Effect of low-frequency repetitive transcranial magnetic stimulation on serum neurotransmitters and cytokines in patients with ischemic poststroke depression

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Abstract
Objective: To study the effect of low-frequency repetitive transcranial magnetic stimulation on serum neurotransmitters and cytokines in patients with ischemic poststroke depression (PSD).

Methods: A total of 68 patients with ischemic poststroke depression who were treated in our hospital between July 2013 and February 2016 were selected as the research subjects and divided into the control group (n=34) who received routine treatment and the observation group (n=34) who received routine treatment + low-frequency repetitive transcranial magnetic stimulation according to the random number table, and the treatment lasted for 4 courses. Immediately after admission and after 4 courses of treatment, the peripheral venous serum was collected from two groups of patients to determine the levels of monoamine neurotransmitters, amino acid neurotransmitters, neurological function indexes and inflammatory cytokines in it.

Results: Immediately after admission, the differences in serum levels of monoamine neurotransmitters, amino acid neurotransmitters, neurological function indexes and inflammatory cytokines were not statistically significant between the two groups of patients. After 4 courses of treatment, serum monoamine neurotransmitters NE, 5-HT and DA levels of observation group were higher than those of control group; serum amino acid neurotransmitters Glu and Asp levels were lower than those of control group while Gly and GABA levels were higher than those of control group; serum neurological function index BDNF level was higher than that of control group while NSE level was lower than that of control group; serum inflammatory cytokines IL-1β, IL-2, IL-6 and TNF-α levels were lower than those of control group.

Conclusion: Low-frequency repetitive transcranial magnetic stimulation can adjust the excitatory/inhibitory neurotransmitter levels, stimulate the monoamine neurotransmitter secretion, optimize the neurological function and reduce the systemic inflammatory response in patients with ischemic poststroke depression.

1. Introduction
The incidence of ischemic stroke is high in clinical practice, depression occurs in some patients after anti-stroke treatment, and it seriously influences the realization of stroke treatment effect, and reduces the patients’ quality of life[1,2]. Both nerve nutrition and microcirculation improvement are the routine therapies for patients with poststroke depression, but the effect is limited, and looking for efficient and safe auxiliary treatment is the key to improving the treatment outcome. Low-frequency repetitive transcranial magnetic stimulation is a method that uses time-varying magnetic field to excite brain tissue, it has regulating effects on the function of the cerebral cortex under different frequency repetitive stimulation, and it has currently been successfully applied in diseases such as Parkinson’s disease with depression and primary depression[3].

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In the study, the low-frequency repetitive transcranial magnetic stimulation was introduced in the treatment of patients with ischemic poststroke depression, and the effect of low-frequency repetitive transcranial magnetic stimulation on serum neurotransmitters and cytokines in patients with ischemic poststroke depression (PSD) was specifically analyzed.

2. Information and methods

2.1. Case information

A total of 68 patients with ischemic poststroke depression who were treated in our hospital between July 2013 and February 2016 were selected as the research subjects, and patients' families signed informed consent. According to the random number table, the included patients were divided into the control group (n=34) who received routine treatment and the observation group (n=34) who received routine treatment + low-frequency repetitive transcranial magnetic stimulation. Control group included 20 male cases and 14 female cases that were 58-76 years old; observation group included 19 male cases and 15 female cases that were 57-74 years old. Two groups of patients were not statistically different in gender and age distribution difference (P>0.05), and the research was approved by the hospital ethics committee.

2.2. Research methods

2.2.1 Therapy

Control group of patients received conventional treatment for ischemic poststroke depression, including improving microcirculation, nourishing brain cells, etc. Observation group, at the same time of conventional treatment, received low-frequency repetitive transcranial magnetic stimulation, specifically as follows: magnetic stimulator (CCY-1 magnetic stimulator from Wuhan Yiruide Company) was used to stimulate patients' left frontal lobe, with 30 s stimulus was as a sequence, frequency was 0.5 Hz, pulse duration was 100 μs, and stimulation intensity was 0.72 T. One sequence of stimulation was completed every day, 5 d was a course of treatment, treatment was stopped for 3 d after one course was completed, and a total of four courses of treatment were completed.

2.2.2 Blood sample preparation methods

Immediately after admission and after 4 courses of treatment, 1.5 mL fasting cubital venous blood was extracted from two groups of patients, joined by anticoagulant, then let stand at room temperature for 24 h and centrifuged at low speed to take supernatant and freeze it in medical cryogenic refrigerator for test.

