

The Effect of Liver-Soothing, Spleen-Strengthening and Mind-Calming Acupuncture on Serum Levels of 5-HT, NE and DA and Sleep Quality in Patients with Insomnia

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Abstract

Objective: To explore the mechanism of soothing liver, strengthening spleen and tranquilizing spirit acupuncture in treating insomnia patients with deficiency of heart and spleen combined with liver depression.

Methods: The 90 patients who met the inclusion criteria were divided into three groups according to the random number table: acupuncture group (soothing liver, strengthening spleen and calming nerves acupuncture), comprehensive group (acupuncture and drug combination therapy), and drug group (oral alprazolam tablets), with 30 patients in each group. The contents of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) in serum were detected by HPLC before and after treatment, and Pittsburgh sleep quality index (PSQI) and TCM syndrome score were observed. Results: After treatment, the contents of NE and DA in the three groups were all decreased ($P < 0.01$), while those in the acupuncture group and the comprehensive group were lower than those in the drug group ($P < 0.01$). The content of 5-HT in both the acupuncture group and the comprehensive group increased ($P < 0.01$), but the change in the drug group was not significant ($P > 0.05$). The total PSQI scores of the three groups were significantly decreased ($P < 0.05$). The score of acupuncture group and comprehensive group was better than that of drug group ($P < 0.05$). After follow-up, the scores of the acupuncture group and the comprehensive group decreased significantly ($P < 0.05$), but there was no difference in the drug group ($P > 0.05$). TCM syndrome score after treatment: acupuncture group and comprehensive group were significantly lower than drug group ($P < 0.01$).

Conclusion: The method of soothing the liver and strengthening the spleen can increase the 5-HT content, reduce the NE and DA content, and improve the sleep quality of insomnia patients.

Keywords: Shugan jianpi anshen acupuncture, insomnia, 5-hydroxytryptamine, Norepinephrine, Dopamine

Introduction

Insomnia is a common disorder with high incidence rate in today's society. It is a subjective perception in which daytime social functions are affected due to poor sleep quality or quantity in meeting normal physiological needs due to difficulty in falling asleep and (or) sleep maintenance. It is the most common sleep disorder (Jia, 2013). In 2002, epidemiological studies on insomnia from 10 countries showed that

45.4% of the Chinese population had experienced different degrees of insomnia in the past month (Soldatos et al., 2004). In an investigation in 2006, proportion of insomnia adults among adult population in China was 57% (Lu, 2011). Incidence rate of insomnia increases with age, which adversely affect daily work and life of individuals (Zhang et al., 2012; Trauer et al., 2015; Li et al., 2015). From July 2017 to November 2019, investigators used liver-soothing, spleen strengthening and mind-calming acupuncture method for treatment of 90 cases of insomnia patients with weakness in heart and spleen, and

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liver depression syndromes. Its efficacy and effects on serum levels of 5-HT, NE and DA in the insomnia patients were observed. The results are reported as follows.

1 Materials and Methods

1.1 General Information

Table 1 Comparison of general data between three groups of insomnia patients

| Group | Cases | Sex | | Years ($\bar{x}\pm s, n$) | Course of Disease ($\bar{x}\pm s, n$) |
|-------------|-------|------|--------|--------------------------------|--|
| | | Male | Female | | |
| Acupuncture | 30 | 12 | 18 | 44.83 \pm 9.76 | 12.6 \pm 7.05 |
| Combination | 30 | 15 | 15 | 44.90 \pm 9.19 | 12.8 \pm 6.94 |
| Medicine | 30 | 13 | 17 | 45.17 \pm 9.56 | 13.0 \pm 7.09 |

1.2 Diagnostic Criteria

Western medicine diagnosis was carried out according to "Chinese Guideline for Diagnosis and Treatment of Insomnia in Adults 2012". Traditional Chinese Medicine diagnosis and syndrome differentiation were carried out according to "Chinese Medicine Clinical Practice Guideline for Insomnia 2016".

