Influence of folic acid and vitamin B12 combined therapy on plasma Hcy, inflammatory factor levels and blood vessels endothelial function in patients with vascular dementia and type H hypertension

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ARTICLE INFO

Objective: To investigate the influence of folic acid and vitamin B12 combined therapy on plasma homocysteine (Hcy) level, blood vessels endothelial function and inflammatory factors in patients with vascular dementia and type H hypertension. Methods: 100 cases of patients with vascular dementia and type H hypertension accorded with the inclusion criteria were selected as research objects. They were randomly divided as the control group and the therapeutic group, 50 cases each. For control group, Enalapril tablets were administered by mouth for treatment. For therapeutic group, folic acid and vitamin B12 treatment were provided on the basis of treatment for control group. Treatments were continued for 12 weeks. Plasma Hcy levels, inflammatory factors [(interleukin-6 (IL-6), interleukin-8 (IL-8) and hypersensitive C reaction protein (hs-CRP)], blood vessels endothelial function indexes variation in patients before and after treatment were observed and detected. Results: Plasma Hcy, IL-6, IL-8 and hs-CRP levels in two groups of patients after treatment were significantly decreased comparing with the same group before treatment, and the above index levels in therapeutic group after treatment were significantly lower than control group (P < 0.05); For comparison of blood vessels endothelial function indexes in the patients, NO levels in two groups after treatment were increased in various degrees, and endothelin-1 (ET-1) were decreased. The differences between levels of the two indexes in therapeutic group before and after treatment were significant, and levels after treatment in therapeutic group were significantly better than in control group (P < 0.05). While variations of the differences in control group before and after treatment were not significant (P > 0.05); After treatment, diastolic pressure and systolic pressure in the two groups of patients were significantly improved comparing with before treatment (P < 0.05). However, after treatment, the differences of levels between therapeutic group and control group were not significant (P > 0.05). MMSE score in therapeutic group after treatment was significantly higher than before treatment, and significantly higher than in control group (P < 0.05). Conclusions: Combined therapy of folic acid and vitamin B12 for treating vascular dementia with type H hypertension could effectively decrease plasma Hcy and inflammatory factor levels, and improve blood vessels endothelial function and dementia degree on patients. It has certain clinical value which deserves to be promoted.

1. Introduction

Vascular dementia (VD) is an acquired intelligence damage syndrome caused by cardiovascular lesion-induced cerebral dysfunction[1]. Homocysteine (Hcy) levels were closely related with vascular diseases, and cerebrovascular disease was a major dangerous factor leading to cognitive decline and dementia. Essential hypertension with high Hcy hyperlipidemia was identified as type H hypertension[2]. Appearance of dementia could be promoted by factors such as high blood pressure, high concentrated plasma Hcy, etc[3]. Therefore, reduction of pressure and plasma Hcy concentration become important measures to prevent vascular
dementia. In our research, folic acid and vitamin B12 (VB12) were provided for patients with vascular dementia and type H hypertension for supplementation, and influences of plasma Hcy levels, inflammatory factors and blood vessels endothelial function on patients were discussed, reported as follows.

2. Materials and methods

2.1. General materials

100 cases of patients treated in our hospital and diagnosed as vascular dementia with type H hypertension from Apr 2013 to Jul 2015 were selected. There were 57 male cases and 43 female cases. They were randomly divided as the control group and the therapeutic group, 50 cases each. In control group, there were 28 male cases and 22 female cases, ages were ranged from 53–72 years old, courses of disease were ranged from 1–7 years; In therapeutic group, there were 29 male cases and 21 female cases, ages were ranged from 52–76 years old, courses of disease were ranged from 2–7 years. No significant difference showed on sex ratios, age compositions and course comparisons between two groups of patients (P>0.05). Our research met medical ethical standards. And it was proved by ethic committee in our hospital. Informed consent forms were accepted and signed by all the patients and relatives. And patients all had complete materials.

2.2. Selection and exclusion standards

Selection standards: (1) All the patients were in accord with NINDS-AIREN diagnosis standards for VD [4]; (2) All the included VD patients were also diagnosed with high type H hypertension (Hcy ≥ 10 µmol/L)[5]; (3) Hypertension diagnose standards were in accord with WHO/ISH hypertension guideline 1999 edition[6]. No calcium ion antagonist, cholinesterase Inhibitor N-methyl-D-aspartic acid (NMDA) receptor antagonist or others had ever been administered during 7 d before treatment.

