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Original Research Article

Effects of electroacupuncture and manual acupuncture treatments on the clinical manifestations and immune-related cytokines in patients with knee osteoarthritis in a randomized and double-blind clinical trial

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Abstract

Objective: Knee osteoarthritis (KOA) is recognized as a degenerative disease. Acupuncture has long been employed for KOA via various techniques. The present study investigated the potential effects of electroacupuncture (EA) and manual acupuncture (MA) on clinical parameters and serum cytokines levels in KOA patients through a three-arm, double-masked, randomized, and sham-controlled clinical trial (IRCT20170305032893N3).

Materials and Methods: Forty-five patients diagnosed with KOA were subjected to MA, EA, or a sham control group (SA) over eight weeks. Pain intensity, quality of life (QoL), stiffness and physical activity were assessed via the Visual Analog Scale (VAS), the SF-12, and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) parameters, respectively. Furthermore, serum levels of interleukin (IL)-4, interferon-gamma (IFN-γ), transforming growth factor-beta (TGF-β), IL-10, and tumor necrosis factor-alpha (TNF-α) were quantified using the enzyme-linked immunosorbent assay (ELISA).

Results: The findings revealed significant improvements in pain, QoL, stiffness, and physical activity parameters with both MA and EA interventions. These benefits were sustained through the 12 and 16-week follow-up assessments compared to the SA group. There were no notable changes in cytokine levels between intervention groups except for the TGF-β as anti-inflammatory cytokine which showed a significant increase following EA treatment (p=0.02).

Conclusion: Both EA and MA methods showed therapeutic effectiveness as a safe complementary method in KOA patients in terms of clinical indexes compared to controls. Ameliorating KOA following the EA method might be due to effects of TGF-β

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Introduction

Knee osteoarthritis (KOA) is acknowledged as a degenerative, chronic, incapacitating musculoskeletal ailment prevalent among adults, particularly older adults. Its escalating incidence has transformed it into a significant global public health concern, with a higher morbidity rate observed in women compared to men. Age, gender, physical mobility, obesity, estrogen and vitamin D deficiency, and smoking represent key risk factors associated with KOA (Shi et al. 2020; Tu et al. 2019; Wang et al. 2020). Patients typically exhibit persistent and severe pain, initially episodic and later continuous, along with crepitation, stiffness, joint disability, decreased range of motion, deformity, and a marked decline in quality of life, imposing substantial social and financial burdens (Atabaki et al. 2022; Litwic et al. 2013).

Inflammation plays a pivotal role in KOA pathogenesis and progression. Synovial and immune cells demonstrate heightened production of inflammatory mediators such as cytokines, chemokines, and matrix metalloproteinases (MMPs), impacting the joint tissue microenvironment, muscle function, and sensory nerves associated with pain perception (Robinson et al. 2016).

Numerous studies have reported elevated concentrations of pro-inflammatory mediators, including interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), in KOA patients, which also serve as predictive markers for disease advancement (Stannus et al. 2013). On the other hand, the role of interleukin (IL)-10 and transforming growth factor-beta (TGF-β) anti-inflammatory cytokines in maintaining chondrocyte homeostasis and cartilage integrity via suppressing inflammatory responses and inhibiting cartilage degradation was reported in recent studies (der Kraan 2018; Lin et al. 2022).

Currently, there is no definitive cure for KOA, and treatment approaches primarily aim to alleviate symptoms, and enhance function and quality of life. Common therapeutic modalities for KOA include exercise therapy, nonsteroidal anti-inflammatory drugs (NSAIDs), and analgesics. However, concerns regarding long-term adherence and potential side effects limit their sustained use. Knee replacement surgery, often recommended for end-stage patients, is a costly intervention requiring meticulous care (Gregori et al. 2018). Nonetheless, current recommendations emphasize the prioritization of research into novel non-pharmacologic therapies to address this unmet medical need (McAlindon et al. 2014).

