

Effect of modified Qing Long Bai Wei needling on the levels of IL-1 β , IL-6 and INF- α in synovial fluid of knee osteoarthritis patients

改良青龙摆尾针法对膝关节炎患者膝关节液IL-1 β 、IL-6和TNF- α 水平的影响

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Abstract

Objective: To compare the modified Qing Long Bai Wei needling method and ordinary acupuncture method in the effects of improving the levels of interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α in the treatment of knee osteoarthritis (KOA), and to determine the advantage of the modified Qing Long Bai Wei needling method for KOA.

Methods: One hundred KOA patients were randomized into a treatment group and a control group by using the random number table, with 50 cases in each group. The treatment group was intervened by the modified Qing Long Bai Wei needling method, and the control group was given ordinary acupuncture. The two groups were observed before and after the treatment to determine the changes in the levels of IL-1 β , IL-6 and TNF- α in synovial fluid, and the clinical efficacies were compared between the two groups.

Results: The total effective rate and clinical recovery rate were 97.9% and 52.1% respectively in the treatment group, versus 85.1% and 25.5% in the control group, and the between-group differences were statistically significant (both $P < 0.01$). After the treatment, the levels of IL-1 β , IL-6 and TNF- α in synovial fluid changed significantly in both groups (all $P < 0.01$); there were significant differences in the levels of IL-1 β , IL-6 and TNF- α in synovial fluid between the two groups (all $P < 0.01$).

Conclusion: The modified Qing Long Bai Wei needling is an effective method for KOA and it can significantly improve the levels of IL-1 β , IL-6 and TNF- α in synovial fluid.

Keywords: Acupuncture Therapy; Osteoarthritis, Knee; Qing Long Bai Wei; Acupuncture Sensation; Interleukin-1beta; Interleukin-6; Tumor Necrosis Factor-alpha

【摘要】目的：比较改良青龙摆尾针法与常规针法对膝关节炎(KOA)患者白细胞介素(IL)-1 β 、IL-6和肿瘤坏死因子(TNF)- α 水平改善情况的差异，明确改良青龙摆尾针法治疗KOA的优势。**方法：**将100例KOA患者按随机数字表法随机分为治疗组和对照组，每组50例。治疗组采用改良青龙摆尾针法治疗，对照组采用常规针法治疗。观察两组患者治疗前后膝关节液IL-1 β 、IL-6和TNF- α 水平的变化情况，并比较两组临床疗效。**结果：**治疗组总有效率和临床痊愈率分别为97.9%和52.1%，对照组分别为85.1%和25.5%，两组差异均具有统计学意义(均 $P < 0.01$)。治疗后，两组膝关节液IL-1 β 、IL-6和TNF- α 水平与同组治疗前比较，差异均具有统计学意义(均 $P < 0.01$)；治疗组膝关节液IL-1 β 、IL-6和TNF- α 水平与对照组比较，差异具有统计学意义(均 $P < 0.01$)。**结论：**改良青龙摆尾针法是一种治疗KOA的有效方法，能明显改善患者膝关节液IL-1 β 、IL-6和TNF- α 水平。

【关键词】 针刺疗法；骨关节炎，膝；青龙摆尾；针刺行气；白细胞介素1 β ；白细胞介素6；肿瘤坏死因子 α

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【文献标志码】 A

Knee osteoarthritis (KOA) is commonly seen in the elderly and tends to affect more and more people every year^[1]. Currently, hyperosteogeny of knee joint and degeneration of the articular surface are considered as the main causing factors^[2]. The most commonly used

drugs for KOA, such as analgesics and non-steroid anti-inflammatory drugs (NSAIDs), can only ease pain but can merely help with the improvement of function, while surgery may cause irreversible damage to the joint tissues. Therefore, conservative treatment is still the first choice for KOA^[3]. Numerous studies at home and abroad have suggested a certain correlation between cytokines and KOA, and it is believed that the interaction among cytokines and the loss of balance will cause the degradation and damage of cartilage matrix

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and then result in osteoarthritis^[4-5]. Acupuncture-moxibustion has been proved effective in treating KOA^[6]. In this trial, we adopted the modified Qing Long Bai Wei needling method to treat KOA and observed the changes in the levels of interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α by taking the ordinary acupuncture method as the control. The report is given as follows.

