Effect of electroacupuncture therapy on serum serotonin levels of patients with anxiety symptoms

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Abstract. Anxiety is a feeling of fear that is accompanied by autonomic symptoms. Acupuncture is one of the alternative therapies to reduce anxiety. The present study aimed to determine the effect of electroacupuncture on serum serotonin levels and anxiety levels of patients with anxiety symptoms before and after therapy. This was a randomized clinical trial that included 38 participants with an (Hamilton Anxiety Rating Scale (HARS) score of 14–27. Participants were divided into the treatment group (n = 19) and control group (n = 19). The HARS score of the treatment group decreased (14 ± 3.62; p < 0.001) with a success rate of 100% and was significantly higher than that of the control group. Meanwhile, the HARS score of the control group also decreased (1.31 ± 1.49; p = 0.001) with a success rate of 63%. Serum serotonin levels of the patients in the treatment group significantly differed before and after therapy [47(-68)-(124); p = 0.005] with a success rate of 100%. However, that of the control group did not significantly differ before and after therapy [49(-92)-(252); p = 0.025] with a success rate of 93% (p = 0.804).

1. Introduction
Anxiety is an affective disorder that is characterized by feelings of fear or deep and prolonged worry. Individuals with anxiety are still oriented to reality, and their personality is intact. The behavior can be disrupted but within normal limits [1-3]. Younger individuals are more likely to present with anxiety. Moreover, women often present with social problems, previous psychiatric problems, and chronic illness [4]. The World Health Organization predicted that psychiatric disorders will account for 15% of the global morbidity in 2020 [5,6]. In the United States, it is the second most common psychiatric disorder (with a 5% prevalence) after depression [7]. According to the Office for National Statistics, anxiety along with depression is the most common mental disorder in the UK. A 1995 survey had found that...
7.7% of the adult population in the UK presented with a combination of anxiety and depression, and in 2000, the incidence was 164 per 1,000 people (1 in 6 adults) [8,9]. The prevalence of anxiety disorder in Indonesia ranged from 6% to 7%, which accounts for 7%–16% of all mental disorders [10]. Based on the Basic Health Research (Risksesdas) of the Indonesian Ministry of Health in 2007, the prevalence of concurrent anxiety disorders and depression was 11.6% in individuals aged >15 years in Indonesia, and it is the second most common mental health disorder (14.1%) after severe mental disorder (2.03%) [11].

Anxiety disorder is a group of mental disorders that include anxiety disorder, panic, phobia, obsessive and compulsive disorder, and post-traumatic stress syndrome [12,13]. In addition to psychiatric symptoms, these disorders also cause physical symptoms [14]. Immunouropathobiologic studies have shown that neurotransmitters are correlated with behavioral and psychiatric disorders. Serotonin is a neurotransmitter associated with the following disorders: anxiety, depression, psychosis, migraine, sexual dysfunction, and sleep, cognitive, and eating disorders. Several treatments for mental disorders affect serotonin levels [15]. The Hamilton scale for anxiety (Hamilton anxiety rating scale, HARS) is the most commonly used tool for assessing anxiety [16]. Psychotherapy and medical therapy are used for the management of these disorders. However, there are constraints in accessing psychotherapy services. Moreover, all pharmacologic treatments have side effects, which cause withdrawal from therapy [12,16]. In 2011, Wu et al. conducted a study on the efficacy, tolerability, and safety of duloxetine in patients with comprehensive anxiety disorder (global anxiety disorder, GAD), and they showed that patients in the treatment group presented with nausea, headache, and somnolence, and they often had suicidal ideations [17]. Thus, patients often seek other therapies.

National surveys in the UK and United States have found that anxiety, depression, and stress are the most common indications for acupuncture therapy (11%) [18]. Acupuncture is an alternative therapy that uses a needle that is jabbed at a particular point on the skin and is performed by a professional. It is a safe procedure with few contraindications or complications [19]. Acupuncture regulates the serotonin and adrenocorticotropic hormone (ACTH) levels, which are associated with anxiety [20]. Electroacupuncture (EA) has a significant anti-anxiety effect, and it decreases serotonin, norepinephrine, and dopamine levels and significantly increases GABA levels. Thus, EA may regulate the positive expression of monoamine and GABA in the central nervous system (CNS) and may restore the balance in these neurotransmitters [21]. Acupuncture increases and prolongs the activity of serotonergic neurons in the reward system of the brain [22]. Other studies on the effect of acupuncture on neurotransmitter levels of individuals with anxiety disorder are as follows:

Yuan et al. in 2007, conducted a study on jin-3-needling acupuncture therapy at the points of Sishenzhen and Dingshenzhen and PC 6 Neiguan, HT 7 Shenmen, and SP 6 Sanyinjiao, with results that showed that patients with GAD presented with high serum platelet, serotonin, and ACTH levels along with improvements in anxiety symptoms. Thus, acupuncture regulates serotonin and ACTH levels, and this is one of the mechanisms associated with the improvements in anxiety [20].