Acupuncture, a traditional therapeutic practice with origins dating back over 3000 years in China and other Asian countries, is emerging as a promising non-pharmacologic intervention for KOA (Corbett et al. 2013). This modality encompasses various techniques, including electroacupuncture (EA) and manual acupuncture (MA), which have shown potential in pain management. Despite a growing body of research investigating the role of acupuncture in KOA treatment and management, the precise efficacy of this approach remains to be fully elucidated (Bannuru et al. 2019; Kolasinski et al. 2020). While systematic meta-analyses have highlighted the effectiveness of acupuncture in reducing chronic pain and improving functional mobility (Vickers et al. 2012), and there is evidence of acupuncture's anti-inflammatory properties in reducing levels of inflammatory cytokines, the underlying mechanisms remain unclear (Shi et al. 2020).

Building upon the background explained above, this study undertook a comprehensive evaluation and comparison of the potential effects of EA and MA on clinical parameters, alongside assessing the levels of selected inflammatory mediators in KOA patients. This investigation was conducted through a rigorous three-arm, double-masked, randomized, and sham-controlled clinical trial design.

Materials and Methods

Study design

This three-arm, randomized, double-blind, sham-controlled clinical trial was conducted at the Acupuncture Clinic of Imam Reza Hospital, affiliated with Mashhad University of Medical Sciences, Mashhad, Iran. The trial protocol was approved by the Ethic committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.1401/371) and registered in Iranian Registry of Clinical Trials (IRCT20170305032893N3).

Patients' recruitment, enrollment, randomization, allocation and blinding

Patients with KOA were diagnosed according to the clinical criteria outlined by the American College of Rheumatology (ACR) (Kellgren and Lawrence 1957). The inclusion criteria encompassed individuals aged between 45 and 70 years, with involvement of at least one knee and a Visual Analog Scale (VAS) pain score of four or greater over the preceding three months. Radiological assessment indicating Kellgren-Lawrence (KL) scores of II or III qualified patients for inclusion. The exclusion criteria comprised a history of joint surgery or arthroscopy within the past year, intra-articular injections or acupuncture treatment within the last six months, or a medical history of inflammatory, autoimmune, cardiac, or coagulation disorders, diabetes, or cancer. Additionally, pregnancy or breastfeeding, a body mass index (BMI) exceeding 30, and participation in concurrent clinical trials were grounds for exclusion.

A total of 93 patients were initially recruited, out of which 45 met the eligibility criteria for participation. All 45 patients provided informed consent and were randomly assigned to three intervention groups in a 1:1:1 ratio, namely manual acupuncture (MA), electroacupuncture (EA), and sham acupuncture (SA). The recruitment and randomization was performed by Acupuncture specialist. Forty-one patients completed the treatment regimen, while four patients withdrew from the study: one due to personal reasons, two due to perceived lack of efficacy, and one due to concurrent use of other treatments. Figure 1 illustrates the flowchart detailing patients' recruitment, enrollment, randomization, intervention, and follow-up. Randomization was performed using a random number generator and implemented in blocks of three, with allocation conducted using sealed envelopes. Blinding of patients and all study personnel, except for the acupuncturists, was maintained throughout the study period until its conclusion.

Interventions

The intervention regimen consisted of two sessions per week over eight consecutive weeks, totaling 16 sessions, for all participants across the MA, EA, and SA groups. Each acupuncture session lasted for thirty minutes. Disposable sterile needles (0.25 × 40 mm, Tony, Republic of China) were utilized for both the MA and EA groups, while an electroacupuncture device (KWD-808 I, Ying Di, Republic of China) was employed solely for the EA group. Straight Berg disposable sterile needles (Asia-med GmbH, Kirchplatz, Germany) were used for the SA group. The acupuncture protocol was developed based on traditional Chinese medicine principles, clinical practices, and expert consensus (Sun et al. 2020). Eleven acupoints were selected uniformly across all groups (MA, EA, and SA) to cover common areas affected by KOA, including ST34, SP10, EX LE2, ST35, EX LE4, GB34, SP9, ST36, LI4, KID3, and ST44. De qi, characterized by a combination of sensations such as soreness, heaviness, numbness, and distention, was elicited during both MA and EA interventions. For ST36 and KID3 acupoints, the stimulation was performed via reinforcing method, while the other points were stimulated by reducing method.

