



# Effects of donepezil combined with folic acid and vitamin B12 on serum levels of inflammatory factors, HCY, NSE and neurotransmitters in elderly patients with Alzheimer's disease complicated with hyperhomocysteinemia

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## ABSTRACT

**Objective:** To investigate the effects of donepezil combined with folic acid and vitamin B12 on the levels of serum inflammatory factors, Hcy, NSE and neurotransmitters in elderly patients with Alzheimer's disease (AD) complicated with hyperhomocysteinemia. **Methods:** A total of 98 elderly patients with AD complicated with hyperhomocysteinemia were randomly divided into control group ( $n=48$ ) and observation group ( $n=50$ ) according to the random data table method. Patients in the control group were treated with donepezil. On this basis, the patients in the observation group were treated with folic acid and vitamin B12, all patients were treated for 3 months. Before and after treatment, the levels of serum inflammatory factors (TNF- $\alpha$ , IL-6 and hs-CRP), Hcy, NSE and brain neurotransmitter (5-HT, NE and DA) were compared between the two groups. **Results:** Before treatment, the levels of TNF- $\alpha$ , IL-6, hs-CRP, Hcy, NSE, 5-HT, NE and DA of the control group and the observation group were not statistically significant. Compared with the group before treatment, the levels of TNF- $\alpha$ , IL-6, hs-CRP, Hcy and NSE in the two groups were significantly lower, and the level of the observation group was significantly lower than those of the control group, the difference was statistically significant; Compared with the group before treatment, the levels of 5-HT, NE and DA in the two groups were significantly increased, and the observation group was significantly higher than that of the control group, the difference was statistically significant. **Conclusion:** Donepezil combined folic acid and vitamin B12 in treatment of AD with hyperhomocysteinemia, which can effectively reduce the body's inflammatory response, reduced Hcy and NSE levels, elevated levels of brain neurotransmitters, has important clinical significance.

## 1. Introduction

Alzheimer's disease (AD) is a common clinical, primary and degenerative nervous system diseases in elderly patients. The main

clinical manifestations are progressive memory loss and cognitive dysfunction[1]. In recent years, the morbidity of AD is rising, which seriously threatens the life quality and health of patients and brings heavy burden to society. Related studies have found that Hyperhomocysteinemia (Hhcy) is relevant to degenerative disease in nervous system, besides cardiovascular and cerebrovascular disease, atherosclerosis and so on, and also involved in the occurrence and development of AD[2-4]. Donepezil is the first and specific drug for the treatment of AD, folic acid and vitamin B12 supplement can delay the further deterioration of AD disease[5]. This study aims to investigate the clinical efficacy of donepezil combined with folic acid and vitamin B12.

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## 2. Research objects and methods

### 2.1 General information

A total of 98 cases of patients with AD complicated with Hcy from Feb. 2016 to Mar. 2017 in our hospital were selected as research objects. All the selected patients satisfied the selection criteria established in this study. The patients were randomly divided into two groups, control group ( $n=48$ ) and observation group ( $n=50$ ). In the control group (48 cases), there were 33 males and 15 females; with ages of 60-76 years; duration of 1-3 years; the mean duration of  $(1.37\pm 0.35)$  years; the average Hamilton Depression (HAMD) scale of  $(12.08\pm 3.14)$ ; the average Mini Mental State examination (MMSE) score of  $(13.88\pm 1.50)$ . In the observation group, there were 34 males and 16 females; with ages of 60-77 years; duration of 1-3 years; the mean duration of  $(1.39\pm 0.42)$  d; the average HAMD scale of  $(12.23\pm 2.95)$ ; the average MMSE score of  $(13.72\pm 1.64)$ . The sex, age, duration, average HAMD scale, and MMSE score of two groups were similar ( $P>0.05$ ). The research content and process of this study complied with the criteria of the hospital ethics committee, and get permission to conduct.

### 2.2 standards of selection

Inclusion criteria: (1) All the patients were in line with relevant diagnostic criteria of AD, diagnosed by clinical manifestation, as well as biochemical and radiological examination[6]; (2) Hcy $>15$   $\mu\text{mol/L}$ , over the age of 65; (3) With the clinical manifestations of significant cognitive decline, memory loss, emotional indifference, etc; (4) Clinical data integrity, all patients and their families were informed consent and signed consent forms, access to treatment voluntarily.

