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# Effects of Some Acupoints (Du-14, Li-11, St-36, and Sp-6) on Serum TNF-α and hsCRP Levels in Healthy Young Subjects

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## Abstract

***Objectives:*** This study was performed to investigate the effect of immune-related acupuncture points on serum tumor necrosis factor-α (TNF-α) and high-sensitivity C-reactive protein (hsCRP) levels.
***Methods:*** Ninety (90) healthy volunteers (aged 20–30 years) were randomly assigned into five equal groups. Acupuncture needles were placed into single acupoints bilaterally in each group. The points were *Da Zhui* (Du-14), *Qu Chi* (Li-11), *Zu San Li* (St-36), *San Yin Jiao* (Sp-6) and a sham point, which is not an acupoint. Manual acupuncture treatment was performed in 6 sessions of 30 minutes each, 3 times per week for 2 weeks. Serum samples were obtained before and after the acupuncture treatments and the serum TNF-α and hsCRP levels were measured.
***Results:*** The TNF-α values (mean ± standard deviation) in the Du-14, Li-11, St-36, Sp-6, and sham acupuncture groups at baseline were $37.63 \pm 10.58$, $37.36 \pm 10.24$, $33.83 \pm 7.36$, $35.73 \pm 6.75$, and $32.05 \pm 5.66$ pg/mL, respectively. After treatment, the mean TNF-α values were $35.89 \pm 11.61$, $34.80 \pm 6.98$, $35.89 \pm 9.22$, $33.30 \pm 5.45$, and $33.78 \pm 5.98$ pg/mL, respectively. In the serum TNF-α levels, no significant change was seen in any of the groups ($p > 0.05$). The mean hsCRP values in the Du-14, Li-11, St-36, Sp-6, and sham acupuncture groups at baseline were $0.90 \pm 0.77$, $1.07 \pm 1.35$, $0.77 \pm 0.54$, $0.75 \pm 0.54$, and $0.94 \pm 0.68$ mg/L, respectively. After treatment, they were $1.09 \pm 1.17$, $0.84 \pm 0.43$, $0.74 \pm 0.49$, $0.80 \pm 0.53$, and $0.62 \pm 0.44$ mg/L, respectively. In the statistical analysis, it was found that hsCRP levels were significantly reduced in the sham acupuncture group ($p < 0.01$). There was not any significant difference between acupuncture and sham groups in terms of serum TNF-α and hsCRP values ($p > 0.05$).
***Conclusions:*** Acupoints, which are considered to have effects on the immune system, may not mediate the immune system via TNF-α, a known inflammatory cytokine, directly in healthy young individuals. However, the changes related to hsCRP values in the sham group need future confirmation studies.

## Introduction

Acupuncture has been used successfully for the treatment of many diseases and symptoms for centuries.[1,2] In the National Institutes of Health Consensus Statement, published in 1997, it is reported that acupuncture can be accepted as an adjunct or alternative treatment for postoperative and chemotherapy-related nausea and vomiting, postoperative dental pain, stroke rehabilitation, headache, menstrual cramps, tennis elbow, fibromyalgia, myofascial pain, osteoarthritis, low back pain, carpal tunnel syndrome, and asthma.[3]

It is stated that acupuncture achieves its influence on pain and depression by using nerotransmitters such as β-endorphin and serotonin and neurogenic mechanisms such as the gate control theory.[1,2,4] Some acupuncture points are widely used to assist in improving pain and inflammation in infections and inflammatory diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), and inflammatory bowel disease.[5,6] However, the mechanisms associated with these acupuncture points that influence the immune system are not yet understood.[5,7]

C-reactive protein (CRP) is an acute-phase protein produced in the liver, which increases due to infection and inflammation. In an inflammatory stimulus, it increases over several hours.[8] Since it increases and decreases according to the disease activities of several inflammatory diseases, such as RA, psoriatic arthritis (PsA), and systemic lupus erythematosus, it is therefore useful in following up the disease activity.[9] The influence of the acupuncture points on the CRP values is not yet well known.