The stimulation in the MA group was applied every ten minutes throughout the session. For the EA group, de qi sensation

was achieved, then electrodes attached to the needles at acupoints utilizing a 2/100 Hz dense-dispersed wave. The current intensity was gradually increased based on individual patient tolerance and then maintained at a fixed level for the duration of the session. In the SA group, non-penetrating straight-berg needles were affixed to the designated acupoints using adhesive bands. In both the MA and SA groups, the electrode wire was connected to the needles, but no current was applied through the wire. Instead, the device timer was activated to produce a sound, creating the illusion of treatment for the patient.

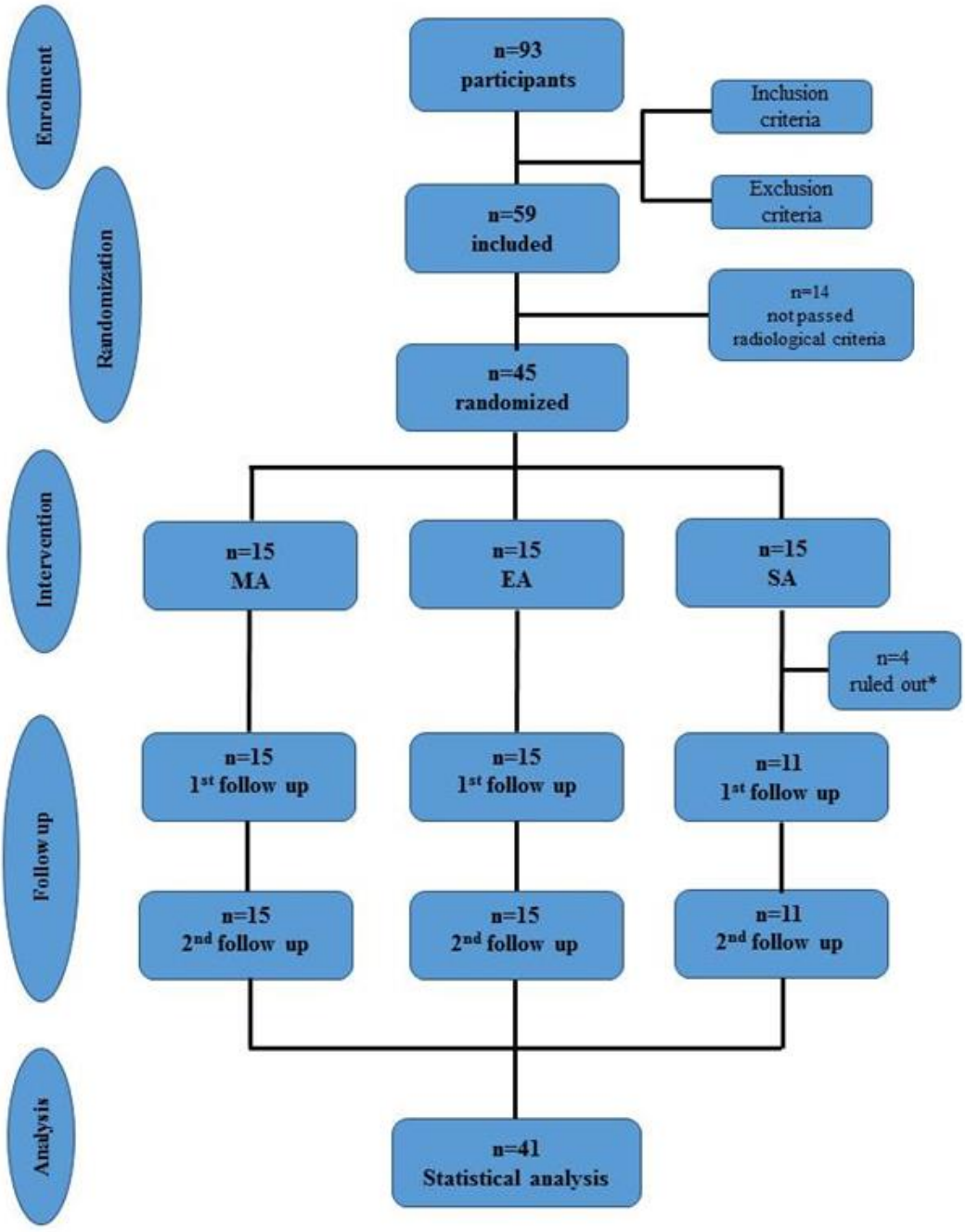

Figure 1. Flowchart of enrollment, randomization, intervention, follow-up, and analysis of patients (*Ruled out; one person due to personal issues, two persons due to not seeing the desired result and one person due to simultaneous use of other treatments)

Baseline demographics

Age, gender, disease duration, BMI, Visual Analog Scale (VAS) pain score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) stiffness score, WOMAC physical activity score, WOMAC total score, Short Form-12 (SF-12) quality of life (QoL) score, and Kellgren-Lawrence (KL) grade were evaluated at baseline before the commencement of the intervention (Table 1).

Table 1. Baseline demographic characteristics of patients enrolled in the clinical trial

Characteristics		MA group (n=15)	EA group (n=15)	SA group (n=15)	p-value
Age (years)		59.80±1.39	62.53±1.02	59.33±1.04	0.124
Gender (n, %)	Female	11 (73.3%)	10 (66.7%)	12 (80%)	0.711
	Male	4 (26.7%)	5 (33.3%)	3 (20%)	
Disease duration (years)		6.00±3.83	9.06±8.39	5.73±5.57	0.27
BMI (kg/m^2)		27.14±2.40	27.50±2.43	26.99±3.06	0.86
VAS score		6.33±1.95	6.86±1.55	6.73±1.57	0.67
WOMAC stiffness score		4.33±0.617	4.53±1.84	4.87±1.50	0.58
WOMAC physical activity score		39.67±10.84	42.40±6.86	40.33±8.82	0.06
WOMAC total score		54.47±12.96	59.33±11.27	57.87±11.32	0.51