NINDS-AIREN diagnosis standards for VD: Patients had clinical and imaging evidence of cerebrovascular disease; Patients had 2 or more cognitive domains disorder and existed memories; Their VD were happened after brain stroke within 3 months.

Excluded standards: (1) Alzheimer’s disease; (2) Depression or secondary intellectual disability induced by other mental dysfunctions; (3) Cognitive disorder induced by head injury; (4) Dementia induced by neuropathic disease, metabolic disease, tumor, hydrocephalus, central nervous system infection, inflammation, poisoning, hypoxia, neurogenetic disease, etc.; (5) Patients who had liver, renal and cardiac functions failure[7].

2.3. Therapeutic methods

Control group: Enalapril tablet (Manufacturer: Shandong Lukangchenxin Pharmaceutical Co. Ltd., strength: 10 mg, approved number: H20083505) was utilized for treatment by mouth, started dosages were ranged from 5–10 mg, administered for one or two doses. The dose of drug could be properly increased basing on blood pressure levels. The everyday maximum dosage did not exceed 40 mg. And one course of treatment was adhered (12 weeks).

Therapeutic group: Basing on Enalapril tablet, 5 mg folic acid tablet (Manufacturer: Tianjin Lisheng pharmaceutical Limited by Share Ltd, approved number: H12020215) was added 1x/day, and 5 mg VB12 (Manufacturer: Shanghai Xinyijifu Pharmaceutical Co., Ltd., approved number: H31022370) was administered by mouth 1x/day. One course of treatment was adhered (12 weeks).

2.4. Detection indexes and methods

4–5 mL venous blood was separately extracted with empty stomach in the early mornings in patients before and after treatment, and it was centrifuged at 3 000 r/min for 10 min. Then serum was extracted and stored at -80 ℃ refrigerator, waiting for detection of inflammatory factors and blood vessels endothelial function indexes. Inflammatory factors included interleukin-6 (IL-6), interleukin-8 (IL-8) and hypersensitive C reaction protein (hs-CRP). ELISA method was used for detection, the kit was provided by Beijing Jingmei Biological Engineering Co., Ltd. Relevant manipulates were conducted strictly following kit instructions; Blood vessels endothelial function indexes: serum MO, endothelin-1 (ET-1). Nitrate reductase colorimetric method was used for NO detection. Radioimmunoassay was used for ET-1 detection. The kits were provided by Shenzhen Jingmei Biological Engineering Co., Ltd. Manipulates were conducted strictly following instructions.

5 mL venous blood was separately extracted from patients with empty stomachs, and infused to anticoagulant tubes with EDTA, shook up and centrifuged at 2 500 r/min for 5 min. Upper plasma was extracted and stored at -20 ℃ low temperature to detect Hcy level. The detection method was method of double reagent enzymatic cycles. The kit was chose from Shanghai Enzyme Linked Biological Technology Co., Ltd. Experimental manipulates were conducted strictly following kit instruction. Blood pressure values and variation of Mini-Mental State Examination (MMSE) evaluations were observed and recorded for two groups of patients before and after treatment.

2.5. Statistical analysis

Quantitative datas were indicated by average number± standard
3. Results

3.1. Variations of plasma Hcy and inflammatory factors levels in two groups of patients before and after treatment

Comparison of plasma Hcy and inflammatory factors IL-6, IL-8, hs-CRP levels between two groups of patients before treatment showed no significant difference ($P>0.05$). After treatment, Hcy, IL-6, IL-8 and hs-CRP levels in two groups were decreased in various degrees comparing with same group before treatment. Hcy levels in therapeutic group after treatment were significantly lower than before treatment, and significantly lower than control group after treatment ($P<0.05$). While in control group, comparison of Hcy levels before and after treatment showed no significant difference ($P>0.05$); After treatment, inflammatory factors IL-6, IL-8, and hs-CRP levels in two groups after treatment were significantly lower than before treatment, and levels in therapeutic group were significantly lower than control group ($P<0.05$) (Table 1).

