• Research Article

Effects of acupuncture treatment on natural killer cell activity, pulse rate, and pain reduction for older adults: an uncontrolled, observational study

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OBJECTIVE: The aim of this study was to examine the changes in natural killer (NK) cell activity, pulse rate, and pain intensity among older adults before and after acupuncture treatment.

METHODS: Fifty-six individuals (16 males and 40 females), aged 60 to 82 years (mean age 72.4 ± 5.0), who were experiencing pain in the shoulder, low back, or knee, participated in the study. NK cell activity, leukocyte differentiation (granulocytes and lymphocytes), pulse rate, and blood pressure values were obtained. Pain intensity was evaluated by using the visual analog scale (VAS). The Wilcoxon test was used to analyze NK cell activity, leukocytes (granulocyte counts and granulocyte-to-lymphocyte ratio), and the VAS score in accordance with the location of pain complaints before and after acupuncture treatment.

RESULTS: NK cell activity decreased after acupuncture treatment for pain in the shoulder-pain and knee-pain groups. Further, the lymphocyte and granulocyte counts increased after acupuncture treatment for the shoulder-pain group. Pulse rate decreased for the shoulder-pain, low-back-pain, and knee-pain groups after acupuncture treatment. The VAS score decreased after acupuncture treatment for the shoulder-pain, low-back-pain, and knee-pain groups.

CONCLUSION: This study showed that in older adults, acupuncture treatment decreases pulse rate, relieves pain in the shoulder, low back, and knee, and reduces NK-cell activity.

KEYWORDS: acupuncture therapy; killer cells, natural; leukocytes; heart rate; pain measurement; clinical trial

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1 Introduction

Over the last few decades, acupuncture has been used in Western countries as a primary modality in complementary and alternative medicine[1-3]. Acupuncture has been known to control pain and modulate the functioning of the autonomic and immune systems. Studies have shown that acupuncture relieves musculoskeletal pain in the shoulder⁴, lower back⁵, and knee⁶. Moreover, acupuncture has been shown to influence the cardiac vagal function⁷. Studies have demonstrated that acupuncture stimulation decreases heart rate⁸, in addition, it reduces heart rate and pupillary constriction simultaneously⁹, responses that are considered signs of parasympathetic activation.

In terms of acupuncture’s influence on the immune system, it has been reported that acupuncture stimulation up-regulates natural killer (NK) cell activity and augments neural-immune...
interaction\textsuperscript{[10]}). Enhancement of spleen NK cell activity was also observed in the wake of electroacupuncture administered to laboratory animals (rats and mice)\textsuperscript{[11,12]}. Hisamitsu et al\textsuperscript{[13]} observed in their experiment on mice that electroacupuncture increased splenic NK cell activity and elevated the concentration of splenic β-endorphin.

Yamaguchi et al\textsuperscript{[14]} reported that after acupuncture stimulation, the function of immune cells (CD2\textsuperscript{+}, CD4\textsuperscript{+}, CD8\textsuperscript{+}, CD11b\textsuperscript{+}, CD16\textsuperscript{+}, CD19\textsuperscript{+}, CD56\textsuperscript{+}, interleukin (IL) 4 and IL-1β), and the number of interferon-gamma levels was strengthened in the peripheral blood of seven healthy male adults. In addition, Yamaguchi et al\textsuperscript{[14]} showed that acupuncture stimulation may regulate immune function and promote the activities of humoral and cellular immunity, as well as NK cell activity. A study by Pavao et al\textsuperscript{[15]} indicated that depression, worry, and stress parameters were improved after acupuncture stimulation, while T-cell proliferation depression, worry, and stress parameters were improved after acupuncture stimulation, while T-cell proliferation was promoted. Further, Mori et al\textsuperscript{[16]} reported that acupuncture may enhance the interactive regulatory function of the autonomic and immune systems.

In this observational study, we attempted to evaluate pain, pulse rate, and immune function. Older adults were examined before and after acupuncture treatment for changes in NK cell activity, pulse rate, and visual analog scale (VAS) score and evaluated in accordance with their complaints.

2 Materials and methods

2.1 Subjects

Subjects were 56 adults (16 males and 40 females) between 60 and 82 years in age (mean age 72.4 ± 5.0). Their complaints were as follows: shoulder pain, 20 (mean age 70.2 ± 5.7); low-back pain, 19 (mean age 73.8 ± 3.4); knee pain, 17 (mean age 73.6 ± 5.0). None of the participants had known immune abnormality, metabolic disorders, contusion, sprains, or fractures.

The subjects were publicly sought out with the cooperation of the Tsukuba Public Health Center, Japan. All participants were informed of the purpose and nature of the study. Written consent was then obtained from each participant, in compliance with the World Medical Association Declaration of Helsinki. The study was approved by the Research Ethics Committee of the Tsukuba University of Technology, Japan.

