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The effect of manual acupuncture on blood neutrophil counts in moderate intensity exercise

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Abstract. Exercise, even though it has a beneficial effect, can cause muscle damage and trigger inflammatory responses, as evidenced by increased neutrophils in the blood. Acupuncture is a therapeutic modality that is expected to reduce acute inflammatory responses due to exercise. Thirty untrained men were divided randomly into two groups. The manual acupuncture group (n = 15) received stimulation at acupoints ST36 and SP6 bilateral by needle insertion, while the placebo group (n = 15) received insertion of needles on plaster without penetrating the skin. Therapy was done once for 30 minutes immediately after the subjects completed the exercise. Blood neutrophil counts were assessed before exercise and one hour after exercise ended. The results show there is a statistically significant difference in the number of neutrophils before and after exercise between the manual acupuncture group and the placebo group (0.08±0.91 and 0.97±0.70; p = 0.006). Acupuncture therapy effectively mitigates the acute inflammatory response triggered by exercise.

1. Introduction

Exercise is an activity that can be done to maintain health. Scientific facts support the positive effects of exercise as part of a healthy lifestyle. One such effect is to mitigate chronic diseases, such as cardiovascular disease, diabetes, malignancy, hypertension, obesity, depression, and osteoporosis. In a world of professional sports, exercise is a means to improve performance as the athlete's main training program because the better an athlete's fitness and health, the higher his physical ability to achieve higher accomplishments [1]. Moderate intensity daily exercise and a total of 150 minutes of exercise per week can decrease the risk of cardiovascular disease and other diseases [2]. But it should be noted that the acute effect of exercise is different from the chronic effect [3]. A moderate intensity workout, if prolonged, can be exhausting or, if undertaken by an untrained individual, can cause muscular damage and initiate an acute inflammation response. Exercise (a workout done during one specific time period), like trauma, is a form of stress on the body that can cause oxidative damage, cytokine secretion, and inflammation, which can lead to systemic inflammation response syndrome [4].

Every leukocyte in the circulatory system will increase during exercise, but neutrophil has the biggest escalation [5,6]. Neutrophil is the first line of defense in the body's immune response [7-9]. In addition to its function as an immune defense system, neutrophil is also involved in a microbicide reaction that releases reactive oxygen species (ROS) molecules, such as O_2- and H_2O_2, which only worsens the muscular damage and inflammation that comes with it [5,8]. In a study by Nieman *et al.* (1991), it was stated that the blood neutrophil count increases from 3.99 ± 0.21 × 109 cell/L to 5.36 ±

0.41 × 109 cell/L after a 45-minute running exercise at 60% of maximum aerobic capacity [2]. Giraldo stated that either moderate intensity exercise or high intensity exercise can cause increasing chemotaxis, phagocytosis, and microbicide capacity from neutrophil, with higher chemotaxis in moderate intensity exercise [10]. Previous study found that the increase in neutrophil was almost twice as high in 12 untrained men who exercised on an ergometer cycle for 40 minutes at 50% VO_2max intensity [11].

Acupuncture is a part of traditional Chinese medicine that involves stimulation of specific points on the skin using needles, pressure, or heat. The United States National Institutes of Health and the World Health Organization (WHO) claim acupuncture to be "a safe and effective procedure to solve pain and injury as well as to support optimum health" [12]. Surveys done in the US and Germany found that 8-9% of the adult population uses acupuncture to prevent and solve many kinds of health problems. The use of general acupuncture is found among college athletes at a rate that is about 12% higher than among the general population [13]. In Asian countries, acupuncture has been used to modulate the physical health of athletes with the goal of achieving a higher level of performance [14].

Itoh *et al*., reported that acupuncture can decrease inflammation and lessen pain in delayed onset muscle soreness (DOMS) [15]. Hutchison *et al*., stated that acupuncture can cause various changes in immune cell status [16]. Matsubara *et al*., did a study of 12 healthy men (age 23.6 ± 3 years) who exercised on an ergometer cycle for 60 minutes at 75% VO_2max. Acupuncture was done at the LU6, LI4, ST36, and ST6 points for 30 minutes after exercise. Blood samples were taken before, right after, and every hour after the exercise for 4 hours, and at 24 hours after exercise. The results show a significant increase in neutrophil in both the acupuncture group and the control group from 1 hour after exercise to 4 hours after exercise ($p < 0.05$). But the neutrophil count was lower in the acupuncture group than in the control group ($p < 0.05$) [17]. Even though scientific facts support the benefits of acupuncture in relation to exercise and general performance, clinical testing is still rare, and the mechanism of acupuncture is not fully understood yet. Therefore, this researcher undertook a study to determine the effect of manual acupuncture on the change in the blood neutrophil count in untrained men who do moderate intensity exercise.

