability in risk of bias across domains. Most studies (1, 3, 5, 6) showed low risk of bias, indicating that randomization was likely performed appropriately. However, a study (4) showed high risk of bias, indicating potential problems with the randomization process. Allocation concealment was at moderate to low risk in most studies. Two studies (3, 5) showed low risk, while others (1, 2, 4, 6, 7) showed moderate risk, indicating some uncertainty about whether

allocation was effectively concealed. Studies 1, 2, 4, 5, 6, and 7 all had high risk of bias, indicating significant challenges in blinding participants and personnel to the intervention, which is particularly difficult in acupuncture studies. Study 3 had moderate risk, still reflecting potential problems with blinding. The risk of bias in outcome assessments was generally moderate, with some studies 1, 3, and 5 showing lower risk, while others studies 2, 4, 6, and 7 had moderate to high risk. This suggests that some outcome assessments may have been influenced by knowledge of the intervention received. All studies had a low risk score in the incomplete outcome data domain, indicating that dropout rates were low or well managed, and missing data were handled appropriately. The risk of selective reporting was moderate in most studies, with one study 4 showing high risk. This suggests that some studies may not have reported all planned outcomes, potentially skewing the findings. The "Other Bias" category, which includes potential biases not captured by the other domains, was generally moderate. Study 3 had a low risk, while the other studies had a moderate risk, suggesting that other factors may have influenced the study results (Figure 2).

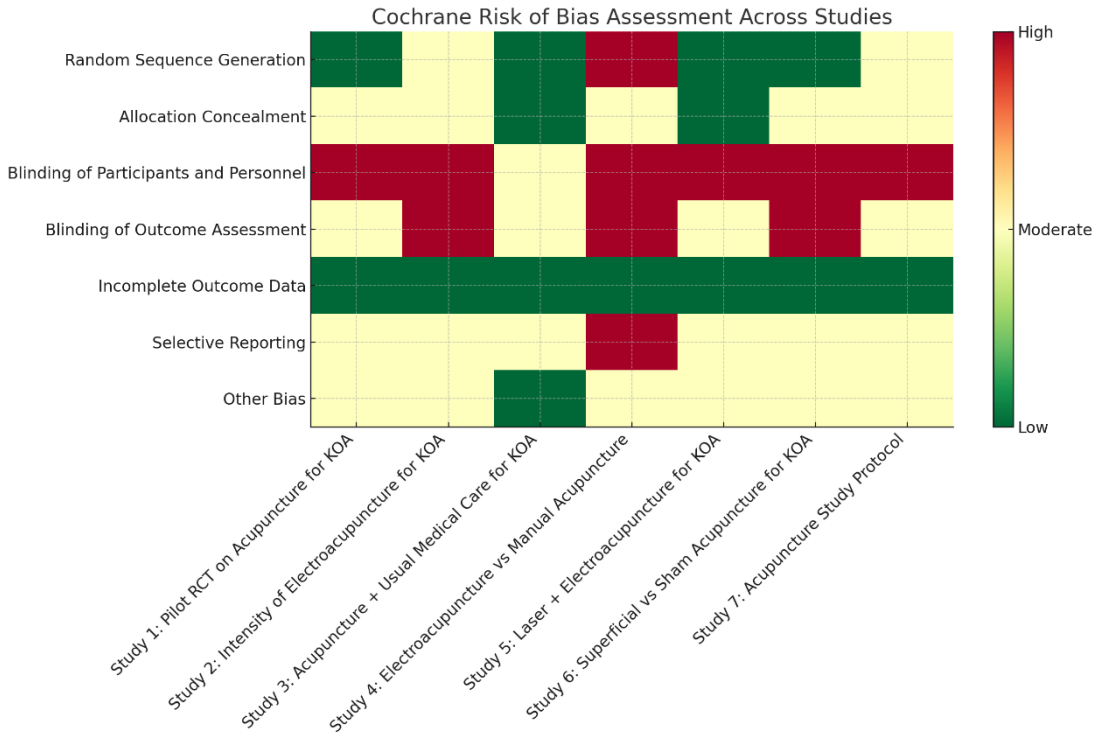

Figure 2. Graphical risk of bias of the included studies

Grading of the quality of evidence

The quality of studies included in this study can be seen in Table 2. All studies have excellent quality because they have a score >10. This means that all articles included in the study have very good quality.