Zhou et al. in 2008, examined the effect of EA at the GV 20 Baihui and SP 6 Sanyinjiao points on the imbalance in monoamine and GABA levels on the SSP of chronic emotional stress-induced mice, and results showed that EA has anti-anxiety effects and significantly decreases serotonin, norepinephrine, and dopamine levels and increases GABA levels. Thus, EA may regulate the positive expression of monoamine and GABA levels in the CNS and may restore balance in these neurotransmitters [21].

Yoshimoto et al. in 2006, examined the neuropharmacological mechanisms of acupuncture on changes in monoamine release in vivo in the brain of 24 mice, resulting in a change in serotonin release in the nucleus accumbens for 20 min and lasted up to 40 min after acupuncture (120 min), and the effect is greater in the bilateral BLEN than in the bilateral Senshu. In conclusion, acupuncture affects the activity of serotonergic neurons in the reward system of the brain. Thus, it may be effective in individuals with emotional disorders [22].

Han, in 1986, found that acupuncture at ST 36 Zusanli and SP 6 Sanyinjiao points accelerated the synthesis and release of serotonin and norepinephrine in the CNS of mice and rabbits [23].
Several therapies are available. However, patients seek other treatments because of the side effects of medications and constraints in accessing psychotherapeutic services. Acupuncture is among these therapeutic or adjunctive therapeutic options for managing anxiety.

2. Methods
This is a single-blinded randomized clinical trial conducted from September 2013 to October 2013. After sample calculation [24], 38 patients with anxiety symptoms in Psychiatry Clinic of Santosa Hospital, Bandung were included in the analysis. This study has been reviewed by the Health Research Ethics Committee of Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital (approval no: 411/H2.P1/ETIK/2013) and Santosa Hospital Bandung (approval no: 046/KM/SHBC/VI/2013). Informed consent was obtained from the participants.

A total of 38 patients with mild and moderate anxiety symptoms were included in the analysis. The participants were randomly divided into treatment and control groups, each with 19 participants. A statistical analysis was conducted. Blood samples were obtained before and after acupuncture (blood plasma) and were analyzed using ELISA at the Clinical Laboratory Prodia, Jakarta.

Male and female patients aged >18 years who were diagnosed with anxiety disorders; who had not received psychotherapy or any medication at least 2 weeks prior to the study; and who were screened by psychiatrists and had an HARS score of 14–27 were included in the study. Individuals with other mental disorders and severe anxiety and those with contraindications for acupuncture were excluded. Moreover, participants were excluded from the study if they did not complete the procedure or they received other anti-anxiety therapies. Samples were randomly allocated using random tables. Participants were divided into treatment and control groups. On day 1, the first HARS score was obtained, and serum serotonin levels of the sham acupuncture (control) and acupuncture (treatment) groups were first obtained while patients were in sitting position. On day 2, EA and sham acupuncture were again performed in the treatment and control groups, respectively. On day 3, the third EA and sham acupuncture were performed in the treatment and control groups, respectively. Moreover, the second HARS scores and serum serotonin levels were obtained.

Dongbang, disposable acupuncture needles (size: 0.25 × 25 mm and 0.18 × 13 mm), and Hwato type SMY-10A as EA brand were used. In the treatment group, stabbing was performed at the GV 20 Baihui, Ex-HN1 Sisenchong, Ex-HN3 Yintang and PC 6 Neiguan, and HT 7 Shenmen points. EA was performed at the following auricular points: 156.C1 depressing, 7.0 tranquilizer, and 9.0 master cerebral points. Two points on the right and left ear were randomly selected, and dense dispersive waves with a frequency of 3/15 Hz and large intensity were used only until felt by the patient. In the control group, the EA pad was placed at points TE 5 Waiguan and LI 14 Binao, which are located bilaterally, and the EA was turned off. The length of action is 30 min [24-30]. Analysis was conducted using the Statistical Package for the Social Sciences software, and a p value of <α or <0.05 was considered statistically significant [31].

