Effect of adjuvant therapy with electroacupuncture on bone turnover markers and interleukin 17 in patients with rheumatoid arthritis

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Supported by funding from the Traditional Chinese Medicine Project of Chongqing to Y.J. (Grant number ZY201602140).
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Accepted: May 21, 2018

RESULTS: In all three treatment groups, serum levels of the bone metabolism markers PICP, N-MID, and B-ALP were elevated and the concentrations of the inflammatory markers β-CTX, IL-17, CRP, and TRACP-5b were reduced after treatment. These differences were significant for the EA group but not the other groups (P < 0.05).

CONCLUSION: EA could effectively reduce the suffering and improve the quality of life of RA patients. It is a promising adjuvant therapy for enhancing the effectiveness of clinical therapeutics.

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Keywords: Electroacupuncture; Rheumatoid arthritis; Bones; Metabolism; Biomarkers; Interleukin-17

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease characterized by abnormalities in the amount and micro-architectural arrangement of the bone tissue.1 It can affect many tissues and organs but it mainly affects the joints. Bone loss is a major unsolved problem in RA that leads to enhanced bone fragility and an increased risk of fractures. The fundamental pathological changes are synovitis characterized by the proliferation of synovial cells, mass infiltration of inflammatory cells, the formation of blood vessels, and the progressive destruction of cartilage and bone. The main complications of RA include marginal and subchondral bone focal erosion, juxtaarticular osteoporosis, and generalized bone loss. Osteoporosis and local bone erosion are common clinical complications of patients with early manifestations of RA and result in
The patients comprised 44 females and 16 males. They were divided into three groups of 20 patients each using a single-blind randomized design: the medication group (also termed the "control" group; MTX: 7.5 mg/week, LEF: 20 mg/d), the medication plus acupuncture group, and the medication plus EA group. The necessary basic acupoints for therapy were the Dazhui point (DU 14), Shenshu point (BL 23), Mingmen point (DU 4), Pi’shu point (BL 20), Kanshu point (BL 18), and Tusunli point (ST 36). Additional acupoints used for different lesions were the Jiandu point (LI 15) and Acromio-illiac point (SI 14) for omarthritis; the Waikuan point (SI 5) and Quchi point (LI 11) for olecranonarthritis; the Hegu point (LI 4), Yangxi point (LI 5), and Yangchi point (SI 4) for carpal arthritis; the Baxie point (EX-UE 9) for metacarpophalangeal arthritis; the Huaniao points (GB 30) for coxarthropy; simultaneous use of the Neixiyan point (EX-LE 4), Dubi point (ST 35), Weizhong point (BL 40), Yanglingquan point (GB 34), and Yinlingquan point (SP 9) for gonarthritis; the Chieh hsi point (ST 41), Kunlun point (BL 60), and Qixu point (GB 40) for ankle arthritis; and the Bafeng point (EX-LE 10) for metatarsophalangeal arthritis.

**Electroacupuncture therapy**

Therapy was performed when the patients were emotion-normal. After disinfecting the necessary basic acupoints, a 0.3 × 50 mm acupuncture needle was inserted perpendicular to the skin to a depth of 1.5-2.5 cm in the Shenshu point (BL 23), Pi’shu point (BL 20), Kanshu point (BL 18), and Tusunli point (ST 36). Combined reinforcing-reduction was achieved by lifting-inserting after De Qi (arrival of Qi). The acupuncture needles were inserted from shallow to deep and left to right with hardly any left turning and later pulled out gently with right turning. The frequency of stimulation was 20-30 times per minute, and needles were retained for 30 min after insertion was complete. For the Mingmen points (DU 4) and Dazhui point (DU 14), 0.3×50 mm acupuncture needles were inserted into the skin to a depth of 1.5-2.5 cm with an approximately 75° upward inclination, and the needles were retained for 30 min after insertion was complete. For points along the target joint, a similar insertion procedure was followed as for the necessary basic acupoints, except that the stimulation frequency was 30-40 times per minute. At the beginning of the retaining period, the needles were energizing. The choice of acupoints for electroacupuncture therapy was flexible; 1-2 points for the necessary basic acupoints and 1 point for the local target joint according to the patient’s condition. The treatment was given once every other day and 10 treatments made up one course over a total period of 8 weeks. EA treatments were performed with the G91-A electroacupuncture instrument (Kangling, Yangzhou, China) and the dilatational wave was allowed to persist for 30 min.