3.2. Comparison of blood vessels endothelial function indexes in two groups of patients before and after treatment

Comparison of blood vessels endothelial function indexes NO and ET-1 levels between two groups of patients before treatment showed no significant difference ($P>0.05$). After treatment, NO and ET-1 levels in control group were not significant different, compared with same group before treatment ($P>0.05$); After treatment, NO and ET-1 levels in therapeutic group were (136.69±17.25) µmol/L and (134.17±30.92) ng/L, respectively. Compared with same group before treatment, NO levels of therapeutic group were significantly higher ($P<0.05$), while ET-1 levels were significantly decreased ($P<0.05$) (Table 2).

3.3. Variations of blood pressures and MMSE evaluations in two groups of patients before and after treatment

Comparison of DBP, SBP and MMSE evaluations between two groups of patients before treatment showed no significant difference ($P>0.05$). SBP and DBP after treatment were significantly decreased comparing with same group before treatment in two groups of patients ($P<0.05$); Comparison of DBP and SBP in two groups after treatment showed no significant difference ($P>0.05$). After treatment, MMSE evaluations in two groups of patients were higher than before treatment in various degrees. Difference of results in control group before treatment and after treatment was not significant ($P>0.05$). After treatment, MMSE evaluations in therapeutic group were significantly higher than before treatment, and significantly higher than in control group ($P<0.05$) (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapeutic time</th>
<th>Hcy (µmol/L)</th>
<th>IL-6 (ng/L)</th>
<th>IL-8 (ng/L)</th>
<th>hs-CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>19.09±3.87</td>
<td>38.95±12.04</td>
<td>73.58±9.28</td>
<td>16.83±6.12</td>
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<tr>
<td></td>
<td>After treatment</td>
<td>18.77±4.47</td>
<td>27.82±9.87</td>
<td>65.43±5.25</td>
<td>12.96±4.41</td>
</tr>
<tr>
<td>Therapeutic group</td>
<td>Before treatment</td>
<td>18.99±3.72</td>
<td>39.22±12.46</td>
<td>74.29±8.86</td>
<td>17.16±4.34</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>11.72±2.96*</td>
<td>20.45±10.8</td>
<td>55.27±5.39*</td>
<td>10.52±3.75*</td>
</tr>
</tbody>
</table>

* indicated that $P<0.05$ compared with same group before treatment; * indicated that $P<0.05$ compared with control group.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapeutic time</th>
<th>DBP (mmHg)</th>
<th>SBP (mmHg)</th>
<th>MMSE evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>77.5±6.1</td>
<td>158.3±11.2</td>
<td>17.06±2.59</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>71.1±6.3*</td>
<td>136.4±10.8</td>
<td>17.62±2.38</td>
</tr>
<tr>
<td>Therapeutic group</td>
<td>Before treatment</td>
<td>78.4±7.1</td>
<td>157.9±8.9</td>
<td>17.01±2.94</td>
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<tr>
<td></td>
<td>After treatment</td>
<td>71.2±6.4*</td>
<td>133.7±10.7</td>
<td>20.32±3.25*</td>
</tr>
</tbody>
</table>

* indicated that $P<0.05$ compared with same group before treatment; * indicated that $P<0.05$ compared with control group.
4. Discussion

In recent years, with the acceleration of population aging, incidence rate of VD has been also increased. According to incomplete statistics, amount of VD patients represented 10%–20% of dementia patients in Europe and America. While in Asia, the incidence of VD was higher. In Japan, amount of senile dementia patients represented 50% of VD patients. Especially for senile patients older than 65 years old, the incidence of dementia was 5%, and about 20% of these senile patients were VD patients. VD seriously affects life quality of patients, and causes huge economic and nursing burdens for families and society[8-10]. Risk factors of vascular dementia include age, gender, place, education, environment, abnormal blood lipid metabolism, hypertension, hyperglycemia, hyperhomocysteinemia, hyperuricemia and so on. Patients with type H hypertension have the above two risk factors[11]. Researches showed that VD patients had higher levels of Hcy than patients with other senile mental diseases, and folic acid, B-group Vitamins like VB12 and VB6 were closely related with Hcy levels[12]. Decompression and reduction of plasma Hcy concentration become important measures to prevent and treat vascular dementia with type H hypertension. In our research, patients who had vascular dementia with type H hypertension were provided folic acid and VB12 suppletions to investigate their influence on plasma Hcy levels, inflammatory factors and blood vessels endothelial function of patients.