1.3 Inclusion Criteria

① Insomnia patients who met the above diagnostic criteria with weakness in heart and spleen combined with liver depression; ② patients aged 18–70 years, non-gender specific; ③ Pittsburgh Sleep Quality Index (Liu et al., 1996) (PSQI>7 points); ④ patients with informed consent to receive acupuncture and/or medicine treatment.

1.4 Exclusion criteria

① Those who did not meet the diagnostic criteria and inclusion criteria; ② pregnant or lactating women; ③ patients with secondary insomnia, such as drug-induced insomnia, insomnia secondary to cardiovascular and cerebrovascular disease, endocrine system disease, tumor, postoperative insomnia, or others; ④ those with other serious life-threatening primary diseases; ⑤ those with anemia and spontaneous bleeding tendency; ⑥ patients with history of mental illness and alcoholism; ⑦ patients who were taking drugs that affect sleep quality.

1.5 Treatment methods

1.5.1 Acupuncture group: acupuncture points of Sishenchong, bilateral Taiyang, Neiguan, Shenmen, Zu Sanli, Sanyin and Taichong were positioned. Acupoint positioning and operation standards were based on the 13th Five-Year Plan textbook of "Acupuncture and Moxibustion" (4th Edition, 2016) edited by Liang Fengrong (Liang and Wang, 2016).

All the 90 cases in this study were outpatients in Acupuncture Department and Neurology Department of our hospital. This study was reviewed and approved by the medical ethics committee of our hospital (batch number: KY2018-004). The three groups of patients had not statistically significant differences in gender, age and disease course ($P>0.05$). See Table 1.

Operation method: Patients took the supine position. The acupoints were positioned. Following routine disinfection, needles were quickly inserted into Sishenchong (insertion of 0.4 inch toward Baihui acupoint), Taiyang (straight insertion of 0.4 inch), Neiguan (straight insertion of 0.3 inch), Shenmen (straight insertion of 0.3 inch), Zusanli (straight insertion of 1 inch), Sanyinjiao (straight insertion of 1 inch) and Taichong (straight insertion of 0.3 inch). Upon needle insertion and arrival of qi, acupoints of Sishenchong, Taiyang, Neiguan and Shenmen points were applied with even-reinforcing-reducing manipulation method. Acupoints Zusanli and Sanyinjiao were applied with lifting-thrusting-reinforcing manipulation method, whereas Taichong acupoint was applied with lifting-thrusting-reducing manipulation method. Acupoints Sishenchong, Taiyang, Zusanli and Sanyinjiao were then connected to electro-acupuncture therapeutic instrument (Qingdao Xin Sheng Industrial Co. Ltd., model G6805-i). Left Sishenchong and left Taiyang were connected, right Sishenchong and right Taiyang were connected, front and rear Sishenchong were connected, Zusanli and Sanyinjiao were connected at both sides, Neiguan and Shenmen were connected at both sides, both Taichong were connected.

Frequency was set at 60 Hz and continuous wave type was adopted. Current intensity was set to level where patient felt distension but not discomfort. The stimulation was performed for 30 minutes. 1.5.2 Medicine group: Patients were given oral medication of Alprazolam tablets (manufactured by Jiangsu Enhua Pharmaceutical, specification: 0.4mg \times 100 tablets, approval number: National Medicine Standard H32020215). Usage method: 2 tablets each time, once a day before bedtime.

1.5.3 Combination group: Patients were given treatment with liver-soothing, spleen-

strengthening and mind-calming acupuncture method combined with oral medication of Alprazolam. Acupuncture operation method and oral medication dosage were as above.

Acupuncture treatment was given once a day from Monday to Friday, and rest on weekend. The treatment was given continuously for 2 weeks. Oral medication treatment was given once a day for 2 weeks. The curative effects in the three groups were observed after 2 weeks of treatment. Follow-up was carried out after 1 month.