**Materials and Methods**

**Patients and treatment procedures**

Sixty RA patients aged between 30 and 60 years old were selected from the First Affiliated Hospital of Chongqing Medical University. The average age of the patients was (50 ± 13) years, and patients were identified based on the American College of Rheumatology revised RA diagnostic criteria in 2009. The mean DAS28 score was 4.4±1.4, which indicated that the patients had, on average, been suffering from pain and distortion of their joints for a long time. The inclusion criteria for patients were a lumbar vertebral density of –1 < T < –2.5, observable osteoporosis in both hands based on imaging examination, CCP positivity, and a disease duration of fewer than 12 months. Patients who (a) had severe cardiac-cerebral, vascular, or hepato renal disease, leukocyte cells < 3 × 10^9/L or other autoimmune diseases; (b) were taking non-steroidal analgesics or glucocorticoids one month before the study; (c) had knee joint infection, joint tuberculosis or a history of operations were all excluded from the study. Research ethics approval was obtained from ethics committee of the first affiliated hospital of Chongqing medical university prior to commencing the study. Written informed consent was obtained from all subjects.

RA treatments targeted toward the inhibition of disease activity or the alleviation of suffering are termed disease-modifying antirheumatic drugs (DMARDs). Traditional therapeutic approaches using DMARDs preferentially initiate treatment with methotrexate (MTX) and leflunomide (LEF). These drugs are extensively applied in the treatment of RA because of their immunosuppressive activity through inhibition of dihydrofolate reductase and dihydroorotate dehydrogenase, respectively. However, the efficacy of traditional therapies is not sufficient to prevent bone destruction. Furthermore, drug toxicity has prevented many RA patients from attaining therapeutic benefit. Mild toxicity reportedly occurs in about 60% of patients, and almost 30% of patients interrupt MTX treatment in the first year because of drug toxicity. However, while biologic agents like interleukin-6 inhibitors (IL-6) can quickly control inflammation and inhibit bone destruction, these often entail exorbitant financial expenditure and high risk of infection.

Electroacupuncture (EA) therapy involves inserting a needle into an acupoint with a trace pulse current to produce synthetic electric and needling stimulation. In this study, we treated patients with a low dose of DMARDs plus EA to investigate the efficacy of this combination therapy.

**Results**

The mean age of the patients was (50 ± 13) years, and patients were identified based on the American College of Rheumatology revised RA diagnostic criteria in 2009. The mean DAS28 score was 4.4±1.4, which indicated that the patients had, on average, been suffering from pain and distortion of their joints for a long time. The inclusion criteria for patients were a lumbar vertebral density of –1 < T < –2.5, observable osteoporosis in both hands based on imaging examination, CCP positivity, and a disease duration of fewer than 12 months. Patients who (a) had severe cardiac-cerebral, vascular, or hepato renal disease, leukocyte cells < 3 × 10^9/L or other autoimmune diseases; (b) were taking non-steroidal analgesics or glucocorticoids one month before the study; (c) had knee joint infection, joint tuberculosis or a history of operations were all excluded from the study. Research ethics approval was obtained from ethics committee of the first affiliated hospital of Chongqing medical university prior to commencing the study. Written informed consent was obtained from all subjects.