3. Results
There were no differences between the treatment and control groups for each characteristic (sex, age, religion, employment status, education and marital status), with a p value > 0.05 (Table 1). No significant difference was observed between the participants based on age and anxiety level. However, the anxiety level of the participants was found to be associated with age (Table 2).

In the treatment group, a statistically significant difference was observed in the HARS scores before and after EA (p < 0.001) (Table 3). Moreover, in the control group, a significant difference was observed in the HARS scores before and after treatment (p = 0.001). The HARS scores of the treatment group (14.83 ± 3.62) of the control group (13.1±1.49) decreased. Thus, the statistical analysis of the changes (delta) in the HARS score showed that the HARS delta score of the treatment group was significantly higher than that of the control group (p < 0.001).
Table 1. Characteristics of the participants

| Variables       | Treatment group, n (%) | Control group, n (%) | p-value |
|-----------------|------------------------|----------------------|---------|
| Sex             |                        |                      |         |
| Male            | 8 (66.7)               | 4 (33.3)             | 0.295   |
| Female          | 11 (42.3)              | 15 (57.7)            |         |
| Age             | 32.37 (SB: 7.98)       | 33.89 (SB: 12.31)    | 0.653   |
| Religion        |                        |                      |         |
| Muslim          | 18 (51.4)              | 17 (48.6)            | 1.000   |
| Christian       | 1 (33.3)               | 2 (66.7)             |         |
| Work            |                        |                      |         |
| Yes             | 19 (55.9)              | 15 (44.1)            | 0.105   |
| No              | 0 (0.00)               | 4 (100.0)            |         |
| Education level |                        |                      |         |
| Junior high school | 0 (0.0)            | 1 (100.0)            | 0.539   |
| Senior high school | 7 (50.0)            | 7 (50.0)             |         |
| College         | 12 (52.2)              | 11 (47.8)            |         |
| Marriage        |                        |                      |         |
| Yes             | 13 (50.0)              | 13 (50.0)            | 1.000   |
| No              | 6 (50.0)               | 6 (50.0)             |         |

Table 2. Characteristics of participants based on age and anxiety level

| Age group      | Anxiety level | p value |
|----------------|---------------|---------|
|                | Mild | Moderate |         |
| >60 years      | 1 (100) | 0 (0.0) | 0.675* |
| 40–60 years    | 6 (75.0) | 2 (25.0) |       |
| <40 years      | 25 (86.2) | 4 (13.8) |       |

* Chi-square test

The initial serotonin levels of the treatment and control groups did not significantly differ (p = 0.919), indicating that both groups are homogeneous. A statistically significant difference was observed in serum serotonin levels of the treatment group (p = 0.005) and control group (p = 0.025) before and after EA (Table 4). However, the delta serum serotonin levels of the treatment and control groups were not statistically significant (p = 0.804).

Table 5 shows that the HARS score of all respondents in the treatment group (n = 19) decreased. In the control group, only 12 (63%) individuals had decreased HARS scores. The success rate of the decreased HARS score in the treatment group was 100%. Moreover, 16 participants had increased serotonin levels after EA (84%), and 3 (16%) had decreased serotonin levels. In the control group, 13 (67%), 1 (5%), and 5 (26%) participants had decreased, constant, and increased serotonin levels, respectively. In the treatment group, changes in serotonin levels of all respondents were observed after EA (Table 6). Thus, the success rate of EA caused changes in serotonin levels in 100% and 93% of the participants in the treatment and control groups. However, the most significant increase in serotonin level was observed in the treatment group. Meanwhile, the control group had the most significant decrease in serotonin level. No significant difference was observed between the groups in terms of age and anxiety level after EA, indicating the relative anxiety level in the different age groups (Table 7).

The serotonin levels after EA in the different age groups did not significantly differ, indicating that the serotonin levels of the different age groups after EA were relatively similar (Table 8). This result indicates that the response to serotonin level changes after EA was not affected by age. A significant increase in serotonin levels after EA was statistically significant in groups with mild or moderate anxiety...
Thus, EA may increase serotonin levels in individuals with mild or moderate anxiety. Changes in serotonin levels in individuals with mild or moderate anxiety did not significantly differ, indicating that serotonin increment in both groups was not significant (Table 10).