1.6 Examination method

1.6.1 For three groups of patients, 4 ml of cubital venous blood was collected at one day before treatment and at 8:00 a.m. (after 12 hours of fasting) after end of treatment, respectively. The blood was centrifuged. Serum was then collected and stored in refrigerator at a temperature of -20° for preservation.

1.6.2 Processing of serum sample: Serum was thawed to room temperature and 0.5 ml of the serum was then transferred into a 10ml centrifuge tube. Subsequently, 0.5 ml of 5% perchloric acid solution was added into the tube. It was then shaken well and subjected to high-speed centrifuge at 12000 r/min for 8 minutes. The supernatant was filtered through 0.45 mm film for further examination.

1.6.3 Chromatography conditions: high performance liquid chromatograph (HPLC) with fluorescence detector (F), C18 chromatographic column (250 mm \times 4.6 mm, 5 μ m). Mobile phase: 0.02% formic acid aqueous solution, methanol gradient elution.

1.6.4 Reference substance: NE, DA, 5-HT were accurately weighed and mixed with 0.02% formic acid methanol into stock solutions with concentration of 100 μ g/ml. Appropriate amount of the stock solution was diluted with 0.02% formic acid aqueous solution to final volume of 10 ml, into working solutions of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 μ g/ml.

1.7 Statistical methods

Statistical software SPSS 21.0 was used for data analysis. Quantitative data were presented in the forms of mean and standard deviation. For comparison between multiple groups, one-way analysis of variance was used for data with normal distribution whereas non-parametric test was used for data with non-normality. Independent t test was used for comparison between two groups, and paired t test was used for comparison within group. Qualitative data were presented in the forms of

frequency and rate, and Chi-squared test was used. For rank data, rank sum test was used. $P < 0.05$ indicated statistically significant difference.

2 Results

2.1 Chromatographic detection of NE, DA and 5-HT using high performance liquid chromatography-fluorescence (HPLC) method.

2.1.1 Chromatogram of NE, DA and 5-HT standards. See Figure 1.

Note: A is chromatogram of NE, DA and 5-HT standards, 1.NE, 2. DA, 3.5-HT.

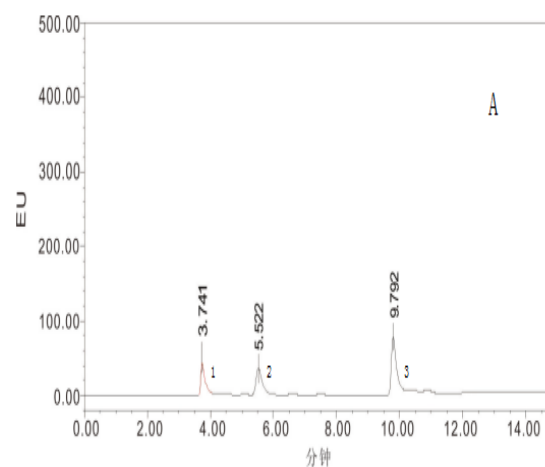


Figure 1 Chromatogram of standards

2.1.2 Chromatograms of serum NE, DA, 5-HT of an acupuncture group patient before and after treatment. See Figure 2.

Note: B is chromatogram of serum before treatment, C is chromatogram of serum after treatment, 1.NE, 2. DA, 3. 5-HT.

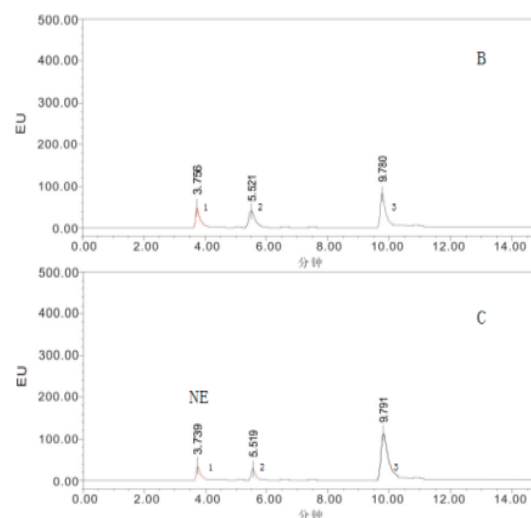


Figure 2 Chromatograms of serum sample from an acupuncture group patient before and after treatment

2.1.3 Chromatograms of serum NE, DA, 5-HT of a combination group patient before and after treatment. See Figure 3.

Note: D is chromatogram of serum before treatment, E is chromatogram of serum after treatment, 1. NE, 2. DA, 3. 5-HT.