Evaluation measurement

We collected 3.2 mL of peripheral blood from each patient on an empty stomach both before treatment and 12 weeks post therapy. The erythrocyte sedimentation rate was determined according to Westergren’s method. The serum was separated to detect the level of bone metabolism markers. The level of C-reactive protein (CRP), tartrate-resistant acid phosphatase-5b (TRACP-5b), bone-specific alkaline phosphatase (B-ALP), and interleukin-17 (IL-17) was monitored by enzyme-linked immunosorbent assay (ELISA, Sangon, Shanghai, China). Furthermore, the levels of carboxyterminal propeptide of type I procollagen (PICP), N-terminal-midfragment of osteocalcin (N-MID), and the β form of the C-terminal telopeptide of type I collagen (β-CTX) were determined by chemiluminescence. Joint hyperplasia was detected by ultrasound. DAS28 grading was performed before and post treatment.

Statistical analysis

All data were analyzed by SPSS 19.0 statistical software (SPSS; IBM Corporation, Armonk, NY, USA) and expressed as the mean ± standard deviation (\( \bar{x} \pm s \)). One-way analysis of variance and independent sample t test were used to analyze the variance. The difference was considered significant at a P value < 0.05 (assigned as b), or less than 0.01 (assigned as c). All experiments were carried out at least three times in triplicate.

Table 1 Comparison of the clinical characteristics of the three treatment groups (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>NNT</th>
<th>Gender (male/female)</th>
<th>Age (years)</th>
<th>Duration (months)</th>
<th>RF (U/mL)</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Knee joint</td>
</tr>
<tr>
<td>Medication</td>
<td>20</td>
<td>6/14</td>
<td>46</td>
<td>5.7±1.4</td>
<td>130.0±7.1</td>
<td>7</td>
</tr>
<tr>
<td>Acupuncture</td>
<td>20</td>
<td>5/15</td>
<td>49</td>
<td>5.3±1.2</td>
<td>132.0±2.4</td>
<td>10</td>
</tr>
<tr>
<td>EA</td>
<td>20</td>
<td>5/15</td>
<td>55</td>
<td>6.0±1.8</td>
<td>126.0±6.6</td>
<td>3</td>
</tr>
</tbody>
</table>

Notes: medication group: treated with MTX (7.5 mg/week) and LEF (20 mg/d); acupuncture group: treated with MTX (7.5 mg/week), LEF (20 mg/d) and acupuncture; EA group: treated with MTX (7.5 mg/week), LEF (20 mg/d) and electroacupuncture. EA: electroacupuncture; NNT: number need to treat; RF: rheumatoid factor.

Table 2 Comparison of the clinical symptom indexes of the three treatment groups (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>NNT</th>
<th>SJC</th>
<th>TJC</th>
<th>VAS</th>
<th>DAS28</th>
<th>Morning stiff duration (min)</th>
<th>CRP (mg/L)</th>
<th>ESR (mm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication</td>
<td>20</td>
<td>13.0±2.4</td>
<td>13.0±1.6</td>
<td>5.8±2.6</td>
<td>6.7±1.9</td>
<td>86.0±22.7</td>
<td>25.6±5.2</td>
<td>58.0±8.2</td>
</tr>
<tr>
<td>Acupuncture</td>
<td>20</td>
<td>12.0±1.2</td>
<td>12.0±1.2</td>
<td>5.4±3.2</td>
<td>6.5±1.3</td>
<td>81.0±28.4</td>
<td>25.2±4.8</td>
<td>57.0±6.9</td>
</tr>
<tr>
<td>EA</td>
<td>20</td>
<td>12.0±2.8</td>
<td>12.0±2.3</td>
<td>5.5±3.6</td>
<td>6.6±1.8</td>
<td>80.0±26.2</td>
<td>24.7±6.6</td>
<td>58.0±7.7</td>
</tr>
</tbody>
</table>

Notes: medication group: treated with MTX (7.5 mg/week) and LEF (20 mg/d); acupuncture group: treated with MTX (7.5 mg/week), LEF (20 mg/d) and acupuncture; EA group: treated with MTX (7.5 mg/week), LEF (20 mg/d) and electroacupuncture. EA: electroacupuncture; NNT: number need to treat; SJC: swollen joint count; TJC: tender joint count; VAS: visual analog scale; DAS28: Disease Activity Score using 28 joint counts; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate. \( P > 0.05 \), \( P < 0.05 \), \( P < 0.01 \), compared with the control group after treatment.