1 Clinical Materials

1.1 Diagnostic criteria

The criteria were made by referring to the *Guideline on the Diagnosis and Treatment of Osteoarthritis* (2007) stipulated by the Chinese Orthopedic Association^[7]: ① recurrent knee joint pain in the previous one month; ② X-ray (in a standing or weight-bearing position) revealed joint space narrowing, subchondral sclerosis and/or subchondral cyst formation, and osteophytes; ③ synovial fluid (checked twice at least) was clear and viscous, white blood cell count <2 000/mL; ④ middle-aged and elderly people (≥ 40 years old); ⑤ morning stiffness ≤ 3 min; ⑥ crepitus on active motion. KOA can be diagnosed when ①+② or ①+③+⑤+⑥ or ①+④+⑤+⑥ were met.

1.2 Inclusion criteria

In conformity with the diagnostic criteria; age at 40-65 years old; joined the trial voluntarily and signed the informed consent form.

1.3 Exclusion criteria

With severe diseases involving the heart, brain, liver, kidney or hematopoietic system; received acupuncture-moxibustion treatment within the previous three months; pregnant or breast-feeding women; those with psychological disorders; those who were still in other clinical trials; those unable to cooperate in or finish the intervention; coupled with diseases that may affect joints, such as psoriasis, metabolic bone disease, knee joint tuberculosis or tumor, septic knee arthritis, and acute trauma (including meniscus injury, collateral ligament injury, cruciate ligament injury, and traumatic synovitis or hematoma).

1.4 Statistical methods

The data were processed by using SPSS version 21.0. Measurement data that conformed to a normal distribution were expressed as mean \pm standard deviation ($\bar{x} \pm s$) and analyzed by *t*-test; enumeration data were analyzed by using Chi-square test. $P < 0.05$ was considered to indicate statistical significance.

1.5 General data

This trial was approved by the Ethics Committee of Hubei Provincial Hospital of Traditional Chinese Medicine (approval number: HBZY2015-C27-01).

One hundred KOA patients were recruited from the outpatient of Acupuncture-moxibustion Department of Hubei Provincial Hospital of Traditional Chinese

Medicine between July 2016 and July 2017. By following their visiting sequence, the patients were randomized into a treatment group and a control group by using the random number table, with 50 cases in each group. Two cases in the treatment group dropped out (one case quitted halfway and the other one lost contact); in the control group, three cases dropped out (two quitted halfway and one lost contact). Eventually, 95 patients completed the study and the whole process is shown in Figure 1.

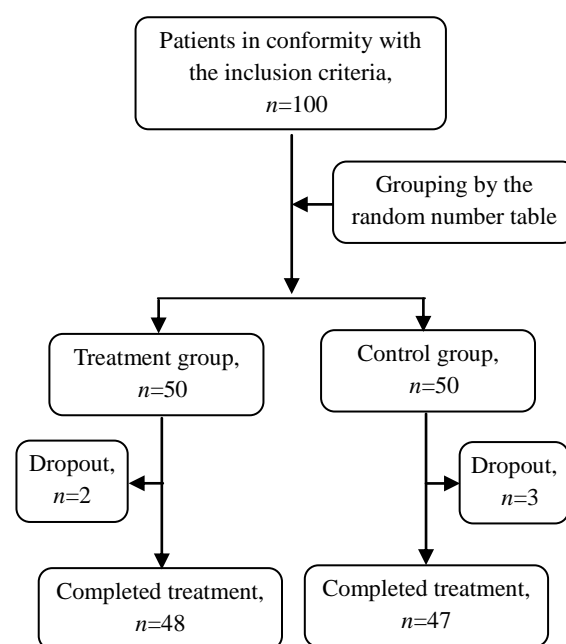


Figure 1. Flow chart of the study

There were no significant differences in the data of gender, age or disease duration between the two groups ($P > 0.05$), indicating the comparability (Table 1).

Table 1. Comparison of the general data

Group	n	Gender (case)		Average age ($\bar{x} \pm s$, year)	Average duration ($\bar{x} \pm s$, month)
		Male	Female		
Treatment	48	15	33	57.1 \pm 9.8	21.4 \pm 4.7
Control	47	17	30	56.5 \pm 11.4	20.3 \pm 5.7

2 Treatment Methods

2.1 Treatment group

Major points: Dubi (ST 35) and Neixiyan (EX-LE 4).

Adjunct points: Zusanli (ST 36), Yanglingquan (GB 34), Xuehai (SP 10), Xuanzhong (GB 39) and Ashi.