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The effects of the immune system-related acupoints on inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and interferon gamma (IFN-γ), have not been researched sufficiently to date. Although several trials in the literature have investigated the influence of some acupoints on the various cytokine levels, there have been no adequately controlled studies comparing the effects of acupoints on the immune system.[10–13]

This study was performed to investigate the effect of immune-related acupuncture points on serum TNF-α and the high-sensitivity C-reactive protein (hsCRP) levels.

## Materials and Methods

Ninety (90) healthy university student volunteers (20–30 years old) from Ataturk University, Turkey participated in this study. People who had any diseases, who take medicine regularly, or who had taken any drugs that might influence the immune system in the last month were not included. Since there may be influences on the immune system and the cytokines, smokers were also excluded from this study.

The 90 volunteers were randomly assigned to five groups: Du-14, Li-11, St-36, Sp-6, and sham acupuncture groups. In each group, bilateral 0.25×25-mm steel acupuncture needles were applied on a point for 30 minutes. These points were *Da Zhui* (Du-14), *Qu Chi* (Li-11), *Zu San Li* (St-36), *San Yin Jiao* (Sp-6) and a sham point, which is not an acupoint. The sham needle point was located 1.5 *cun* lateral to *Cheng Jin* (Bl-56) in the sham group. Manual acupuncture treatment was performed in six sessions of 30 minutes duration each, three times per week for 2 weeks. Needles were not manipulated. *De qi* sensation was obtained in acupuncture sessions. Volunteers were not allowed to take any medicine during the study.

Blood samples were obtained at the baseline (just before the first acupuncture session) and after the sixth acupuncture session. Serum samples were stored at –80°C until analyses was performed, and the serum TNF-α values were measured with commercial kits (Cat. No: KHC3011, Biosource, Belgium) using the enzyme-linked immunosorbent assay method. A nephelometric method was used to assay hsCRP levels in the blood serums. The intra-assay and interassay coefficient of variation percentages were 4.4% and 7.5% for TNF-α, and 2.3% and 3.1% for hsCRP, respectively.

The study was approved by the local ethics committee of Ataturk University. Volunteers were informed about the study protocol but not about the groups that they would be assigned to. Written informed consent, according to the Declaration of Helsinki, was obtained from all participants.

## Statistical Analysis

Sample size calculation was based on the outcome of TNF-α compared between the five groups using the one-way ANOVA test. With a significance level of 0.05, standard deviation of 5 pg/mL, an average sample size of 18 cases in each of the five groups gives a power of 83% to detect an effect size of 0.39.

Statistical analysis was performed using the packet program, SPSS 11.0 for Windows. The values of measurable parameters were checked for normal distribution by means of the Kolmogorov-Smirnov test prior to statistical analysis. One-way ANOVA, paired-samples *t* and Wilcoxon rank tests were used for the statistical analysis. Only *p* values of 0.05 and below were considered as statistically significant.

## Results

Eighty-seven (87) volunteers completed the study. The volunteers were distributed into the following groups: 18 volunteers in the Du-14 group, 16 in the Li-11 group, 18 in the St-36 group, 18 in the Sp-6 group, and 17 in the sham group. A subject in the sham group left the study voluntarily because of a respiratory tract infection that developed in the second session. One (1) volunteer, who took nonsteroidal anti-inflammatory drugs due to a headache, and another who did not attend the last sessions of the treatment in the Li-11 group, were excluded from the study.

The demographic properties and laboratory test results are shown in Table 1. The mean TNF-α values at baseline in the Du-14, Li-11, St-36, Sp-6, and sham acupuncture groups were $37.63 \pm 10.58$, $37.36 \pm 10.24$, $33.83 \pm 7.36$, $35.73 \pm 6.75$, and $32.05 \pm 5.66$ pg/mL, respectively. After six sessions of acupuncture, the mean TNF-α values were $35.89 \pm 11.61$, $34.80 \pm 6.98$, $35.89 \pm 9.22$, $33.30 \pm 5.45$, and $33.78 \pm 5.98$ pg/mL, respectively. In the serum TNF-α levels, no significant changes were seen in any of the groups ($p > 0.05$) (Table 1).