The effectiveness and safety of acupuncture

Traditional types of acupuncture therapy such as warm acupuncture, manual acupuncture and needle acupuncture require a long time to evaluate their effectiveness, usually with an intensity of 45 minutes at 3 sessions per week for 4-8 weeks. Based on the research results, all types of acupuncture therapy are safe in KOA patients to reduce knee pain intensity and assess other parameters with few side effects. Some mild side effects can occur after therapy, including pain or discomfort at the puncture site, bruising and blueness, feeling tired and sleepy, dizziness, redness and swelling. Serious complications rarely occur when performed by health workers who are skilled in providing acupuncture therapy. The results of the study showed that all types of acupuncture therapy are effective and safe in KOA patients. There was a decrease in the pain scale in KOA patients (p=0.001-0.008) after being given acupuncture therapy as measured by the VAS

(27-29) and showed functional improvement (p=0.001-0.027) with decreased scores on the WOMAC assessment (30). These two measurement tools are the main results in all studies included in this review. In the secondary outcome, acupuncture was also effective in improving the quality of life of KOA patients (p=0.001-0.057) by measuring SF-12 and AqoL-SF36 (30-32).

Table 2. Reporting quality grading of included SR

Author (Year)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Overal Quality
Lin et al., 2018 [27]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Excellent Quality
Lv et al., 2019 [32]	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Excellent Quality
Zhang et al., 2019 [38]	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Excellent Quality
Wang et al., 2020 [30]	Y	Y	Y	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	Excellent Quality
Wu et al., 2020 [29]	Y	Y	UC	Y	Y	Y	Y	Y	Y	Y	Y	UC	Y	Excellent Quality
Lam et al., 2021 [28]	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Excellent Quality
Chang et al., 2022 [34]	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Excellent Quality

Note: Q1: Was true randomization used for assignment of participants to treatment groups?; Q2: Was allocation to treatment groups concealed?; Q3: Were treatment groups similar at the baseline?; Q4: Were participants blind to treatment assignment?; Q5: Were those delivering treatment blind to treatment assignment?; Q6: Were outcomes assessors blind to treatment assignment?; Q7: Were treatment groups treated identically other than the intervention of interest?; Q8: Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?; Q9: Were participants analyzed in the groups to which they were randomized?; Q10: Were outcomes measured in the same way for treatment groups?; Q11: Were outcomes measured in a reliable way?; Q12: Was appropriate statistical analysis used?; Q13: Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for conducting and analysis of the trial?; Y: Yes; N: No; Uc: Unclear.

Acupuncture therapy is also able to improve several indicators of physical ability assessment which include: balance, muscle strength, range of motion, gait and even the mental status of KOA patients (33). Acupuncture as a traditional treatment showed more effective results compared to usual medical care in KOA patients. Based on the results of an analysis traditional acupuncture and electroacupuncture which coupled with usual medical care showed a very significant increase in functional improvement in KOA patients and a decrease in pain intensity as measured using the VAS. In this review, the participants' VAS scores for pain, stiffness, and function and SF-12 in the EA (electro-acupuncture) and MA (manual acupuncture) groups decreased after 8 weeks compared to those before therapy, indicating that both EA and MA are effective for treatment of KOA.

Discussion

This systematic review of seven studies on acupuncture for KOA revealed mixed results, but overall acupuncture therapy has positive efficacy in the treatment of KOA. The majority of studies showed that acupuncture, whether manual, electroacupuncture, or other acupuncture techniques performed alone or in combination with other treatments, significantly reduced pain and improved knee function in patients with KOA. However, efficacy varies depending on the type of acupuncture performed, the intensity of treatment, and the specific patient population. These findings are promising as an alternative treatment for patients with KOA, but potential bias and unclear reporting may influence the findings in these studies.

The finding of the current study revealed that acupuncture therapy appears to be more effective when given together with usual medical care for KOA, but the effect may appear smaller when used in conjunction with other alternative therapies (30). All the research reports presented in this study stated

that acupuncture has a significant impact on KOA, however further research is still needed regarding several factors that may influence the treatment of KOA.

It is recommended that an analysis be carried out to determine the effect of acupuncture based on the characteristics of the respondents (age, gender, severity, length of suffering). In this review, we found that no research reported the results of an analysis of subgroups to find out whether certain patients could respond better to this form of acupuncture therapy. There were several questions related to the applicability of acupuncture therapy, whether acupuncture is the best treatment for bilateral symptoms, or cases where osteoarthritis can affect several joints (3, 36).