Exclusion criteria: (1) Excluding delirium, vascular dementia, depression and other mental illness; (2) Accompanied by severe organic disease, coronary heart disease, cerebral thrombosis, liver and kidney dysfunction and other diseases; (3) Accompanied by other diseases which can affect cognitive dysfunction; (4) Severe aphasia, disturbance of consciousness; (5) Patients with incomplete clinical data after admission; (6) Do not accept the study of treatments.

### 2.3 Therapeutic method

Both groups keep controlling the diet, adequate sleep and proper activity. Based on this, the patients of control group were treated by donepezil hydrochloride tablets (Eisai, Manufacturer: Eisai Co Ltd., byua powerfulco H20070181), 1 time/d; 10 mg/time. The patients of observation group were treated by folic acid tablets (Tianjin Lisheng Pharmaceutical Co., Ltd., byua powerfulco H12020215, specification: 5 mg 100 s), 10 mg/d, 1 time/d; Vitamin B12 treatment (Manufacturer: Shanghai Xinyi Jiufu Pharmaceutical Co., Ltd., byua powerfulco H31023025, specification: 50  $\mu\text{g}$ ), 0.5 mg/d, 1 time/d; oral administration of donepezil hydrochloride tablets at the same time, the specific method was the same as control group, two groups of patients were treated for 3 months.

### 2.4 Observational indexes

5 mL of fasting peripheral venous blood of two group patients were collected before treatment and after 3 months treatment, and divided into two tubes. For one of the tubes, blood serum was collected by centrifugation and stored at  $-80$   $^{\circ}\text{C}$  for further test. The main outcome measures were serum inflammatory factors, homocysteine (Hcy) and neuron-specific enolase (NSE) levels. Serum inflammatory cytokines include tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP). The levels of TNF- $\alpha$ , IL-6, Hcy and NSE were detected by ELISA, using the corresponding ELISA kit (purchased from Shanghai enzyme biotechnology Co., Ltd.). Hs-CRP was detected by particles enhance transmission immunoturbidimetry assay (PETIA). The kit was provided by Shanghai Xinyu Biotechnology Co., Ltd. Another tube was used to test the brain neurotransmitter index of plasma, which includes serotonin (5-HT), norepinephrine (NE) and dopamine (DA) via radioimmunoassay. The kit was provided by Shanghai Yinmian Biotechnology Co., Ltd., and the specific operation in strict accordance with the kit instructions.

### 2.5 Statistical analysis

SPSS 17.0 statistical package was conducted for statistical analysis. The level of each indicator in this study was in line with the normal distributions, which were described as (Mean  $\pm$  SD). t test was conducted to group comparison as well as between comparison, values of  $P<0.05$  were considered to be statistically significant.

## 3. Results

### 3.1 Changes of inflammatory cytokines levels before and after treatment

The changes of TNF- $\alpha$ , IL-6, and hs-CRP levels in two groups before and after treatment are shown in Table 1. Before treatment, TNF- $\alpha$ , IL-6, and hs-CRP levels in two groups were close and not statistically significant ( $P>0.05$ ). After treatment, TNF- $\alpha$ , IL-6, and hs-CRP levels in control group were  $(100.47\pm 10.95)$  ng/L,  $(154.09\pm 59.77)$  ng/L and  $(8.16\pm 3.39)$  mg/L, respectively, which were significantly decreased compared with that before treatment, it was considered to be statistically significant ( $P<0.05$ ). After treatment, TNF- $\alpha$ , IL-6, and hs-CRP levels in observation group were  $(88.42\pm 7.04)$  ng/L,  $(74.99\pm 32.08)$  ng/L and  $(5.32\pm 2.42)$  mg/L, respectively, significantly decreased and lower than that before treatment, as well as lower than that in control group, which was considered to be statistically significant ( $P<0.05$ ).

Table 1.