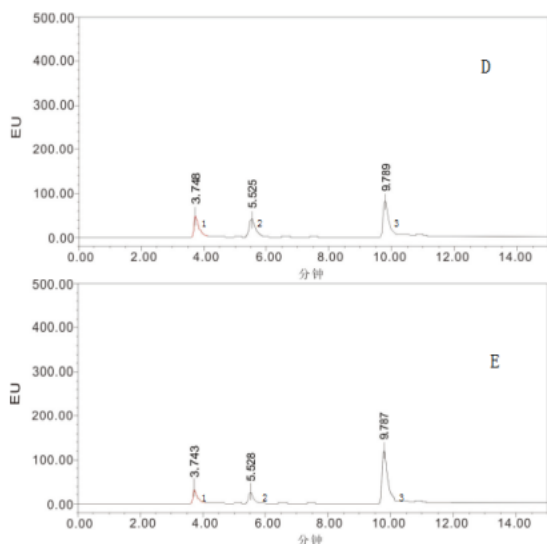


Figure 3 Chromatograms of serum sample of combination group patient before and after treatment

2.1.4 Chromatograms of serum NE, DA, 5-HT of a medicine group patient before and after treatment. See Figure 4.

Note: F is chromatogram of serum before treatment, G is chromatogram of serum after treatment, 1. NE, 2. DA, 3. 5-HT.

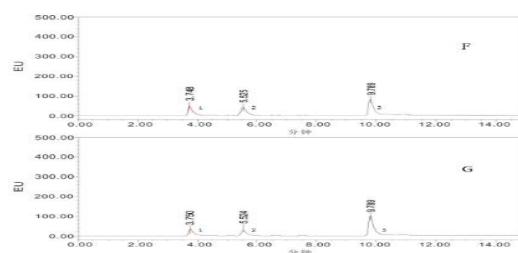


Figure 4 Chromatograms of serum sample of medicine group patient before and after treatment

2.2 Comparison of serum NE level before and after treatment in three patient groups

In the three groups of patients, serum NE level was significantly lower than before treatment ($P < 0.01$). Post-treatment serum NE levels of acupuncture group and combination group were significantly lower than that of medicine group ($P < 0.05$). There was no significant difference in post-treatment level between acupuncture group and combination group ($P > 0.05$). See Table 2.

2.3 Comparison of serum DA level before and after treatment in three patient groups

In all three patient groups, serum DA level decreased after treatment ($P < 0.01$). The decrease of serum DA level in insomnia patients of acupuncture group and combination group was more significant than medicine group ($P < 0.01$). There was no significant difference in decrease of serum DA level in insomnia patients between acupuncture group and combination group ($P > 0.05$). See Table 3. **Table 2 Comparison of serum NE level before and after treatment in three patient groups ($\bar{x} \pm s$, $\mu\text{g/L}$)**

| Group | n | Before Treatment | After Treatment | F | P |
|-------------|----|-------------------|---------------------------------|------|------|
| Acupuncture | 30 | 58.10 \pm 11.63 | 45.47 \pm 8.77 ^{Δ#} | | |
| Combination | 30 | 58.30 \pm 11.34 | 43.53 \pm 11.08 ^{Δ*} | 6.18 | 0.00 |
| Medicine | 30 | 58.20 \pm 10.41 | 52.20 \pm 10.08 ^Δ | | |

Note: Δ comparison with before treatment, $P < 0.01$; ∇ comparison with combination group and medicine group, $P > 0.05$; # comparison with combination group, $P > 0.05$; comparison with medicine group, $P < 0.05$; * comparison with medicine group, $P < 0.05$.