2. Materials and Methods

This single randomized clinical test with placebo control was performed in December 2016 at the Indonesia Sports Medicine Center facility in Jakarta. The inclusion criteria were men age 20–35 years, capable of physical training based on anamnesis, vital signs, and general physical examination, who agreed to be involved until the end of the study. The exclusion criteria were having a history of cardiovascular, lung, metabolic, or malignant diseases; having experienced a musculoskeletal injury in the past 6 months; consuming non-steroid anti-inflammatory, glucocorticoid, vitamin supplements, orantioxidants in the past week; participating in a work out or exercise in any form in the 72 hours prior to the intervention; exercising once a week for the past 3 months, or having a scar at the acupuncture point where the therapy would take place. The subjects were randomly allocated using a computer-based random numeric table to divide them into 2 groups—the manual acupuncture group and the placebo acupuncture group. Exercise in this study was running for 30 minutes nonstop on a treadmill. As preparation prior to exercising, subjects were instructed to get enough sleep the night before, to eat breakfast, to drink at least 200–400 ml of water 1 hour before starting the exercise, and to wear sports clothing (short-sleeved shirt, workout shoes, and pants) comfortable for running.

Before beginning to exercise, the subjects warmed up with a slow-paced walk around the exercise area for 3–5 minutes and 7stretching motions involving the trunk of the body and the big muscles (hamstring, quadriceps, and calf). During the exercise, the researcher observed the subjects' pulses at moderate intensity (64–76% HR max). In the first 5 minutes, the speed of the treadmill was gradually increased to6 km/h and kept that way until minute 30. For the subjects that didn't reach 64% HR max by minute 10, the speed of the treadmill was increased to 7 km/h, and so on, and kept at that level until minute 30. Exercise was stopped when the intensity surpassed 76% HR max or the subject was too exhausted to continue the protocol. Exercise ended with the same stretching motions as in the warm-

up. For the manual acupuncture group, the subjects lay their backs. Before acupuncture point stimulation, aseptic and antiseptic procedures were done, along with the plastering of Plesterin that had been perforated at the ST36 Zusanli and SP6 Sanyinjiao areas. After that, acupuncture needles were inserted until the subjects reported a needling sensation (stiff, rheumatism, electrocuted-like sensation). The needles were left in place for 30 minutes and then taken out and discarded. Subjects in the placebo group also lay on their backs. Aseptic and antiseptic procedures were done, and acupuncture needles were inserted in the plaster but did not touch the subjects' skin. The needles were left for 30 minutes and then taken out and discarded.

The subjects' blood neutrophil was analyzed twice. The first time was after the subjects were divided into the manual acupuncture group and the placebo group but before exercising, while the second time was one hour after exercising and receiving either the manual acupuncture or placebo treatment. Acupuncture points used in this study were ST36 Zusanli, which is located on the anterior lower limbs, on the line that connects ST35 and ST41; 3 B-cun, which is below ST35; and SP6 Sanyinjiao, which is located on the lower limbs, posterior from medial tibial border, 3 B-cun above the prominent medial malleolus [18]. Data were processed using the statistics program SPSS 20. A normality test was used for all the numeric data with Saphiro-Wilk. Numerical data with normal randomization is presented in average value and deviation standard, while data with abnormal randomization is presented in median value or range. Statistical analysis of the blood neutrophil count before and after intervention for each group was done using a paired t-test if the data randomization was normal or Wilcoxon if the randomization was not normal. Statistical analysis for difference comparisons between the blood neutrophil counts of each group was performed using an unpaired t-test for normal data distribution or a Mann-Whitney test if the distribution was not normal. Based on the comparative hypothesis test, if the value of p obtained is $p < \alpha$ ($p < 0.05$), then it can be stated that there is a statistically significant difference between the variables compared.

3. Results and Discussion

3.1 Results

This study is the first acupuncture research in Indonesia that assesses the blood neutrophil count in subjects who perform exercise. A single randomized clinical test with a placebo control group was done with30 subjects who met the exclusion and inclusion criteria, and who were then divided randomly into two groups—the manual acupuncture group and the placebo group. There were no drop-out subjects in this study.

Table 1. Characteristic of subjects

Characteristics	Manual acupuncture	Placebo	p value
Age	23.13 ± 3.482	23.53 ± 2.722	0.556*
Smoking habit			
Yes	12 (80%)	13 (86.7%)	1.000**
No	3 (20%)	2 (13.3%)	
Body mass index	20.26 ± 3.161	20.42 ± 2.409	0.561*
Systolic blood pressure	128.47 ± 12.129	128.60 ± 10.225	0.974***
Diastolic blood pressure	75.00 ± 13.180	73.20 ± 10.150	0.678***
Pulse	83.33 ± 11.108	81.53 ± 13.223	0.690***
Initial blood neutrophil count (x 10^3/ml)	5.347 ± 1.742	5.06 ± 1.891	0.534*

*Mann-Whitney **Fisher's Exact ***Independent samples t-test

Fisher's Exact Test shows there is no significant difference ($p = 1.000$) for smoking habit. The Mann-Whitney test shows there is no significant difference between the two groups for age variable ($p = 0.556$) or body mass index ($p = 0.561$). An unpaired t-test shows there is no significant difference between the groups in terms of systolic blood pressure variable ($p = 0.974$). From the data mentioned

above, in general there is no significant difference ($p > 0.05$) for the characteristics of subjects in the two groups; therefore it is reasonable to compare the groups.