Changes of inflammatory factors levels before and after treatment.

| Groups            | n  | Time             | TNF- $\alpha$ (ng/L) | IL-6 (ng/L)         | hs-CRP (mg/L)     |
|-------------------|----|------------------|----------------------|---------------------|-------------------|
| Control group     | 48 | Before treatment | 126.25 $\pm$ 13.38   | 230.47 $\pm$ 120.59 | 14.12 $\pm$ 4.05  |
|                   |    | After treatment  | 100.47 $\pm$ 10.95*  | 154.09 $\pm$ 59.77* | 8.16 $\pm$ 3.39*  |
| Observation group | 50 | Before treatment | 125.96 $\pm$ 13.55   | 241.69 $\pm$ 124.63 | 14.28 $\pm$ 4.19  |
|                   |    | After treatment  | 88.42 $\pm$ 7.04*#   | 74.99 $\pm$ 32.08*# | 5.32 $\pm$ 2.42*# |

Note: compared with group before treatment, \* $P$ <0.05; compared with group after treatment, # $P$ <0.05.

Table 2.

Changes of Hcy and NSE levels in serum before and after treatment.

| Groups            | n  | Time             | Hcy ( $\mu$ mol/L) | NSE (U/mL)        |
|-------------------|----|------------------|--------------------|-------------------|
| Control group     | 48 | Before treatment | 25.52 $\pm$ 3.02   | 14.67 $\pm$ 2.63  |
|                   |    | After treatment  | 18.91 $\pm$ 3.43*  | 10.72 $\pm$ 1.87* |
| Observation group | 50 | Before treatment | 25.81 $\pm$ 4.17   | 14.58 $\pm$ 2.49  |
|                   |    | After treatment  | 12.18 $\pm$ 2.62*# | 8.17 $\pm$ 1.56*# |

Note: compared with group before treatment, \* $P$ <0.05; compared with group after treatment, # $P$ <0.05.

Table 3.

Change of brain neurotransmitter levels in plasma before and after treatment.

| Groups            | n  | Time             | 5-HT (ng/L)         | NE (ng/L)            | DA (ng/mL)           |
|-------------------|----|------------------|---------------------|----------------------|----------------------|
| Control group     | 48 | Before treatment | 58.49 $\pm$ 15.47   | 84.31 $\pm$ 11.08    | 93.74 $\pm$ 19.56    |
|                   |    | After treatment  | 74.62 $\pm$ 10.28*  | 117.63 $\pm$ 16.78*  | 116.27 $\pm$ 13.18*  |
| Observation group | 50 | Before treatment | 58.51 $\pm$ 16.46   | 84.36 $\pm$ 11.54    | 93.87 $\pm$ 19.54    |
|                   |    | After treatment  | 92.75 $\pm$ 11.62*# | 134.92 $\pm$ 17.06*# | 142.76 $\pm$ 15.27*# |

Note: compared with group before treatment, \* $P$ <0.05; compared with group after treatment, # $P$ <0.05.

### 3.2 Changes of Hcy and NSE levels before and after treatment

The test results of Hcy and NSE levels in two groups before and after treatment are shown in Table 2. Before treatment, there was no significant difference in Hcy and NSE levels between the two groups ( $P$ >0.05). After treatment, Hcy and NSE levels in control group were (18.91 $\pm$ 3.43)  $\mu$ mol/L and (10.72 $\pm$ 1.87) U/mL, respectively, which were significantly decreased compared with that before treatment, it was considered to be statistically significant ( $P$ <0.05). After treatment, Hcy and NSE levels in observation group were (12.18 $\pm$ 2.62)  $\mu$ mol/L and (8.17 $\pm$ 1.56) U/mL, respectively, significantly decreased and lower than that before treatment, as well as lower than that in control group, which was considered to be statistically significant ( $P$ <0.05).

### 3.3 Changes of plasma brain neurotransmitter levels before and after treatment

There was no significant difference in 5-HT, NE and DA levels in two groups before treatment. After treatment, 5-HT, NE and DA levels in control group and observation group were (74.62 $\pm$ 10.28) ng/L, (117.63 $\pm$ 16.78) ng/L, (116.27 $\pm$ 13.18) ng/mL, (92.75 $\pm$ 11.62) ng/L, (134.92 $\pm$ 17.06) ng/L and (142.76 $\pm$ 15.27) ng/mL, respectively. The levels of them were significantly increased compared with that before treatment, and the levels in observation group were higher than that in control group, the difference was statistically significant ( $P$ <0.05). As shown in Table 3