Table 3 Comparison of serum dopamine level before and after treatment in three patient groups [M(Q), $\mu\text{g/L}$]

| Group | n | Before Treatment | After Treatment | Z | P |
|-------------|----|------------------|---------------------------|--------|---------|
| Acupuncture | 30 | 38.50 (12.25) | 10.00 (6.25) * \diamond | -4.874 | P=0.000 |
| Combination | 30 | 36.00 (12.00) | 9.00 (7.25) * \diamond | -4.874 | P=0.000 |
| Medicine | 30 | 39.50 (15.25) | 25.00 (9.00) * \diamond | -4.704 | P=0.000 |

Note: *comparison of level after treatment within group, $P < 0.01$; comparison between group: \diamond comparison between acupuncture group and medicine group, $P < 0.01$, comparison between combination group and medicine group, $P < 0.01$.

2.4 Comparison of serum 5-HT level before and after treatment in three patient groups

The levels of serum 5-HT in acupuncture group and combination group were significantly higher than before treatment ($P<0.01$), whereas medicine group showed no difference compared with before

treatment ($P>0.05$). Levels of 5-HT in acupuncture group and combination group after treatment were significantly higher than that of medicine group ($P<0.01$), and the difference between the two groups was not significantly different ($P>0.05$). See Table 4.

Table 4. Comparison of serum 5-hydroxytryptamine level before and after treatment in three patient groups ($\bar{x}\pm s$, $\mu\text{g/L}$)

| Group | n | Before Treatment | After Treatment | t | P |
|-------------|----|-------------------|---|--------|-------|
| Acupuncture | 30 | 94.70 \pm 22.04 | 163.23 \pm 16.52 $\Delta\blacktriangle$ | -13.43 | 0.000 |
| Combination | 30 | 95.20 \pm 18.73 | 164.83 \pm 27.36 $\Delta\blacktriangle$ | -11.66 | 0.000 |
| Medicine | 30 | 94.27 \pm 17.79 | 100.27 \pm 16.03 $\nabla\blacktriangle$ | -1.51 | 0.143 |

Note: Δ comparison between before and after treatment in acupuncture group and combination group respectively, $P<0.01$, ∇ comparison between before and after treatment in medicine group, $P>0.05$; \blacktriangle comparison of post-treatment level between acupuncture group and combination group, $P>0.05$, comparison of post-treatment level between medicine group and acupuncture with comprehensive group respectively, $P<0.01$.

2.5 Comparison of total PSQI scores before and after treatment in three patient groups

All three groups showed significantly reduced total PSQI scores after treatment ($P<0.05$). Acupuncture group and combination group showed comparable improvement in sleep quality ($P>0.05$), and the curative effect on the two groups was more significant than that on medicine group ($P<0.05$). The follow-up carried out after 1-month showed

significantly better long-term curative effect in acupuncture group and combination group when compared with the scores before treatment ($P<0.01$); long-term curative effect in medicine group was not significant ($P>0.05$). Comparison showed no significant difference in post-treatment scores between acupuncture group and combination group ($P>0.05$), where the total score of medicine group increased ($P<0.01$). See Table 5.

Table 5. Comparison of total Pittsburgh Sleep Quality Index scores before treatment, after treatment and after follow-up among three patient groups ($\bar{x}\pm s$, n)

| Group | n | Before Treatment | After Treatment | After follow-up |
|-------------|----|------------------|----------------------------|---|
| Acupuncture | 30 | 13.6 \pm 2.20 | 5.63 \pm 2.33 Δ # | 6.12 \pm 2.39 $\diamond\blacktriangle\blacklozenge$ |
| Combination | 30 | 14.10 \pm 2.51 | 5.93 \pm 1.95 Δ # | 6.53 \pm 2.05 $\diamond\blacktriangle\blacklozenge$ |
| Medicine | 30 | 13.00 \pm 2.15 | 9.40 \pm 3.67 Δ | 12.40 \pm 3.67 $\diamond\blacktriangle$ |