Method: The modified Qing Long Bai Wei needling was used. After standard sterilization, the patient was asked to calm down and regulate the breath. Filiform needles of 30 mm in diameter and 40 mm in length were used for acupuncture. The patient was then asked to give a quiet cough when the doctor punctured the

needles with hand-pressing insertion method. When the needle reached to certain depth and caused numb or distending feeling, it was lifted up to subdermal layer. Then, the needle body was pressed down with the needle tip pointing towards the affected area. The handle of the needle was held to slowly wag in every direction, but the needle was maintained in the same layer. The insertion and removal of the needles were operated by layers and the handle of the needle was wagged for different times in different layers: three times for the layer of heaven (superficial layer), nine times for the layer of human (middle layer) and six times for the layer of earth (deep layer) at the insertion; nine times for the layer of earth (deep layer), three times for the layer of human (middle layer) and six times for the layer of heaven (superficial layer) at the removal. The above performance was conducted three times respectively at the insertion and removal. During the process, the patient needed to inhale through the nose and exhale through the mouth. The needle was inserted at exhalation while the needle handle was wagged all around at inhalation after the arrival of needling qi, which is considered as reinforcing method. The patient inhaled through the mouth and exhaled through the nose, and the needle was inserted at inhalation while the needle handle was wagged all around at exhalation after the arrival of needling qi, which is called reducing method. The reinforcing method was performed at Zusanli (ST 36) and Xuanzhong (GB 39); the reducing method was used for Dubi (ST 35) and Neixiyan (EX-LE 4); even reducing-reinforcing method was applied to the rest points. The needles were retained for 30 min. The treatment was performed once every day, 6 sessions as a course of treatment at a one-day interval, for 4 courses in total.

2.2 Control group

Major points: The same as the treatment group.

Adjunct points: The same as the treatment group.

Method: Ordinary acupuncture method was used. After standard sterilization, filiform needles of 30 mm in diameter and 40 mm in length were adopted for acupuncture treatment. When needling qi was obtained, even reducing-reinforcing lifting-thrusting method was performed. The needles were retained for 30 min and manipulated once every 15 min during the retaining. The treatment was conducted once daily, 6 sessions as a course of treatment at a one-day interval, for 4 courses in total.

3 Observation of Therapeutic Efficacy

3.1 Observation indicators

Before and after the intervention, 3 mL knee joint fluid was drawn by using a 5 mL syringe under aseptic condition and then centrifuged at 3 000 rpm for 5 min. Then, the supernatant was collected and stored at -70°C . Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of IL-1 β , IL-6 and TNF- α in the joint fluid.

3.2 Criteria of the therapeutic efficacy

By referring to the Nimodipine method, the therapeutic efficacy was determined based on the index of severity for osteoarthritis (ISOA)^[8-9]. Therapeutic efficacy index = (Pre-treatment score – Post-treatment score) \div Pre-treatment score $\times 100\%$.

Clinical recovery: The symptoms disappeared, functional activities went normal, and the therapeutic efficacy index $\geq 75\%$.

Markedly effective: The symptoms substantially disappeared, normal activities and work were possible, and the therapeutic efficacy index $\geq 50\%$ and $< 75\%$.

Effective: Pain was almost gone, extension and flexion of the joint was substantially normal, activities or work abilities were partially regained, and the therapeutic efficacy index $\geq 25\%$ and $< 50\%$.

Invalid: Failed to reach the effective standard, and the therapeutic efficacy index $< 25\%$.

3.3 Treatment result

3.3.1 Comparison of the clinical efficacy

The total effective rate and clinical recovery rate were respectively 97.9% and 52.1% in the treatment group, versus 85.1% and 25.5% in the control group, and there were significant differences between the two groups (both $P < 0.01$), indicating that the treatment group produced a more significant efficacy than the control group (Table 2).

3.3.2 Comparison of the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid

There were no significant differences in the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid between the two groups before the treatment (all $P > 0.05$). After the treatment, the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid changed significantly in both groups (all $P < 0.01$); the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid in the treatment group were significantly different from those in the control group (all $P < 0.01$), (Table 3).