RESULTS

As shown in Table 1, there were no statistical differences in the demographic data between the three groups of patients. However, the clinical symptoms changed significantly between treatment initiation and the post-treatment follow-up after 12 weeks for the EA group: the DAS29 and VAS scales and the swollen and tender joint count and morning stiffness duration were all significantly decreased compared with the control (medication only), which indicated a major improvement in the quality of life of these patients. Thus, the EA group (treated with MTX, LEF, and EA) showed that this approach had a superior therapeutic effect when compared with medication alone (Table 2).

As shown in Table 3, the concentrations of serum PICP, N-MID, and B-ALP were increased after treatment, while the concentrations of serum β-CTX and TRACP-5b decreased. The differences in these indexes were significantly greater for the EA group compared with the medication (control) group. The concentration of serum IL-17 in the EA group was also dramatically reduced after treatment compared with the control.

DISCUSSION

RA is characterized by the aggregation of T cells, B cells, macrophages, and synovial fibroblasts within the synovial membrane, which eventually leads to joint de-
In conclusion, our findings suggest that electroacupuncture combined with MTX and LEF could significantly reduce suffering and improve the quality of life of RA patients. The concentrations of various bone metabolism biomarkers tested were closely linked with measures of therapeutic efficacy, indicating that the current approach was suitable for investigating the effect of electroacupuncture as an adjuvant therapy.

**REFERENCES**


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**Table 3 Comparison of laboratory indexes among the three treatment groups (x ± s)**

<table>
<thead>
<tr>
<th>Group</th>
<th>PICP (ng/mL)</th>
<th>N-MID (ng/mL)</th>
<th>β-CTx (pg/mL)</th>
<th>IL-17 (ng/mL)</th>
<th>B-ALP (µg/L)</th>
<th>TRACP-5b (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication</td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
<td>Posttreatment</td>
</tr>
<tr>
<td></td>
<td>88.0±22.3</td>
<td>10.6±6.4</td>
<td>511.0±59.4</td>
<td>188.0±38.2</td>
<td>16.0±1.9</td>
<td>4.6±0.5</td>
</tr>
<tr>
<td>Acupuncture</td>
<td>98.0±32.5</td>
<td>14.0±4.1</td>
<td>405.0±43.8</td>
<td>157.0±24.3</td>
<td>18.0±6.2</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>EA</td>
<td>79.0±25.5</td>
<td>10.0±5.2</td>
<td>498.0±39.9</td>
<td>190.0±43.5</td>
<td>15.0±2.1</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td></td>
<td>102.0±18.2²</td>
<td>15.0±6.2'</td>
<td>375.0±28.4²</td>
<td>150.0±39.4²</td>
<td>20.0±3.2'</td>
<td>3.2±0.2'</td>
</tr>
<tr>
<td></td>
<td>86.0±20.0</td>
<td>10.0±3.3</td>
<td>508.0±42.5</td>
<td>185.0±44.2</td>
<td>16.0±2.7</td>
<td>4.2±0.6</td>
</tr>
</tbody>
</table>

Notes: medication group: treated with MTX (7.5 mg/week) and LEF (20 mg/d) and acupuncture group: treated with MTX (7.5 mg/week), LEF (20 mg/d) and acupuncture; EA group: treated with MTX (7.5 mg/week) and electroacupuncture. EA: electroacupuncture; PICP: carboxyterminal propeptide of type I procollagen; N-MID: N-terminal-midfragment of osteocalcin; β-CTx: β form of the C-terminal telopeptide of type I collagen; B-ALP: bone-specific alkaline phosphatase; TRACP-5b: tartrate-resistant acid phosphatase-5b. *P > 0.05, †P < 0.05, ‡P < 0.01, compared with the control group after treatment.


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