At the beginning of the study, the mean hsCRP values in the Du-14, Li-11, St-36, Sp-6, and sham acupuncture groups

Table 1. Demographic Properties and Laboratory Characteristics of Groups (Mean ± SD)

| | *Du-14 group* (n = *18*) | *Li-11 group* (n = *16*) | *St-36 group* (n = *18*) | *Sp-6 group* (n = *18*) | *Sham group* (n = *17*) |
|---|---|---|---|---|---|
| Sex (female/male) | 8/10 | 5/11 | 7/11 | 6/12 | 6/11 |
| Age (years) | 21.56 ± 2.25 | 22.50 ± 2.66 | 22.39 ± 1.91 | 22.89 ± 3.20 | 21.29 ± 1.83 |
| TNF-α (pg/mL) (baseline) | 37.63 ± 10.58 | 37.36 ± 10.24 | 33.83 ± 7.36 | 35.73 ± 6.75 | 32.05 ± 5.66 |
| TNF-α (pg/mL) (after the acupuncture) | 35.89 ± 11.61 | 34.80 ± 6.98 | 35.89 ± 9.22 | 33.30 ± 5.45 | 33.78 ± 5.98 |
| Δ TNF-α | -1.74 ± 9.88 | -2.57 ± 8.16 | 2.06 ± 6.8 | -2.43 ± 6.31 | 1.73 ± 6.91 |
| hsCRP (mg/L) (baseline) | 0.90 ± 0.77 | 1.07 ± 1.35 | 0.77 ± 0.54 | 0.75 ± 0.54 | 0.94 ± 0.68 |
| hsCRP (mg/L) (after the acupuncture) | 1.09 ± 1.17 | 0.84 ± 0.43 | 0.74 ± 0.49 | 0.80 ± 0.53 | 0.62 ± 0.44* |
| Δ hsCRP | 0.19 ± 1.37 | -0.23 ± 1.15 | -0.03 ± 0.25 | 0.05 ± 0.34 | -0.32 ± 0.44 |

*$p < 0.01$: Compared with baseline values.
SD, standard deviation; TNF-α, tumor necrosis factor–α; hsCRP, high-sensitivity C-reactive protein; Δ, post-treatment baseline change score.

were $0.90 \pm 0.77$, $1.07 \pm 1.35$, $0.77 \pm 0.54$, $0.75 \pm 0.54$, and $0.94 \pm 0.68$ mg/L, respectively, and $1.09 \pm 1.17$, $0.84 \pm 0.43$, $0.74 \pm 0.49$, $0.80 \pm 0.53$, and $0.62 \pm 0.44$ mg/L after the acupuncture sessions, respectively. In the statistical analysis, it was found that hsCRP levels were significantly reduced in the sham acupuncture group ($p < 0.01$). No significant changes were seen in the other groups ($p > 0.05$).

There was no significant difference between acupuncture and sham groups in terms of serum TNF-α and hsCRP values ($p > 0.05$).

## Discussion

In this study, four of the most common acupoints (Du-14, Li-11, St-36, and Sp-6) used conventionally in infections and inflammatory diseases were chosen and their influences on proinflammatory cytokines and acute phase proteins were compared. A non-acupoint in the sham acupuncture group was used to observe the effect of a prick.