### Table 3. Comparison of HARS scores before and after EA

|            | Pre       | Post      | Delta    | p value   |
|------------|-----------|-----------|----------|-----------|
| Treatment  | 19.21 (SB: 3.42) | 4.47 (SB: 1.61) | -14.84 (SB: 3.62) | <0.001# |
| Control    | 15.95 (SB: 1.75) | 14.63 (SB: 1.12) | -1.31 (SB: 1.49) | 0.001# |

*p value <0.001*

* t-test, dependent test, *Uji t-test, independent test

### Table 4. Comparison of serum serotonin levels before and after EA

|                   | Pre       | Post      | Delta    | p Value   |
|-------------------|-----------|-----------|----------|-----------|
| Treatment group   | 229 (127–470) | 274 (114–559) | 47 (--68)--(124) | 0.005** |
| Control group     | 202 (101–402) | 268 (118–464) | 49 (--92)--(252) | 0.025** |

*p value 0.919^  0.804^*

* Wilcoxon signed-rank test, ^Uji Mann–Whitney U test (distribusi data tidak normal); **p<0.05

### Table 5. Changes in HARS scores

| HARS score | Treatment group | Control group | Total N (%) |
|------------|-----------------|---------------|-------------|
| Decrease   | 19 (100)        | 12 (63)       | 31 (82)     |
| Constant   | 0 (0)           | 7 (37)        | 7 (18)      |
| Increase   | 0 (0)           | 0 (0)         | 0 (0)       |

### Table 6. Changes in serum serotonin levels

| Serotonin levels | Treatment group | Control group | Total n (%) |
|------------------|-----------------|---------------|-------------|
| Decrease         | 3 (16)          | 13 (67)       | 16 (42)     |
| Constant         | 0 (0)           | 1 (5)         | 1 (3)       |
| Increase         | 16 (84)         | 5 (26)        | 21 (55)     |

### Table 7. Comparison of age groups and anxiety level after EA

| Age group | Anxiety       | p value   |
|-----------|---------------|-----------|
|           | Mild          | Moderate  |
| >60 years | 0 (0.0)       | 1 (100.0) | 0.464*     |
| 40–60 years | 5 (62.5) | 3 (37.5)  |
| <40 years | 14 (48.3)     | 15 (51.7) |

*Uji Chi-square test
### Table 8. Serotonin levels by age group after EA

| Serotonin level after EA | >60 years old | 40–60 years old | <40 years old | p value |
|-------------------------|---------------|-----------------|---------------|---------|
|                         | 126.0         | 227.12          | 300.38        | 0.057*  |

*ANOVA test

### Table 9. Comparison of serum serotonin levels before and after EA in terms of anxiety level

| Variables          | Serotonin level | p value |
|--------------------|-----------------|---------|
|                    | Pre             | Post    |         |
| Mild anxiety       | 244.31 (SB: 96.08) | 284.62 (SB: 107.61) | 0.002* |
| Moderate anxiety   | 185.33 (SB: 60.28) | 257.67 (SB: 65.51) | 0.004* |

*p<0.05

### Table 10. Changes in serum serotonin levels according to anxiety level

| Variable            | Anxiety         | p value |
|---------------------|-----------------|---------|
| Delta serotonin level | Mild (45.5–92) | 45.5 (–92)–(252) | 76.0 (18–118) | 0.123* |

*Mann–Whitney U test

### 4. Discussion

The study was conducted at Psychiatric Clinic of Santosa Hospital Bandung on 38 patients with mild (HARS score 14–20) and moderate anxiety (HARS score 21–27) who fulfilled the inclusion criteria and were divided randomly into two groups: treatment and control groups, each consisting of 19 respondents. Acupuncture and EA were performed on the treatment and control groups, respectively, once daily for 3 consecutive days. The HARS score of the patients was obtained by a psychiatrist before the first and after the third acupuncture. Blood samples were obtained before the first and immediately after the third acupuncture (maximum 30 min after stabbing), and blood sampling was performed at 07.00–09.00 am after the serotonin circadian rhythm. Plasma samples were analyzed using ELISA at the laboratory clinic of Prodia, Jakarta. During the study, no positive effects were observed from needle stabbing, electrostimulator stimulation, or blood sampling.

In the treatment group, acupuncture was performed at the GV 20 Baihu, Ex-HN1 Sisenchong and Ex-HN3 Yintang, PC 6 Neiguan, and HT 7 Shenmen points, and electroacupuncture was conducted on two of three ear acupuncture points (156.C1 depressing, 7.0 tranquilizer, 9.0 master cerebral points) using dense dispersive wave with a frequency of 3/15 Hz and large intensity until it has been felt by the patient. The needle was left for 30 min. In the control group, the electrostimulator pad was placed at TE 5 Waiguan and LI 4 Binao points, which are located bilaterally. The electrostimulator was turned off and left for 30 min.