Note: Δ comparison between before treatment and after treatment, $P<0.01$ for all the three groups; \diamond comparison between post-follow-up and before treatment, $P<0.01$ in acupuncture group and combination group, $P>0.05$ for medicine group; \blacktriangle comparison between post-follow-up and after treatment, $P>0.05$ in acupuncture group and combination group, and $P<0.01$ in medicine group; # Comparisons of post-treatment level between medicine group and acupuncture group with combination group respectively, $P<0.01$; \blacklozenge comparisons of post-follow-up level between medicine group and acupuncture group with combination group respectively, $P<0.01$.

2.6 Comparison of TCM syndrome scores before and after treatment in three patient groups

In all three groups, post-treatment TCM syndromes scores were significantly lower than that of before treatment ($P<0.01$). Acupuncture group and combination group showed no significant different in improvement in insomnia symptoms ($P>0.05$), and the two groups showed more significant improvement in insomnia symptoms than medicine group ($P<0.05$).

Follow-up was carried out after 1-month. Follow-up scores of acupuncture group and comprehensive group were significantly lower than those before treatment ($P<0.01$), and comparison with the score after treatment showed no significant difference ($P>0.05$). In medicine group, there was no difference between scores after follow-up and before treatment ($P>0.05$), and there was significant increase when compared with after treatment ($P<0.05$). See Table 6.

Table 6. Comparison of TCM syndrome scores before treatment, after treatment and follow-up among three patient groups ($\bar{x}\pm s$, n)

| Group | n | Before Treatment | After Treatment | After follow-up |
|-------------|----|------------------|--------------------------|----------------------------|
| Acupuncture | 30 | 22.1±10.21 | 7.87±10.17 ^{△#} | 8.23±9.31 ^{◇▲◆} |
| Combination | 30 | 23.1±10.11 | 8.13±10.80 ^{△#} | 8.55±10.19 ^{◇▲◆} |
| Medicine | 30 | 22.6±10.20 | 14.63±13.88 [△] | 21.63±10.57 ^{◇▲◆} |

Note: Δ comparison between before and after treatment, $P < 0.01$; \diamond comparison between after follow-up and before treatment, $P < 0.01$ in acupuncture group and combination group, and $P > 0.05$ in medicine group; \blacktriangle comparison between after follow-up and after treatment, $P > 0.05$ in acupuncture group and comprehensive group, and $P < 0.01$ for medicine group; # Comparisons of post-treatment between medicine group and acupuncture group with combination group respectively, $P < 0.01$; \blacklozenge comparisons of post follow-up scores between medicine group and acupuncture group with combination group respectively, $P < 0.01$.

3 Discussions

In traditional Chinese medicine, insomnia belongs to the categories of 'sleepless', 'insomnia' and 'restless'. This disorder is commonly due to factors such as anxiety and fatigue, internal injury of heart and spleen, disharmony of yang and yin, disharmony of heart and kidney, heatiness due to yin deficiency, disturbance of liver yang, deficiency of qi in heart and gallbladder, and disharmony within stomach, which bring disturbance to mind and lead to insomnia. According to "Lingshu Dahuo Theory", when protective qi does not enter yin meridians but stays in those of yang, one will be full of yang vital. When vital of yang is full, so as the Yangqiao meridian. With that, one will be deficit in yin and not be able to fall in sleep. "Book of Jingyue, Insomnia" records: 'Sleep is about calmness in which spirit plays the main role. One sleeps when the spirit is calm and not when the spirit is anxious. Anxiety is either caused by disturbance by pathogenic qi or insufficiency of nutritional energy. In case of disturbance by pathogenic qi, it is due to struggles between pathogenic qi and body's resistance. If insomnia occurs in the absence of pathogenic qi, it is due to deficiency of vital energy.' A normal sleep relies on human body's harmony dynamics of yin and yang, harmony between internal organs, adequacy of qi and blood, calmness in mind and spirit, calmness of heart-blood, and entry of yang energy into yin meridian. "Classification of Symptoms, Theory on Insomnia" records: 'Anxiety injures spleen. Spleen blood will loss and become deficit which lead to persistent insomnia.'