SF-12 quality of life score		30.07±7.63	28.20±7.33	28.00±5.21	0.65
KL grade (n, %)	Grade 2	6 (40%)	4 (26.7%)	7 (46.7%)	0.51
	Grade 3	9 (60%)	11 (73.3%)	8 (53.3%)	

Assessing the clinical parameters

To assess the impact of interventions on clinical parameters, patients completed questionnaires measuring VAS score, WOMAC stiffness score, WOMAC physical activity score, and Short Form-12 (SF-12) quality of life score at baseline (week 0), the conclusion of the intervention (week 8), and during two follow-up periods (weeks 12 and 16). All these assessment and follow-ups were supervised by the rheumatologist.

Measurement of pro-inflammatory and anti-inflammatory cytokines

To evaluate the impact of interventions on serum inflammatory and anti-inflammatory cytokine levels, blood samples were collected at baseline (prior to initiating intervention) and at the conclusion of the treatment period (week 8). Serum samples were isolated, and the levels of interferon-gamma (IFN-γ), interleukin 4 (IL-4), interleukin 10 (IL-10), transforming growth factor-beta (TGF-β), and tumor necrosis factor-alpha (TNF-α) were quantified using the enzyme-linked immunosorbent assay (ELISA) method. This analysis was conducted in accordance with the manufacturer's instructions (ZellBio GmbH, Germany).

Statistical analysis

Statistical analysis and graph generation were performed using SPSS software version 22 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8 (GraphPad, CA, USA), respectively. Data are expressed as Mean ± Standard Deviation (SD). The normality distribution of data was assessed using the Shapiro-Wilk test. Subsequently, unpaired t-tests and one-way analysis of variance (ANOVA) were employed for normally distributed data, while the Wilcoxon and Kruskal-Wallis tests were utilized for non-normally distributed data.

Results

Patients' characteristics

Table 1 presents the baseline characteristics of patients across different treatment groups. No significant differences were noted in baseline demographic parameters among the MA, EA, and SA groups.