Table 2.Comparison of before and after exercise blood neutrophil average count between groups

Blood neutrophil count (x 10^3/mL)	Before exercise	After exercise	Difference	p
Manual acupuncture group	5.35 ± 1.743	5.43 ± 1.561	0.08 ± 0.906	0.683*
Placebo group	5.06 ± 1.891	6.03 ± 2.111	0.97 ± 0.702	0.000*

*Wilcoxon

There is no significant difference between the blood neutrophil count before and an hour after exercise in the manual acupuncture group ($p = 0.683$), but there is a significant difference in the blood neutrophil count before and an hour after exercise in placebo group ($p = 0.000$). The average change in the blood neutrophil count before and an hour after exercise in the manual acupuncture group was 0.08 x 10^3/ml, while the average change in the blood neutrophil count before and an hour after exercise in the placebo group was 0.97 x 10^3/ml. This unpaired t-test result shows that there is a significant difference in the blood neutrophil count before and after exercise between the manual acupuncture group and the placebo group ($p = 0.006$).

3.2 Discussion

The age of subjects of this study ranged from 20 to 35 years. Previous study by Liu and Simmon showed that there is no significant change in blood leukocyte count, blood neutrophil count, malondialdehyde serum level, and elastaseal serum level after exercise in subjects under 20 years old [8]. Meanwhile, in people over 35, there are usually physiological changes in the body's biological systems that can affect the body's response to exercise. The subjects in this study were limited to males because there are several differences in terms of body physiology between males and females, especially related to hormones [19]. The series of research activities required 1 hour and 50 minutes for each subject. The exercise and acupuncture therapy were only done once since the aim of this study was to assess the direct effect of acupuncture therapy on the change in the blood neutrophil count that is initiated by a one-time exercise session during a specific time frame. A single exercise session causes cellular homeostatic damage and activates immune cell and inflammation mediators even in healthy individuals. Radom-Aizik found that exercise causes not only an increase in neutrophil count in the blood circulation, but also a substantial change in genetic functional expression as well as in the genetic signaling pathway [20]. Neutrophil is an important component of the innate immune system and its defense against bacterial and fungal infection by a quick response to potential pathogens. In contrast to its beneficial effects, neutrophil is also the main contributor to tissue damage during inflammation [21]. Chronically activated neutrophils can give rise to disease [20].

Several studies have shown that exercise has negative as well as positive effects on immunity. Exercise results in the formation of ROS. Increased ROS production can exceed antioxidant capacity, leading to oxidative stress. Oxidative stress is related to damage to proteins, lipids, and DNA, and it has a cause-and-effect connection to the aging process, cancer development, and cardiovascular disease. Exercise also induces systemic inflammation response that is accompanied by an increase in acute-phase proteins and cytokine. The exercise-induced increase of IL-6 concentration in plasma originates from cells in the contracting muscle fibers [20]. The effect of exercise depends on its type, intensity, and duration. Generally, the decreasing body defense system is found mostly in cases of prolonged, continuous exercise (>1.5 hours) of moderate to high intensity (70% to 77% VO_2max) that is without energy supply. A hard training schedule or an endurance competition, such as a marathon or long-distance cycling, can cause extreme physical stress and lead to depression of immunity in athletes, a condition that is related to a high risk of infection, especially upper respiratory tract infections. The increase in neutrophil after exercise, even though high in level, reflects the decreasing response to lipopolysaccharide bacterial stimulation that can occur for hours [22]. Neutrophil