Hcy is an intermediate product generated in process of Methionine metabolism. It is synthesized by liver and kidney. Researches of epidemiology indicated that increase of Hcy in heart and brain vessels and peripheral vessels were closely related with vascular diseases. People who had higher concentration of plasma Hcy showed higher incidence rates of cerebral apoplexy and myocardial infarction than people who had normal concentration of Hcy. Meanwhile, researches pointed out high concentration of plasma Hcy was also important risk factor on appearance of dementia. Patients with type H hypertension could more easily experience damage of cognitive function, and damage degree was positively correlated with plasma Hcy levels, its action mechanism might be injury of vessel endothelial cells, promotion of vascular smooth muscle cells generation and acceleration of appearance and development of vessel walls immune inflammatory reactions[13]. In addition, researches also confirmed that high concentration of plasma Hcy had close relationship with decrease of cognitive function and appearance of dementia. Patients with type H hypertension could more easily experience damage of cognitive function, and damage degree was positively correlated with plasma Hcy levels, its action mechanism might be injury of vessel endothelial cells, promotion of vascular smooth muscle cells generation and acceleration of appearance and development of vessel walls immune inflammatory reactions[14]. The main reasons for inducing the increase of Hcy concentration are: (1) Nutritional factors, for instance, diets contain rich methionine; (2) Decomposition of Hcy was blocked since lack of folic acid, VB12 and VB6; (3) Activation of heterozygous or homozygous Cystathionine B-synthase (CBS); (4) Kidney cleared obstacles for Hcy[15,16]. Hcy could induce peripheral blood mononuclear cells to secrete MCP-1 and inflammatory factor (IL-8) by regulating reduced enzyme II (NADPH) to promote production of oxygen free radicals, and increase single peripheral blood mononuclear cell cultured in vitro to release IL-6. IL-8 is a cytokine with divide function. Increasing level of it could induce infiltration of cerebral parenchymal white blood cells and lipid peroxidation, cause injury of neurons, thus to induce cognitive dysfunction. Researches already verified that serum inflammatory factors IL-6 and IL-8 were also involved in progression of vascular dementia besides plasma Hcy[17]. Relevant researches in recent years indicated that folic acid and VB12 supplementation could effectively diminish and control Hcy levels[18]. Results of our research showed that plasma Hcy, inflammatory factors IL-6, IL-8 and hs-CRP levels in patients after folic acid and VB12 treatment were significantly decreased comparing with prior treatment. After treatment, improvement of each index level in therapeutic group was obviously better than in control group. Results were consistent with relevant reports composed by predecessors[19,20]. Results further proved that folic acid and VB12 could effectively regulate plasma Hcy and inflammatory factors levels of patients.

High concentration of Hcy could damage endothelial functions of capacitance and resistance vessels, and diminish generation of vasodilation factors derived from endothelium, especially for behaviors of patients with senile hypertension and high Hcy hyperlipidemia[21]. NO is a main vasodilatation factor. It could suppress platelet aggregation, sustain vessel vasodilation status and suppress proliferation of smooth muscle cells, etc. ET-1 is a kind of contractor peptide secreted by vessel endothelial cells which could contract vessels and promote proliferation of cells. Imbalance of the two factors levels would lead to imbalance of angiotsis regulation, and further induce endothelial dysfunctions[22]. Researches indicated that after combined adjuvant therapy of folic acid and VB12 on treating patients with senile hypertension, dysfunction of brachial flow-mediated dilatation (FMD) before and after reactive hyperemia could be improved, and body NO and ET-1 levels could be improved[23]. Results of our research showed that after folic acid and VB12 suppletion, NO levels in patients of therapeutic group were significantly increased. ET-1 levels were significantly decreased. Differences showed statistical significance on the two levels comparing with control group. Diastolic and systolic pressure after treatment were significantly lower than before treatment. MMSE evaluation were obviously better than before treatment after folic acid and VB12 suppletion provided, and were significantly better than control group. Results of our research are consistent with researches processed by predecessors[24,25], which indicated that folic acid and VB12 combination could effectively improve endothelial function of patients, diminish mean systematic
arterial blood pressure to keep it on a normal level, thus to effectively improve degree of dementia for patients.

Above all, combined therapy of folic acid and VB12 for treating vascular dementia with type H hypertension could effectively decrease plasma Hcy and inflammatory factor levels, improve blood vessels endothelial function, diminish mean systematic arterial blood pressure and effectively improve dementia degree on patients. It has certain clinical value which deserves to be promoted.

References