2.2.3 Observation index detection methods

(1) Monoamine neurotransmitters: high performance liquid chromatography (HPLC) method was used to determine serum norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (DA) contents. (2) Amino acid neurotransmitters: high performance liquid chromatography was used to determine serum glutamic acid (Glu), aspartate (Asp), glycine (Gly) and γ-aminobutyric acid (GABA) contents. (3) Neurological function indexes: ELISA kits were used to determine the serum brain-derived neurotrophic factor (BDNF) and neuron-specific enolase (NSE) levels. (4) Inflammatory markers: ELISA kits were used to determine serum interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) contents.

2.3 Statistical methods

Personnel (1-2) with professional statistical knowledge were selected to record and calculate the data obtained in the study, measurement data was in terms of (mean ± SD), comparison between groups was by grouping t test and P<0.05 was set as the standard of statistical significance in differences.

3. Results

3.1. Monoamine neurotransmitters

Before and after treatment, comparison of serum monoamine neurotransmitters NE (ng/L), 5-HT (μg/L) and DA (μg/L) levels between two groups of patients was as follows: immediately after admission, the differences in serum NE, 5-HT and DA levels were not statistically significant between the two groups of patients (P>0.05); after 4 courses of treatment, serum NE, 5-HT and DA levels of both groups were higher than those immediately after admission (P<0.05). After 4 courses of treatment, serum NE, 5-HT and DA levels of observation group were higher than those of control group (P<0.05), shown in Table 1.

Table 1.

Comparison of serum monoamine neurotransmitter levels before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>NE</th>
<th>5-HT</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>34</td>
<td>Immediately after admission</td>
<td>30.28±4.12</td>
<td>219.26±25.77</td>
<td>120.37±15.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 4 courses of treatment</td>
<td>61.09±6.57*</td>
<td>327.45±38.66*</td>
<td>192.63±25.82*</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>Immediately after admission</td>
<td>30.41±3.99</td>
<td>218.74±27.49</td>
<td>121.16±16.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 4 courses of treatment</td>
<td>48.73±5.62*</td>
<td>269.36±30.84*</td>
<td>157.88±19.64*</td>
</tr>
</tbody>
</table>

Note: compared with same group immediately after admission, *P<0.05; compared with control group after 4 courses of treatment, †P<0.05.
3.2. Amino acid neurotransmitters

Before and after treatment, comparison of serum amino acid neurotransmitters Glu, Asp, Gly and GABA levels between two groups of patients was as follows: immediately after admission, the differences in serum Glu, Asp, Gly and GABA levels were not statistically significant between the two groups of patients \( (P>0.05) \); after 4 courses of treatment, serum Glu and Asp levels of both groups were lower than those immediately after admission while Gly and GABA levels were higher than those immediately after admission \( (P<0.05) \). After 4 courses of treatment, serum Glu and Asp levels of observation group were lower than those of control group while Gly and GABA levels were higher than those of control group \( (P<0.05) \), shown in Table 2.

3.3. Neurological function indexes

Before and after treatment, comparison of serum neurological function indexes BDNF (ng/mL) and NSE (g/L) levels between two groups of patients was as follows: immediately after admission, the differences in serum Glu, Asp, Gly and GABA levels were not statistically significant between the two groups of patients \( (P>0.05) \); after 4 courses of treatment, serum BDNF levels of both groups were higher than those immediately after admission \( (P<0.05) \). After 4 courses of treatment, serum BDNF levels of observation group was higher than that of control group \( (P<0.05) \), shown in Table 3.

3.4. Inflammatory cytokines

Before and after treatment, comparison of serum inflammatory cytokines IL-1\( \beta \), IL-2, IL-6 and TNF-\( \alpha \) levels between two groups of patients was as follows: immediately after admission, the differences in serum IL-1\( \beta \), IL-2, IL-6 and TNF-\( \alpha \) levels were not statistically significant between the two groups of patients \( (P>0.05) \); after 4 courses of treatment, serum IL-1\( \beta \), IL-2, IL-6 and TNF-\( \alpha \) levels both groups were lower than those immediately after admission \( (P<0.05) \). After 4 courses of treatment, serum IL-1\( \beta \), IL-2, IL-6 and TNF-\( \alpha \) levels of observation group were lower than those of control group \( (P<0.05) \), shown in Table 4.