Clinical outcomes

Figure 2 displays the effects of interventions on clinical parameters across different groups. As illustrated, the VAS pain scores exhibited a significant reduction in all MA ($p<0.001$), EA ($p<0.001$), and SA ($p=0.006$) groups following eight weeks of intervention. In the case of two follow-up points, reduced VAS score was statistically significant in EA group both at week 12 ($p<0.001$) and week 16 ($p=0.0007$). Similar results were observed in the case of MA treatment and both week-12 ($p<0.001$) and week-16 ($p=0.003$) follow-ups showed a significant reduced VAS score compared to the 0-point. In the case of SA intervention, the results are different and no significant differences were observed at week 12 ($p=0.03$) and week 16 ($p=0.31$) compared to the before of intervention.

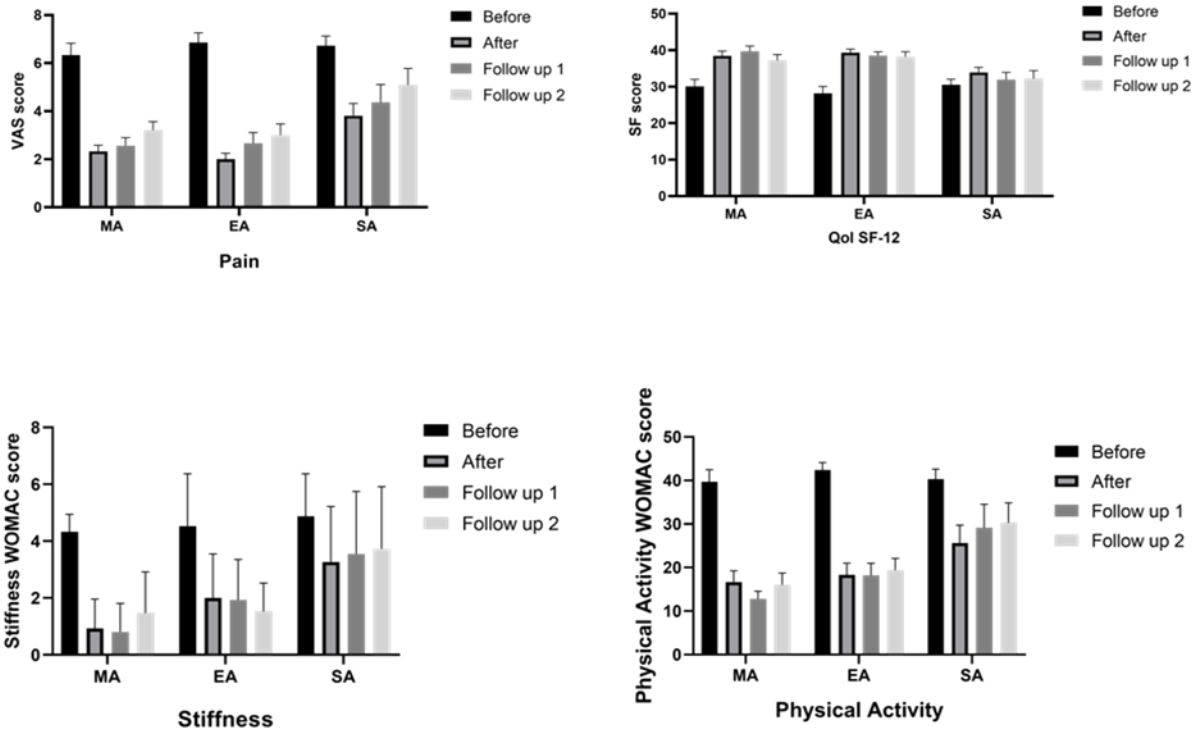

Figure 2. Comparison of the scores of VAS, QoL SF, Stiffness WOMAC, and Physical Activity WOMAC parameters in different intervention groups (manual acupuncture (MA), electro acupuncture (EA), and sham acupuncture (SA)) between before and after the intervention and two follow-ups

The Short Form-12 (SF-12) quality of life parameter demonstrated significant improvement in both MA and EA groups, extending to the second follow-up at week 16. Notably, the magnitude of change was more pronounced in the EA group. In EA group, SF-12 parameter was significantly improved after the intervention ($p=0.0004$), and at week 12 ($p=0.001$) and week 16 ($p=0.003$) as follow-up points. Also, analysis of MA group data showed significantly improved SF-12 levels after the intervention ($p=0.002$), and at week 12 ($p=0.0006$) and week 16 ($p=0.01$) as follow-up points. In the case of SA group, no significant changes were observed in the SF-12 levels after the intervention ($p=0.54$), and at week 12 ($p=0.94$) and week 16 ($p=0.89$) as follow-up points.