Simple comparative research can be done to find out the most efficient offer for acupuncture therapy (36-38). An interesting finding in this structured review is that a sham control study (placebo) is actually no use for acupuncture therapy in KOA, perhaps it is unethical to deny the benefits of acupuncture to KOA patients. Acupuncture has a significant effect on KOA patients compared to sham acupuncture, perhaps some studies in our findings reported a combination of acupuncture with additional treatment but the reported results showed significant changes in reducing pain and improving functional joints. As a consideration in the use of non-steroidal anti-inflammatory drug therapy (NSAIDs), we found that acupuncture has much fewer side effects during treatment. NSAID therapy may have a rapid effect on pain reduction. However, it cannot provide a significant improvement in functional status as a result of KOA. Prolonged use of NSAIDs will cause a significant response severity in the gastrointestinal, cardiovascular and renal systems.

The effects of acupuncture therapy on pain compared to sham acupuncture therapy was small (mean difference and standard deviation) 0.37 (0.12, 0.61) and compared to the effect of NSAID treatment 0.33 (0.24, 0.39) versus placebo (39). The results of the average comparison are more meaningful comparing to non-extra treatment, because it will reflect the benefits in practice, and include beneficial effects which are varied for acupuncture therapy. In this finding the effect of acupuncture was 0.78 (0.57, 1.02), which was considered huge. The change in pain scale improvement after being given acupuncture therapy was 3.2 WOMAC points, one-third reducing baseline pain's average. The mean functional improvement was 11.5 WOMAC points, meaning the effect is promising increasing overall disability by about one third of the average. The benefits obtained are significant, especially when one considers the known safeness level of acupuncture therapy. A research (38) reported the adverse events at acupuncture group which is similar to those sham acupuncture with untreated group. One patient at acupuncture group reported dying caused of complications, this event was not related to the acupuncture therapy given but more likely to the refecoxib taking 4 weeks before the start of the study.

The findings of this review underscore the need for more detailed research to better understand the role of acupuncture in treatment of KOA. Consistency is needed in acupuncture therapy including needle placement location, treatment duration, and number of treatments per session. These factors will allow for more accurate comparisons across studies and may help determine the most effective acupuncture practice for KOA. Many studies have been conducted with short-term follow-up periods limited to weeks or months. Studies with long-term follow-ups are essential to determine the sustained effects of acupuncture on KOA, particularly in terms of pain management and functional improvement over time. In addition, any outcomes obtained during treatment should be recorded in detail, whether positive or negative, regarding the effects of each treatment. This is done to avoid or reduce bias during the treatment. Further research into the biological mechanisms underlying the effects of acupuncture on KOA may provide a clearer basis for its use and help to identify which patients are most likely to benefit from this therapy.

This systematic review had some limitations. The published results only explain the positive results of acupuncture therapy, the negative results are not clearly conveyed in the research results because they were not studied in depth. Moreover, the quality of randomization in data collection is not explained in detail, so it can cause bias in the results obtained. Also, most studies in this study were conducted in China, so that many people accept and practice acupuncture, however in some countries they are still not familiar with this therapy, so there will be cultural influences that can be an obstacle to patient trust and expectations. The study was not conducted in a cohort or over a long period of time, so we cannot find out the possible side effects of acupuncture therapy. The population or respondents who were used as research subjects were not diverse in terms of age so they could not be studied further. This review summarizes the evidence findings in a narrative manner, because the interventions

included in this review varied, and success rates were assessed based on different criteria and cannot be done through quantitative summary. Moreover, the success rate of acupuncture therapy needs to be reviewed in more depth, because several studies did not provide exclusion criteria for the severity of KOA. Finally, because of the small number of studies included, it was not possible to perform subgroup analyzes stratified by factors such as length of treatment, different types of acupuncture, and KOA stage. Therefore, continuous comprehensive and in-depth studies were needed to verify the effectiveness and safeness of acupuncture in KOA patients.

Implications for practice

Healthcare providers should consider integrating acupuncture into comprehensive treatment protocols for knee osteoarthritis, alongside conventional therapies, due to its demonstrated effectiveness in pain relief and functional improvement. Continued research efforts are needed to further elucidate the acupuncture's mechanism of action and to explore its long-term efficacy and safety profiles in diverse patient populations with knee osteoarthritis.

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Conflicts of interest

The authors declared no conflict of interest.

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Authors' Contributions

Ni Made Dwi Yunica Astriani contributed to protocol development, search strategy, study selection, editing and formatting the manuscript. Putu Indah Sintya Dewi and Aditha Angga Pratama performed data extraction, risk of bias assessment, data synthesis, interpreted the results and implications. Mochamad Heri supervised the overall conduct of systematic review. All authors read and approved the final version of the manuscript.

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