4. Discussion

The incidence of depression after ischemic stroke treatment is up to 30%, this is associated with brain injury, individual mental change and so on, and with the disease progression, patients’ independent living ability and quality of life both reduce significantly[4]. The curative effect of neurotrophy and other conventional therapies is limited for patients with poststroke depression, and the low-frequency repetitive transcranial magnetic stimulation, as a new therapy for secondary depression/primary depression, has received extensive attention at present. Certain intensity of time-varying magnetic field can excite brain tissue and make it produce induced
current, and low-frequency repetitive transcranial magnetic stimulation can promote sensorimotor synaptic function, promote the purposeful motion reflex arc and actively improve the intracerebral physiological process in patients with depression\cite{12,13}. The macro effect of low-frequency repetitive transcranial magnetic stimulation treatment of various depressive diseases has been confirmed, but the resulting serological index change and related mechanism are less covered at present, and the microscopic effect and possible mechanism of the therapy was elaborated in the study from serum neurotransmitters, neurological function indexes and inflammatory factors.

Stroke lesions directly damage neurons and lead to the reduced secretion of monoamine neurotransmitters such as NE, 5-HT and DA, and after their levels reduce to a certain degree, the patients can show tension, anxiety, fear, depression and other negative emotions\cite{17,18}. In the study, monoamine neurotransmitter levels were compared between the two groups of patients, and it was found that serum NE, 5-HT and DA levels of observation group after treatment were higher than those of control group, showing that the low-frequency repetitive transcranial magnetic stimulation can promote the monoamine neurotransmitter levels in body fluids, raise patients’ spirits and improve the symptoms of depression. Many studies have shown that there is excitatory/inhibitory amino acid expression imbalance around the lesions in patients with stroke, it is mainly characterized by the increased excitatory amino acids Glu and Asp levels, and the decreased inhibitory amino acids Gly and GABA levels, and the imbalance degree is consistent with brain injury degree and is also one of the main causes of depression after treatment\cite{9,10}. It was found in the study that serum Glu and Asp levels of observation group were lower than those of control group while Gly and GABA levels were higher than those of control group, showing that the low-frequency repetitive transcranial magnetic stimulation can also adjust the balance of amino acid neurotransmitter expression, and further promote the recovery of brain function.

Neurologic injury is the main consequence of the expression imbalance of a variety of neurotransmitters, and also the direct cause of depression after brain injury treatment, patients’ emotional center may be affected after severe brain injury, and meanwhile, all kinds of body language damage results in the decrease of patient’s independent living ability and quality of life, and further induces the subjective perception of depression\cite{11}. BNDF can nourish neurons and prompt the damaged synaptic regeneration, its content is negative correlated with brain injury, and studies have shown that BNDF is intrinsically related to depression\cite{12,13}. NSE specifically exists in neurons under physiological condition, it is released after neuron injury and cell membrane damage, then enters into the cerebrospinal fluid, further enters into the circulating blood through the damaged blood brain barrier and is detected, so the NSE levels in peripheral blood are positively correlated with the degree of brain injury and depression\cite{14}. In the study, nerve function index levels were compared between two groups of patients, and it was found that serum BDNF level of observation group after treatment was higher than that of control group while NSE level was lower than that of control group, further illustrating that the low-frequency repetitive transcranial magnetic stimulation can optimize neural function in patients with ischemic poststroke depression, and is expected to ease patients’ depression.

The pathogenesis of poststroke depression is not clear, but many studies believe that it is closely related to the imbalance of inflammatory factor expression\cite{15}. The study of NIU Hailing\cite{16} shows that serum inflammatory factors IL-1 and IL-6 levels in patients with poststroke depression are higher than those in cerebral infarction patients without depression, explaining that the high expression of these inflammatory factors is involved in the occurrence of poststroke depression. Nerve - endocrine - immune system is of great significance in maintaining the body homeostasis, brain and immune system balance is broken after the occurrence of stroke, massive immune cell activation can induce inflammatory infections, and some scholars think that the immune system in patients with mild-to-moderate depression may be suppressed\cite{17,18}. In the study, serum inflammatory factor levels were compared between two groups of patients, and it was found that serum IL-1, IL-2, IL-6 and TNF-α levels of observation group after treatment were lower than those of control group, showing that the low-frequency repetitive transcranial magnetic stimulation can reduce the patient’s systemic inflammatory reaction, it may be because that it optimizes patients’ immune status, and this is one of the fundamental mechanisms of the therapy to relieve ischemic poststroke depression.

To sum up, it is concluded that low-frequency repetitive transcranial magnetic stimulation can equalize the secretion of serum monoamine neurotransmitters and amino acid neurotransmitters in patients with ischemic PSD, optimize neural function and reduce systemic inflammatory response, and it is expected to become the reliable way for long-term PSD treatment. In this paper, the number of included patients is limited, it may cause a certain error to the research results, and subsequent study on large sample is to be conducted to further confirm the clinical application of the technology.

**References**

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