For the stiffness WOMAC score, both EA and MA interventions resulted in significant reductions following the 8-week

intervention ($p<0.001$ in both MA and EA groups), whereas no significant changes were observed in the SA group after the intervention ($p=0.44$), and at week 12 ($p>0.99$) and week 16 ($p>0.99$) as follow-up points. Importantly, the effects of MA and EA persisted significantly until the 12-week ($p<0.01$ in both MA and EA groups) and 16-week ($p<0.001$ in both MA and EA groups) follow-ups, respectively.

Additionally, the physical activity WOMAC score exhibited significant improvement in both MA and EA groups, continuing until the second follow-up at 16^{th} week . Although, significant improvements in EA and MA interventions were reported after the intervention ($p<0.001$ in EA and $p=0.0005$ in MA), at week 12 ($p<0.001$ in EA and $p<0.001$ in MA), and week 16 ($p<0.0001$ in EA and $p=0.0002$ in MA), no significant changes were observed following SA treatment, after the intervention ($p=0.05$), and on two follow-up points ($p=0.18$ and $p=0.27$, respectively).

Inflammatory and anti-inflammatory cytokines

Table 2 and Figure 3 depict the effects of interventions across different groups on the concentration of pro-inflammatory cytokines in serum. Moreover, Table 2 and Notably, the levels of the anti-inflammatory cytokine TGF-β were significantly increased following EA intervention ($p=0.02$). However, the levels of IFN-γ exhibited a reduction following EA intervention, and IL-4 levels decreased in the MA group, though these fluctuations were not statistically significant.

Table 2. Comparison of the serum levels of various cytokines in different intervention groups at before, after intervention, and two follow-ups.

The inflammatory and anti-inflammatory cytokines		**Intervention group (Mean ± SEM)**			**p-value between intervention groups**
		MA	**EA**	**SA**	
IFN-γ (ng/ml)	Before	49.30±4.29	50.52±5.26	51.34±4.29	0.10
	After	49.2±5.98	44.49±4.13	63.91±8.60	
	p-value	0.96	0.37	0.45	
IL-4 (pg/ml)	Before	4.11±0.28	4.34±0.27	4.05±0.27	0.55
	After	3.54±0.32	4.40±0.51	3.94±0.45	
	p-value	0.19	0.92	0.82	
TNF-α (ng/ml)	Before	14.23±1.77	14.93±2.14	17.78±1.75	0.08
	After	20.69±2.79	12.17±2.10	16.90±3.44	
	p-value	0.06	0.36	0.8	
IL-10 (ng/ml)	Before	62.14±13.00	68.07±14.17	57.90±8.44	0.33
	After	67.46±15.89	46.73±8.32	36.32±5.0	
	p-value	0.79	0.22	0.06	
TGF-β (ng/ml)	Before	292.2±31.6	211.0±24.8	282.8±25.2	0.52
	After	273.4±21.1	314.7±35.0	235.0±41.9	
	p-value	0.61	0.02	0.32	

Manual acupuncture (MA), electro acupuncture (EA), and sham acupuncture (SA)