In the sham group, serum hsCRP levels were found to be decreased in 15 of 17 cases (88%) after acupuncture when compared to baseline values. This observation may show that, when an acupuncture needle is inserted into a place that is not an acupoint on the body, after only 15 days the hsCRP levels are decreased dramatically. CRP is an acute phase reactant that increases in a short time during inflammation. It may react to changes in the inflammation process at the short time of 6–12 hours.[14] CRP is a sensitive, nonspecific inflammatory mediator, which can affect local inflammation as well as systemic inflammation.[15] Local inflammation occurs via the prick in that area. During this time, the increasing but small CRP values can be determined by the measurements of hsCRP levels. In this study, blood tests were not performed in the early periods. At 4–6 days, hsCRP may be reduced due to the decrease in the local inflammation, which is related to the improvement of the pricked area. In this study, since the control blood sample was taken 12 days after the first session (at the sixth session), hsCRP may have decreased in nearly all cases of the sham group. On the other hand, the expected decreases of hsCRP values were not observed in all individuals of the true acupuncture groups (Du-14, Li-11, St-36, and Sp-6 groups). This interesting finding may be related to an unknown interaction, which we could not interpret now, between these acupoints and local inflammation.

Inflammation is a protective reaction that develops against tissue injury, infection, or an immune stimulus.[16] Local invasions and injuries begin with a localized inflammatory reaction that causes the release of proinflammatory cytokines.[17] TNF-α is a proinflammatory cytokine that is very effective in local and systemic inflammations.[18] In addition, TNF-α increases the production of other inflammatory cytokines such as IL-1β, IL-6, and IL-18 during the inflammatory process. Therefore, it brings infections under control, produces coagulation in the damaged tissue, and stimulates tissue improvement.[15] An excessive increase of TNF-α levels causes an extreme immune reaction and contributes to the development of atherosclerosis, shock, endotoxemia, and chronic inflammatory diseases such as RA, AS, and inflammatory bowel disease.[19–21]

Yim and colleagues[22] reported a decrease in increased serum TNF-α, IL-6, and IFN-γ levels as a result of 3 weekly sessions of electroacupuncture treatment, a total of 5–9 weeks on the St-36 point in collagen-induce arthritic mice. In this study, it was shown that acupuncture could provide a reduction in serum TNF-α levels, which were already high in inflammatory arthritis. Likewise, Tian et al.[12] also found that serum TNF-α levels increased in rats with ulcerative colitis compared to normal rats. After that, they performed electroacupuncture on the St-36 point once a day for 10 days on these rats. As a result, they reported that there was a decrease in TNF-α levels compared to the control rats. Using electric stimulation in these two studies with a long treatment period (5–9 weeks) or intensive (consecutive 10 sessions) treatment being applied to animals may have produced these results.

On the other hand, Jong and his team were unable to find any changes in IL-4, IL-6, IL-10, soluble interleukin-2 receptor, and IFN-γ levels in 9 healthy individuals in whom they had performed electroacupuncture on the Li-11 point.[23] In the current study, TNF-α levels that were already expected to be within normal levels in healthy individuals were not affected by the acupuncture treatment in all acupuncture groups in which St-36 and Li-11 were also included.

From this outcome it may be understood that the acupoints that are thought to be related to the immune system do not affect the immune system via TNF-α, which is an inflammatory cytokine. The fact that there were no changes in the sham acupuncture group may show that a needle prick cannot produce a strong enough stimulation to change the TNF-α levels. This study was performed among young healthy individuals. It is known that many acupuncture points more likely have influence as regulators. In most of the rheumatic diseases, serum TNF-α levels are high, and successful results are determined via anti-TNF-α treatments in inflammatory diseases such as RA, AS, Crohn disease, and PsA.[10,15,24–26] These acupoints, which do not affect inflammatory cytokines in healthy subjects, may reduce TNF-α levels because of their regulatory properties when performed in the case of rheumatic diseases, so that they may contribute to the treatment of the disease.

## Conclusions

As a result, acupuncture therapy performed on the Du-14, Li-11, St-36, and Sp-6 acupoints does not seem to affect the immune system via TNF-α inflammatory cytokines in healthy subjects. However, the changes related to hsCRP values in the sham group needs future confirmation studies. Placebo-controlled, point-by-point comparative studies using more volunteers should also be conducted in a manner similar to our study with inflammatory diseases such as RA and AS with high TNF-α levels to provide important additional information.

## Disclosure Statement

No competing financial interests exist.

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