homeostasis is a balance that is managed between series of processes, including granulopoiesis inside the bone marrow, the release of neutrophil into the blood, the migration of neutrophil from blood to tissue, and apoptosis as well as neutrophil clearance either in a normal or inflamed condition. The tight control of neutrophil in normal and inflamed conditions is an important component of innate immunity. Therefore, the failure of neutrophil regulation can contribute to inflammatory diseases or the worsening of infection. Recent advances in identifying the feedback control mechanism that manages production and release of neutrophil show the importance of path IL-17 / G-CSF [21], which stimulates the production and maturation of neutrophil by stimulating the proliferation and differentiation of myeloid progenitor. In addition, G-CSF also causes neutrophil to be released from the bone marrow into the circulatory system by induction ligand CXCR2 expression or inhibiting the expression and signaling from SDF-1 and CXCR4. Stark *et al*. state that neutrophil apoptosis by tissue phagocytes will decrease the expression of G-CSF, limiting the stimulus to produce neutrophil when the neutrophil is already available in the tissue. But in the oxidative stress condition, it is found that the peripheral blood neutrophil count is related to increased IL-23 and IL-17 expression because there is no inhibiting of the signal related to neutrophil phagocytosis. Furthermore, IL-17 release by the Th17 cell can also be initiated by pro-inflammatory cytokine, such as IL-6. The increase of IL-23 and IL-17 will also lead to a systemic G-CSF increase, which contributes to excessive neutrophil production and its mobilization from bone marrow [21]. The involvement of the IL-23/IL-17 axis of G-CSF in granulopoiesis regulation is confirmed in several murine independent models. The interaction of the Th17 cell and neutrophil is also found in humans [23].

Acupuncture originated in China more than 5,000 years ago. These days the practice of acupuncture has expanded to Western countries and is used to medicate many kinds of diseases as well as to serve as anesthesia during surgery. Researchers have determined that the meridian and acupuncture points in traditional Chinese medicine actually correlate with the anatomical system and neurophysiology (Natural Standard, 2013). Several studies have proved that acupuncture stimulation of some areas of the upper brain stem and hypothalamus can release opioid and serotonin [24]. The stimulation technique used in this study was manual acupuncture. The reason for choosing this technique was that it is simple, economical, practical, safe, and effective. Acupuncture intervention can be practiced in first-degree health care facilities. The acupuncture points used in this study were ST36 Zusanli and SP6 Sanyinjiao. According to the Guo *et al*. study, acupuncture at ST36 can decrease IL-17 and IL-23 serum levels, which leads to a decrease in G-CSF. This may happen because acupuncture can regulate T activities in cells, especially Th17, which decreases excessive expression of IL-17 [25]. Moreover, ST36 is also related to the vagal activities that are negotiated by acetylcholine (ACh). An ACh bond with receptor α7nAChR that is expressed in tissue macrophages can inhibit pro-inflammatory cytokine synthesis, which in turn decreases the inflammation process and stimulus to the neutrophil necessity [26]. Acupuncture at the SP6 Sanyinjiao point inhibits leukocyte infiltration as well as myeloperoxidase in peritonitis rat model, which has a pattern of inflammation similar to the one initiated by exercise. An increase in the level of IL-10, which is an anti-inflammatory cytokine, also plays a role in the path IL-7/G-CSF path [27,28].

The results of this study show that there is a significant difference in the change in blood neutrophil count before and after exercise between the manual acupuncture group and the placebo group ($p = 0.006$). In the group analysis, subjects that received manual acupuncture treatment did not experience a significant change in their blood neutrophil count before and one hour after exercise (0.080 x 103/ml, $p = 0.683$), with a 1.5% increase from the initial blood neutrophil count, which shows that acupuncture is effective in resolving the inflammatory response and oxidative stress that are initiated by exercise and in regulating neutrophil so that homeostasis is maintained. This effect was not found in the placebo group. This study is expected to be practiced in other specific populations, such as elite athletes who are frequently faced with tight exercise schedules and competitions of high intensity and long duration. This condition can instigate a stronger inflammatory response even though the athletes' bodies have adapted physiologically. The inflammatory response and the complaints that come with it, such as pain and decreased muscle strength or acute respiratory tract infection incidence, can affect

athletes' performance during competition. Therefore, acupuncture can be an alternative for enhancing athletic performance. The limitation of this study was that the blood neutrophil count examination wasn't done over a longer duration, such as 2, 3, 4, and 24 hours after exercise, because of a limited research budget and lack of time.

4. Conclusion

The results of this study show that manual acupuncture has a significant effect on the blood neutrophil count in untrained men that perform moderate intensity exercise. There is no significant increase in the average blood neutrophil count in untrained men before doing the moderate intensity exercise and after moderate intensity exercise with a placebo acupuncture treatment (5.06 ± 1.891 and 6.03 ± 2.111, $p = 0.000$). There is a significant change in the average blood neutrophil count in untrained men after doing moderate intensity exercise with manual acupuncture compared to a placebo treatment (0.08 ± 0.906 and 0.97 ± 0.702; $p = 0.006$). Manual acupuncture can be a supportive therapy in resolving the acute inflammatory response initiated by exercise. Further research should be undertaken using more sensitive biomarkers (such as IL-23, IL-17, G-CSF, MPO), evaluating subjective complaints (such as muscle pain) and after-therapy follow-up, comparing the effectiveness of various acupuncture therapy modalities, and using other populations such as athletes as the subjects to assess effectiveness and to determine the optimal dose and acupuncture technique for each individual.

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