A rapid science that deepens ansietas-based neurobiological science explained that specific neurochemical and neuropeptide systems are associated with fear and stimuli that cause anxiety and affect the cortex and subcortical regions of the brain. Long-term dysregulation of these systems contributes to the development of anxiety disorders. The development of molecular genetics also helps in identifying genes that cause neurobiological disorders that result in increased vulnerability to anxiety disorders [32]. The imbalance among GABA, serotonin, norepinephrine, and dopamine in the CNS is among the mechanisms associated with anxiety. An increase in abnormal levels of monoamine neurotransmitters in the limbic system leads to anxiety. Damage in the GABAergic neurons that produce GABA inhibition neurotransmitters leads to an imbalance between excitation and inhibition, resulting...
in abnormal emotions, such as excessive excitation and anxiety, and subsequently, the GABAergic pathways may inhibit the excitation of serotonin and norepinephrine transmission from the mesencephalon to the cerebral cortex via the limbic system [21]. Serotonin is a neurotransmitter that inhibits the amygdala, and antidepressants can increase serotonin level by blocking serotonin transporters [33].

Acupuncture induces therapeutic effects by increasing serotonin system activity in the CNS and inhibits the process of serotonin degradation. Serotonin, which is regulated by acupuncture, has a neuroprotective effect, and it prevents synaptic plasticity, suppressing artificially induced apoptotic processes in the hippocampus region of the brain’s corpus striatum [34,35]. EA stimulation at the acupuncture points can activate TRPV1 in the afferent nerve fibers (primarily expressed on the projection of nerve fibers Aδ and C) leading to the depolarization of the neural membrane, which subsequently activates the sodium and calcium ion channels. EA caused a signal transduction to the CNS that modulated the CNS response [36]. Serotonin, norepinephrine, dopamine, and GABA regulation also regulates the positive expression of monoamine and GABA neurotransmitters in the CNS and restores their balance. Moreover, it enhances and prolongs the activity of serotonergic neurons in the reward system of the brain [20-22].

Statistical analysis was performed, and no significant difference was observed in terms of participant characteristics (sex, age, religion, employment status, and education and marital status) in the treatment and control groups. Thus, the two groups were homogenous. A significant difference was observed in terms of the HARS score of the treatment and control groups before and after EA. However, the decrease in the HARS score of the treatment group was significantly higher than that of the control group. The HARS score of the treatment group decreased to 14.83 ± 3.62, whereas that of the control group decreased to 1.31 ± 1.49. This result showed that EA (treatment group) was more effective in decreasing the HARS score than the sham acupuncture (control group). However, the HARS score of the control group still decreased before and after therapy. Baseline serotonin levels of the treatment and control groups did not significantly differ. Thus, the two groups were homogeneous. After acupuncture, a significant difference was observed between the treatment and control groups in terms of serum serotonin levels before and after EA. The delta serotonin levels of the treatment and control groups were not significantly different, indicating significant changes in serotonin levels at baseline and after treatment and control measures. However, the difference was not significant between the two groups.

Changes in HARS scores and serotonin levels were observed. All participants in the treatment group had decreased HARS scores, whereas 62% of participants in the control group had decreased HARS scores. In the treatment group, approximately 84% of the participants had increased serotonin levels, and 16% had decreased serotonin levels after EA. Thus, the success rate of EA in inducing serotonin changes in the treatment group was 100%, whereas that in the control group was 93%. No statistically significant difference was observed between the intervention groups. However, a different trend was observed. That is, the serotonin levels only increased by 26%, remained constant at 5%, and decreased by 67%. The anxiety level of the different age groups did not significantly differ before or after EA. This suggests that anxiety level is relatively similar across different age groups. The increase in serotonin levels was more significant after EA than before EA in individuals with mild and moderate anxiety. However, the increase was not significant. The results of this study indicate that EA is superior to sham acupuncture in terms of improving anxiety symptoms, as supported by the decrease in HARS scores. Changes in the serotonin levels of the treatment and control groups were not statistically significant. In the present study, side effects from acupuncture were not observed. Thus, acupuncture is a relatively safe therapy [37]. Moreover, EA improves the clinical symptoms of individuals with anxiety, as supported by the decrease in HARS scores. In this case, EA can still be beneficial for individuals with anxiety.
5. Conclusion
EA was more effective in decreasing HARS scores and inducing changes in serum serotonin levels than sham acupuncture in individuals with anxiety symptoms.

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