2.2 Treatment methods

Acupuncture treatments were administered by three licensed acupuncturists who had a minimum of 5 years of clinical experience in treating pain conditions in the shoulder, low back, and knee.

Disposable stainless-steel needles (40 mm in length, 0.16 mm and 0.20 mm in diameter, SEIRIN, Japan) were used. Eighteen needles were used for each acupuncture session. Electroacupuncture and in situ needle techniques were used. Electroacupuncture was used in order to improve local muscular blood circulation and decrease pain, while the in situ needle technique was administered to induce a generalized systemic effect.

For the electroacupuncture technique, the following acupuncture points were used depending on the complaints of the participants: Tianzhu (BL10) and Jianjing (GB21) for shoulder pain, Jianjing (GB21), Shenshu (BL23) and Dachangshu (BL25) for low-back pain, and Xuehai (SP10) and Zusanli (ST36) for knee pain. All acupuncture points were stimulated bilaterally. Electrical stimulation was initiated at the intensity level at which muscle twitching movements occur with a frequency of 1 Hz for 10 min.

For the in situ needle technique, the following acupuncture points were used regardless of the participants’ complaints: Ganshu (BL18), Pishu (BL20), Weishu (BL21), Shenshu (BL23), Jiuewei (CV15), Zhongwan (CV12), Tianshu, (ST25), and Daju (ST27). All acupuncture points were stimulated bilaterally except the Jiuewei (CV15) and Zhongwan (CV12) points. Acupuncture needles were inserted to a depth of 10 to 15 mm and manually stimulated to induce the deqi sensation; then the needles were left in situ for 10 min.

The entire treatment session, including the electroacupuncture and in situ needle technique, lasted approximately 30 min. During the course of the study, the subjects took no medications for their pain.

2.3 Outcome measures

Peripheral blood (NK cell activity, leukocyte differentiation), pulse rate, and blood pressure were obtained, and pain intensity was evaluated by using a VAS before and after acupuncture treatment.

2.4 Measuring method

Prior to acupuncture treatment, a VAS score was obtained from each subject. Pulse rate and blood pressure were then measured using a wrist-type automatic blood pressure monitor (HEM-630, OMRON Corp, Kyoto, Japan) and stabilized at the level of the heart in a sitting position.

A 7-milliliter blood sample was then collected from each subject, from the cubital fossa. The collected blood was sent to an independent laboratory (Koto Biken Medical Laboratory, Tsukuba, Japan) for analysis of leukocyte counts, granulocyte-to-lymphocyte ratio, and NK cell activity. For NK cell activity, K-562 chronic myelogenous leukemia cells were cultivated with target cells. Free cells that were injured by this target cell were labeled with a beta radioactive isotope of hydrogen, from which the cell activity level was calculated. Peripheral blood was collected between 09:00 and 12:00.

The VAS score, pulse rate, blood pressure, and blood samples were also obtained following the acupuncture treatment, using the same sequence and procedures used for the pre-treatment assessments.
Acquisitions of the VAS score, pulse rate, blood pressure, and blood samples were done by two registered nurses who were blind to the details of this study.

### 2.5 Statistical analysis

Leukocyte differentiation (granulocytes and lymphocytes), pulse rate, blood pressure, and VAS score were compared via the Wilcoxon test before and after acupuncture treatment, in accordance with each of the subjects’ complaints. The ages of the subjects in the shoulder-pain, low-back-pain, and knee-pain groups were evaluated via the Sidak multiple comparison test.

SPSS Advanced Model 15.0 was used as the statistical analysis software. A P-value of less than 0.05 was considered significant; a P-value of less than 0.1 was considered a tendency.

### 3 Results

#### 3.1 Baseline data

No difference was observed among the three groups (shoulder pain, low-back pain, and knee pain) in comparison by age.

#### 3.2 Shoulder pain

NK cell activity decreased after acupuncture treatment compared to before the treatment (P = 0.049). Granulocyte counts increased after the acupuncture treatment (P = 0.01). Leukocyte counts showed a tendency to increase after the acupuncture treatment (P = 0.064). Lymphocyte counts showed a tendency to increase after the acupuncture treatment (P = 0.079). No change was observed in the leukocyte ratio or lymphocyte ratio.

Pulse rate decreased after the acupuncture treatment (P = 0.002). Systolic blood pressure increased after the acupuncture treatment (P = 0.011), while no notable changes occurred in diastolic blood pressure. The VAS for shoulder pain lowered after the acupuncture treatment (P = 0.018).

#### 3.3 Low-back pain

Granulocyte counts showed a tendency to increase after acupuncture treatment (P = 0.053). No change was observed in NK cell activity, the lymphocyte ratio, lymphocyte counts, the granulocyte ratio, or leukocyte counts.

Pulse rate decreased after acupuncture treatment (P = 0.007). No change was observed in either systolic or diastolic blood pressure. The VAS for low-back pain decreased after acupuncture treatment (P = 0.005).