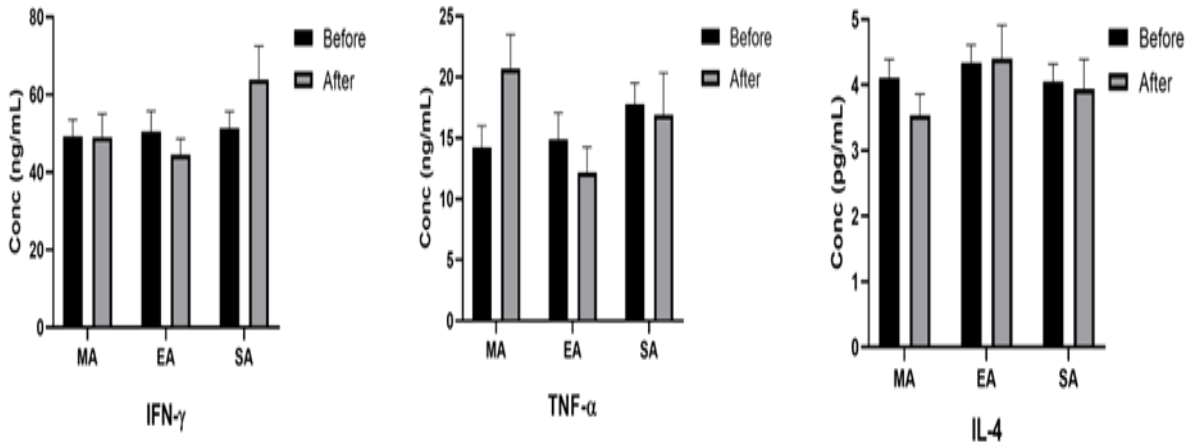

Figure 3. The serum levels of IFN-γ, TNF-α, and IL-4 inflammatory cytokines among different intervention groups (manual acupuncture (MA), electro acupuncture (EA), and sham acupuncture (SA)) in different interventions

Discussion

The current study explored the therapeutic effects of MA and EA as two acupuncture modalities on clinical indices and the concentrations of pro-inflammatory and anti-inflammatory cytokines in KOA patients, by employing a three-arm, double-masked, and randomized clinical trial design alongside a sham group. The findings revealed that both MA and EA interventions significantly improved pain VAS scores and SF-12 QoL parameters, reflecting reductions in pain intensity and enhancements in overall well-being, respectively. Additionally, stiffness and physical activity WOMAC scores, indicative of clinical manifestations in patients, showed significant improvement following the 8-week intervention period. Subsequent monitoring of clinical parameters at weeks 12 and 16 demonstrated the sustained efficacy of MA and EA interventions in improving these parameters, aligning with findings from similar studies reporting maintenance of improvement up to week 26 (Tu et al. 2019; Tukmachi et al. 2004) and even up to one year when patients completed the treatment period and continued monthly acupuncture sessions (Christensen et al. 1992).

In line with findings reported by Manheimer et al. (Manheimer et al. 2006), our study observed an improvement in pain outcomes in the SA group, which persisted until week 12, likely due to the placebo effect. However, this improvement diminished by week 16 compared to the EA and MA treatments. When comparing the efficacy of EA and MA groups, both methods demonstrated comparable efficacy. Yet, follow-up assessments until weeks 12 and 16 revealed a decline in the VAS pain index within the MA group. Consistent with our results, studies by Liu et al. (Liu et al. 2021), Tu et al. (Tu et al. 2019), and Ahsin et al. (Ahsin et al. 2009) reported higher efficacy of EA over MA in improving and maintaining pain (VAS), stiffness (WOMAC), and physical activity (WOMAC) parameters. Additionally, Ahsin et al. demonstrated that sham acupuncture reduced pain severity; however, no improvements were observed in stiffness or physical activity, mirroring our findings (Ahsin et al. 2009).

Our strategy for choosing acupuncture points was based on the previous studies (Jin and Guan 2022; Liu et al. 2024) and the experiences of acupuncture specialists. In this case, we tried to manipulate points that could potentially influence the pathways which control pain and promote intracellular signaling pathways toward a state that lead to suppressed production of pro-inflammatory cytokines such as IL-1β, Il-6, and TNF-α and increased secretion of anti-inflammatory and immunomodulatory

mediators such as TGF-β and IL-10 (Wu et al. 2020). Fortunately, our study showed promising results in the case of successful choosing acupuncture points, as improved VAS pain score and increased levels of TGF-β cytokine were reported.