## 4. Discussion

In recent years, epidemiological studies have shown a significant decrease in mortality rate caused by heart disease and acquired immunodeficiency syndrome, but a 68% increase in AD-related mortality. AD has become the fourth fatal disease after cancer, stroke and heart[7,8], it is also a major cause of disability and dependency in the elderly[9]. The main pathological changes of AD are senile plaques and nerve fiber tangles, the main pathogenesis is the decline of cholinergic neuron level in central nervous system[10]. Foreign related researches pointed out that Hhcy is the independent risk factor of cognitive dysfunction and AD. General, There always shows a high Hcy level in patients with AD, and also, Hcy level is significantly associated with the risk of AD[11,12]. Cholinesterase inhibitor is the first approved drug with special effect for the treatment of AD, it can reverse the pathological process in varying degrees and delay the development of AD. Donepezil belongs to a relatively specific acetylcholinease inhibitor, which is the second approved drug for the treatment of AD. The therapeutic effect of donepezil is mainly inhibit the degradation process of acetylcholine, improve memory nerve conduction, so as to improve the memory function of patients[13,14]. At present, a large number of studies have confirmed that supplementation of folic acid and vitamin B12 can effectively reduce the level of Hcy, which is an important measure for the treatment and prevention of Hhcy[15,16]. This study aim to investigate the effects of donepezil combined with folic acid and vitamin B12 for AD with Hhcy treatment, by exploring the levels of inflammatory factors, Hcy, NSE, and brain neurotransmitters.)

In recent years, a large number of studies have shown that there are significant inflammatory stress and obvious high level of serum

TNF- $\alpha$ , IL-6 and other factors in AD patients, but the pathogenesis are still unclear. However, some researchers have confirmed that high levels of inflammatory response can lead to the degeneration of cognitive function-related neuronal as well as the damage of cholinergic nerve path, which can further aggravate the deterioration of the disease[17,18]. Therefore, levels of serum inflammatory cytokines have great value in evaluating the efficacy and prognosis for the treatment of AD with Hcy patients. The results of this study showed that both regimens were effective in reducing serum TNF- $\alpha$ , IL-6, and hs-CRP levels in patients, and the inhibitory effect of combined folic acid and vitamin B12 on inflammatory factors was more significant. The results were consistent with previous reports that folic acid and vitamin B12 supplementation can reduce the patient's inflammatory response[19], but the specific mechanism remains to be further studied.)

In addition to inflammatory response, there are still Hcy, NSE and brain neurotransmitter levels abnormalities in AD with Hcy patients. Hcy is the intermediate product of methionine metabolism, and the high level of Hcy is closely related to cognitive function and cardiovascular and cerebrovascular diseases[20]. It is ultimately lead to cognitive dysfunction mainly through the regulation of peripheral blood mononuclear cells secrete MCP-1 and inflammatory factors, reduce oxidative stress and antioxidant stress activity, damage vascular endothelium and blood-brain barrier integrity[21]. Vascular dementia and AD patients have high NSE level. Under normal circumstances, the level of NSE in blood is very low, NSE is mainly spread over neuron and cytoplasm of neuroendocrine cells, when nerve cells damaged, NSE substantial into blood circulation, leading to an elevated level of it, which is an important indicator of brain injury assessment[22]. 5-HT, NE and DA are important neurotransmitter markers, and the level of 5-HT, NE and DA in AD patients is decreased, the change of their levels is considered to be an important basis for assessing the therapeutic effect of drugs[23]. This study found that the two groups of Hcy, NSE levels were significantly reduced and neurotransmitter index levels were significantly increased after treatment, the difference was statistically significant compared two groups after treatment. The results of this study show that donepezil is an effective drug for the treatment of AD with Hcy, the specific mechanism may be that donepezil is related to the reduction of Hcy and NSE levels, as well as the recovery of neurotransmitters and so on. Basis on this, supplemented with folic acid and vitamin B12 can further promote the transformation of hcy in vivo, reduce hcy level, down-regulate the level of NSE, and increase neurotransmitter markers. The main reasons may be that folic acid and vitamin B12 treatment can effectively inhibit the apoptosis of nerve cells, enhance the activity of free radical scavenging enzyme, reduce the body's lipid peroxidation, so as to reduce the brain injury.

In summary, donepezil monotherapy as well as combined with folic acid and vitamin B12 are effective treatment of AD with Hcy, which can effectively reduce the inflammatory response reduce Hcy and NSE levels, elevate brain neurotransmitter index levels. However, combination therapy showed better efficacy on the above indicators, which is significant for the prognosis of AD with Hcy.

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