Fast-paced and high-stress lifestyle of the modern society lead to increased physiological and psychological stress among people. At the same time, people's dietary habit also changed. Fatigue, anxiety, emotional frustration and improper diet have become the important causes of insomnia (Birling et al., 2017; Tan et al., 2016). Anxiety and fatigue harm spleen and heart, whereas emotional

frustration causes heaviness of liver qi. When there is depression in liver, it subsequently causes impaired function of spleen. The interaction between liver and spleen leads to weak qi and blood, which causes difficulties in nourishment of heart and calming of mind, leading to insomnia. Therefore, treatment should focus on nourishment of blood and heart, soothing of liver and regulation of qi, soothing of mind and promotion of sleep. Brain is the house for vital and spirit. Regulation of brain and mind, and calming of spirit can be done through exterior acupoints of Sishencong and Taiyang. Spleen and stomach are the foundation for vital activities and replenishment source of qi and blood. It is known that 'acupoints of He regulate internal organs. The combination of acupoints He and Zusanli of stomach, and acupoints He and Yinlingquan of spleen jointly play roles in tonification of qi and blood. Sanyinjiao is the key acupoint where the three meridians of liver, spleen and kidney meet. It controls invigoration of kidney, strengthening of spleen, soothing of liver and regulation of qi. Acupoint Taichong is one of the Yuan points located at liver, which has the function in soothing liver and regulation of qi. When all these acupuncture points play roles together, it gives the curative effect in nourishment of qi and blood, soothing of liver and regulation of qi, calming of heart and mind, will then relieve insomnia. (Liu and Wei, 2013)

Modern researches shown that dopamine is one of the important neurotransmitters involved in sleep-wake system. It plays a very important role in sleep-wake system in which stimulation of dopaminergic nervous system can trigger awakening state. Acupuncture has been proved to inhibit the synthesis of dopamine and other excitatory neurotransmitters and improve insomnia symptoms. Acupuncture at Baihui and Shenmen acupoints of insomnia rats reduced serum levels of monoamine neurotransmitters DA and NE, and effectively improved sleep structure of the rats

(Guo et al., 2018). The central neurotransmitter 5-HTergic neurons of sleep-wake system can inhibit wakefulness when they are discharged and active, while increased discharge in NE neuron will prolong duration of wakefulness (Adell, 2004). Reduction of NE in brain will accelerate the accumulation of 5-HT in forebrain, which reduces the inhibitory effect of 5-HT and thereby regulating and improving sleep (Jiang, 2001). Previous study showed that stimulation of Baihui and Shenting acupoints with electric acupuncture could effectively regulate sleep-wake rhythm and alleviate sleep disturbances in sleep-deprived rats following cerebral ischemia. The mechanism of action might be related to up-regulation of 5-HT and 5-HIAA and down-regulation of DA and NE (Li et al., 2018).

Experimental results of this study showed reduced levels of NE and DA after treatment in all the three patient groups ($P < 0.01$). The levels in acupuncture group and combination group were lower than medicine group ($P < 0.01$). Both acupuncture and combination groups showed increased level of 5-HT ($P < 0.01$), while the level changes in medicine group was not significant ($P > 0.05$). The total PSQI scores of the three groups were significantly reduced ($P < 0.05$). The scores of acupuncture group and combination group were better than those of medicine group ($P < 0.05$). After follow-up, acupuncture group and combination group showed significant score reduction ($P < 0.05$), there was no difference for medicine group ($P > 0.05$). Post-treatment TCM syndrome scores of acupuncture group and comprehensive group were significantly lower than medicine group ($P < 0.01$). These demonstrated that liver-soothing, spleen-strengthening and mind-calming acupuncture method can increase 5-HT level and reduce NE and DA levels, and improve sleep quality of insomnia patients.

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