Inflammation plays a pivotal role in the initiation and progression of KOA, with increased levels of IL-1β and TNF-α in synovial fluids highlighted in KOA pathogenesis (Shi et al. 2020). In our study, we assessed the levels of both pro-inflammatory and anti-inflammatory cytokines in serum following various acupuncture methods. The results revealed no significant associations between IFN-γ, TNF-α, IL-4, and IL-10 serum levels. However, EA intervention significantly increased the levels of the anti-inflammatory cytokine TGF-β. Consistent with our findings, a study by Shi et al. reported that acupuncture improved clinical parameters alongside the suppression of TNF-α and IL-1β inflammatory cytokines (Shi et al. 2020). Furthermore, Liu *et al.* and some other researchers highlighted the efficacy of EA in clinical improvement due to suppressed inflammatory cytokines (Kang et al. 2018; Kang et al. 2020; Liu et al. 2021; Yue et al. 2016). The conflicting results between improved clinical symptoms and insignificant changes in cytokine levels in our study may be attributed to the limited sample size and heterogeneity of disease severity among recruited patients. Additionally, Wu et al. reported improved clinical symptoms and suppressed TNF-α and IL-1β levels due to complementary acupuncture in conjunction with *Duhuo Jisheng* supplementation, a Chinese traditional herbal medicine (Wu et al. 2020).

Overall, acupuncture, whether administered through MA or EA methods, emerges as an efficient, potent, and safe complementary therapeutic approach for KOA. The short-term efficacy of SA intervention on pain (VAS) without significant impact on quality of life (SF-12), stiffness (WOMAC), and physical activity (WOMAC) parameters emphasizes the effectiveness of MA and EA. Recent studies have emphasized the association of clinical improvement following acupuncture in KOA with changes in cytokine profiles; however, our study only demonstrated significant fluctuations in TGF-β levels following EA. Important limitations include the small sample size, short follow-up periods, limited number of measured cytokines, and source of cytokines. Furthermore, all recruited patients in our study exhibited KL scores of 2-3, indicating mild to moderate forms of the disease, whereas severe patients may exhibit different outcomes. Additionally, MRI analysis of involved joints may provide further insights into the effects of MA and EA on tissue changes. The strengths of this study include the absence of prior acupuncture history among patients, which ensures effective blinding throughout the study period for both patients and other study personnel, except the acupuncturist; in addition, the present study attempted to simultaneously assess both the efficacy and mechanism of action of acupuncture in KOA patients.

In summary, our study demonstrates that both MA and EA significantly alleviate clinical parameters including stiffness WOMAC and physical activity WOMAC, and improve quality of life indices in KOA patients. However, no clear associations were observed between serum levels of pro-inflammatory and anti-inflammatory cytokines (except for TGF-β in the EA group which was significantly increased compared to MA group) and improvements in clinical manifestations following different acupuncture interventions. Overall, acupuncture emerges as an effective, safe, and cost-effective complementary method, offering KOA patients an alternative to reduce medication consumption and mitigate associated side effects. Furthermore, future investigations exploring the underlying mechanisms involved could provide valuable insights

into the therapeutic effects of acupuncture in KOA.

Conflicts of interest

All authors declare no potential conflict of interests.

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Data availability statement

Data are available on request from corresponding authors.

Ethical Considerations

The trial protocol was approved by the Ethic committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.1401/371) and registered in Iranian Registry of Clinical Trials (IRCT20170305032893N3).

Code of Ethics

IR.MUMS.1401/371

Authors' Contributions

Ahmad Hamze: help for providing the hypothesis, accomplished all the project steps and visit all the patients, help in preparing the manuscript.

Kamilla Hashemzadeh: provided the hypothesis and supervise all the study, preparing the manuscript.

Ali Khorsand Vakilzadeh, Hamid Reza Bahrami-Taghanaki, Sajad Dehnavi, Mojgan Mohammadi, Negar Morovatdar, and Mohammad Ali Khodadoust: help in visiting the patient, accomplishing the acupuncture procedure, immunology tests, and preparing the manuscript.

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