#### 3.4 Knee pain

NK cell activity decreased after the acupuncture treatment (P = 0.029). Lymphocyte counts showed a tendency to increase after the acupuncture treatment (P = 0.084). No change was observed in the lymphocyte ratio, granulocyte counts, the granulocyte ratio, or leukocyte counts.

Pulse rate decreased after the acupuncture treatment (P = 0.00). No change was observed in systolic or diastolic blood pressure. The VAS for knee pain lowered after the acupuncture treatment (P = 0.00). Table 1 summarizes the changes in peripheral blood (NK cell activity and leukocyte differentiation), pulse rate, blood pressure, and the VAS score before and after the acupuncture treatment for each complaint.

### 4 Discussion

This study showed that subjects’ pulse rates and pain in the shoulder, low back, and knee decreased after acupuncture treatment. In addition, acupuncture influenced immune function.

Previous studies have suggested that pulse rate reduction occurs with acupuncture treatment; such a response is

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<td>29.6±16.9</td>
<td>27.6±15.8*</td>
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<td>Leukocyte CNT (μL)</td>
<td>5 975.0±1 535.8</td>
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<td>5 878.9±1 438.2</td>
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<td>Lymphocyte CNT (μL)</td>
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<td>857.1±842.7</td>
<td>1 036.7±968.1</td>
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<td>Granulocyte (%)</td>
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<td>66.2±9.4*</td>
<td>63.3±8.8</td>
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<td>Granulocyte CNT (μL)</td>
<td>3 944.4±1 283.7</td>
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<td>VAS value (mm)</td>
<td>41.8±20.3</td>
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<td>37.2±24.1</td>
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VAS: visual analog scale; NK: natural killer; CNT: count; BP: blood pressure. *P<0.05, **P<0.01, vs pre-treatment.
considered to be associated with parasympathetic activation\([14,17]\). We hypothesized that the pulse-rate-reduction response observed in this study was also elicited by the activation of parasympathetic nerve function. The present study also demonstrated decreased NK cell activity in older adults following acupuncture treatment. The previous study by Yamaguchi et al\([14]\) showed that an increase in NK cell activity (CD16 and CD56 subsets) in the peripheral blood of humans was observed at 1, 2, and 8 d following acupuncture stimulation. In addition, Arranz et al\([17]\) observed that NK cell activity among anxious female patients was lower compared to that of healthy people, and that the impaired NK cell activity among the patient groups increased 72 h after acupuncture treatment.

We observed decreased NK cell activity, increased leukocyte counts, and a tendency toward increasing leukocyte counts and lymphocyte counts following acupuncture treatment among the shoulder-pain sufferers. We hypothesize that the observed response was associated with the modulatory effects of acupuncture on NK cell activity and other immune functions. It should be noted that the post-acupuncture decrease in NK cell activity observed in our study contrasts with the findings of previous studies by Yamaguchi et al\([14]\) and Arranz et al\([17]\).

At least a few explanations exist for the contradicting outcomes of the studies. First, the study participants’ demographics differed. While our study observed the response in an older adult population, the studies by Yamaguchi et al\([14]\) and Arranz et al\([17]\) included younger adults. In addition, the NK cell-enhancing effects observed in the study by Arranz et al\([17]\) took place among anxious females who had shown impaired immune function prior to the treatment. Second, a difference existed in the timing of the outcome measurements. We collected post-acupuncture blood samples 30 min following acupuncture, while the first post-treatment samples were collected 24 h\([14]\) and 72 h\([17]\) following the acupuncture stimulation in the previous studies. In other words, our study demonstrated the immediate immunomodulatory effects of acupuncture, while the previous studies\([14,17]\) showed the delayed immunological responses to acupuncture.

The major limitation of this study is the absence of a control group. Thus, the possibility cannot be denied that the responses observed in this study involved unspecific, placebo, or natural alterations of immune parameters. In this study, we collected blood samples before and after acupuncture treatment. Although no adverse reactions occurred during or after the blood sample collections, an invasive examination procedure took place prior to the treatment that may have served as a stressor for the participants. Without a control group, the possible confounding variables associated with the invasive blood collection procedure cannot be excluded. In addition, the acupuncture treatment administered in this study was a single treatment, so the cumulative effect of repetitive treatments could not be determined.

In both our study and previous studies\([14,17]\), the acupuncture session was conducted once only. However, in clinical acupuncture, multiple treatments are administered on a regular basis, such as weekly or biweekly, in an attempt to produce cumulative effects when treating patients with immune-related disorders. In future studies, it will be important to elucidate the immune-modulation effects of multiple acupuncture sessions with a well-designed controlled study.

5 Conclusion

Acupuncture treatment reduced pulse rate, relieved shoulder, low back, and knee pain, and decreased NK cell activity for older adults.

6 Acknowledgement

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7 Competing interests

The authors declare that they have no competing interests.

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Submission Guide

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