Table 2. Comparison of the clinical efficacy (case)

Group	<i>n</i>	Clinical recovery	Markedly effective	Effective	Invalid	Clinical recovery rate (%)	Total effective rate (%)
Treatment	48	25	15	7	1	52.1 ¹⁾	97.9 ¹⁾
Control	47	12	16	12	7	25.5	85.1

Note: Compared with the control group, 1) $P < 0.05$

Table 3. Comparison of the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid ($\bar{x} \pm s$, ng/mL)

Group	n	Time	IL-1 β	IL-6	TNF- α
Treatment	48	Pre-treatment	118.24 \pm 13.02	45.36 \pm 11.24	66.24 \pm 12.38
		Post-treatment	68.32 \pm 11.24 ¹⁾²⁾	21.43 \pm 8.15 ¹⁾²⁾	33.26 \pm 9.43 ¹⁾²⁾
Control	47	Pre-treatment	117.64 \pm 13.58	46.74 \pm 10.35	64.34 \pm 13.57
		Post-treatment	79.53 \pm 12.27 ¹⁾	31.62 \pm 9.46 ¹⁾	57.42 \pm 10.76 ¹⁾

Note: Intra-group comparison, 1) $P < 0.01$; compared with the control group after the treatment, 2) $P < 0.01$

3.4 Safety evaluation

Some adverse events which are common in acupuncture treatment occurred, including hematoma, topical bleeding and needling pain, and were properly treated. There were no other severe adverse events occurred during the 4-week study.

4 Discussion

KOA belongs to the scope of bone Bi-impediment and is often caused by liver-kidney insufficiency, cold-damp invasion and blocked meridians and collaterals. The main symptoms include swelling, pain and limited range of motion of the knee joint. Acupuncture-moxibustion is a traditional Chinese medicine (TCM) therapy. Acupuncture at the corresponding points can unblock and regulate the local qi and blood, restore the internal balance of knee articular cartilage and improve the symptoms to effectively treat KOA^[10].

Zhen Jiu Da Quan (*The Complete Compendium of Acupuncture and Moxibustion*) in the Ming Dynasty mentions several complex needling manipulations, such as Shao Shan Huo (Mountain-burning Fire), Tou Tian Liang (Heaven-penetrating Cooling), Qing Long Bai Wei (Green Dragon Wagging Its Tail) and Bai Hu Yao Tou (White Tiger Shaking Its Head). In this book, the author Xu Feng vividly describes the Qing Long Bai Wei needling manipulation, one of the four Fei Jing Zou Qi (Meridian Transmission and Qi Action) methods, 'to perform Qing Long Bai Wei is like to control rudder, not inserting deeper or withdrawing the needle, but slowly wagging it left and right'^[11]. Professor Li Jia-kang, a famous TCM expert working in our department, has modified the performance of Qing Long Bai Wei through constant clinical practice for boosting the treatment efficacy. Besides the three original elements, as controlling rudder, wagging left and right and wagging slowly, he has combined the reinforcing-reducing methods of respiration and nine-six numerical method, as well as needling based on heaven-human-earth layers. According to years' clinical practice and study^[12-13], it has been found that the modified Qing

Long Bai Wei needling method not only can achieve satisfactory qi-obtaining, qi-guiding and qi-activating effects, but also can fortify the needling sensation, the effect of regulating and unblocking meridians and collaterals, and qi activities.

Osteoarthritis is also known as degenerative joint disease, majorly manifested by cartilage degradation, osteophyte formation and subchondral sclerosis^[14]. The development of osteoarthritis involves a variety of cytokines and inflammatory mediators, including IL-1 β , IL-6 and TNF- α in serum^[15]. Among which, IL-1 β , a polypeptide and a subtype of IL-1, is closely associated with cartilage damage^[16]. IL-6 is a pro-inflammatory cytokine secreted by T lymphocytes and mainly works to inhibit the synthesis of glycoprotein in articular cartilage and promote the degradation of fibroblasts and matrix micromolecules, which will destroy the structure and function of the cartilage^[17-18]. TNF- α , a subtype of TNFs, can accelerate the proliferation of fibroblast-like synoviocytes, resulting in fibrinoid degeneration of synovial tissues and enhancing the degradation of cartilage matrix^[19]. Marks PH, *et al*^[20] found that the levels of IL-1 β and TNF- α in joint fluid of osteoarthritis patients increased significantly, which was positively correlated with the damage of joint cartilage.

This study observed the changes in the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid of KOA patients after the treatment with the modified Qing Long Bai Wei acupuncture method and also discussed the efficacy of this treatment for KOA. The results suggested that the treatments adopted in both treatment and control groups managed to down-regulate the levels of IL-1 β , IL-6 and TNF- α in knee joint fluid of KOA patients, while the improvements in the treatment group were more significant than those in the control group (all $P < 0.01$). Moreover, we found that the modified Qing Long Bai Wei needling method produced a stronger needling sensation which can rapidly reach to the affected site and release pain. Since this treatment method can swiftly and effectively improve the knee joint motor function of KOA patients and produce valid therapeutic efficacy, it is worth further study.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Informed Consent

Informed consent was obtained from the patients in this study.

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