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Original Article

Serum homocysteine, folic acid and $VitB_{12}$ in patients with mild cognitive impairment

Bo Jiang, Guo-En Yao, Cheng-Yun Ding, Cun-Shan Yao, Jun-Ling Ge, En-Chao Qiu, Yue-Qi Guo, Yun-Xia Wang

Department of Neurology, The First Hospital Affiliated to the Chinese PLA General Hospital, Beijing 100048, China

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Abstract: Objective: This study aims to investigate the levels of serum homocysteine (Hcy), folic acid and $VitB_{12}$, as well as their correlations with cognitive function in patients with mild cognitive impairment (MCI). Methods: The levels of serum homocysteine, folic acid and $VitB_{12}$ in 128 patients with MCI were retrospectively studied, and compared with 112 healthy controls. The Montreal cognitive assessment (MoCA) scale was used to assess intelligence status, and the event-related potential of P300 examinations were performed. Results: The level of Hcy in the MCI group was higher than in the healthy control group. Folic acid level was lower than in the healthy control group, and there was no significant difference in $VitB_{12}$ level between these two groups. The latency of P300 in the MCI group was significantly longer than in the healthy control group. In the MCI groups, MoCA score in the moderately elevated Hcy group was lower than that in the normal Hcy group; and the latency of P300 was prolonged. There was no significant difference in MoCA score and P300 latency between the mildly elevated Hcy group and normal Hcy group. MoCA score was negatively correlated with the level of serum Hcy and P300 latency. Conclusion: The level of Hcy in patients with MCI was increased, and there may be correlations between the increase in Hcy level and cognitive impairments.

Keywords: Cognitive impairment, neuropsychology, P300, homocysteine, folic acid, $VitB_{12}$

Introduction

Mild cognitive impairment (MCI) is a mild memory or cognitive impairment, which is not in conformity with educational level and age, and does not affect the ability of daily life. It is a transitional stage between normal aging and Alzheimer's disease (AD) [1]. The present study revealed that the incidence rate of MCI in a population of over 65 years old is 19% [2], in which 46% of MCI patients will convert into dementia within three years. However, dementia conversion rate is only 3% in the normal population of the same age [3]. Hence, it is of important clinical significance to identify factors associated with the onset of MCI and intervene in the early stage. Homocysteine (Hcy) is a kind of amino acid containing sulfhydryl group generated in the metabolism of methionine, and is related to many nervous system diseases [4]. At present, research results on the relationship between Hcy and cognitive function are different. Some scholars believed that there are clear correlations between these two [5, 6], while other scholars believe that there are no correlations between these two [7]. The purpose of this study was to observe the serum levels of Hcy, folic acid and vitamin B12 ($VitB_{12}$), and explore their correlations with cognitive function in MCI patients; in order to provide evidence for clinical intervention.

Materials and methods

General materials

In this study, all the MCI patients were amnestic MCI (aMCI), who visited the Neurology Department of the First Affiliated Hospital of PLA General Hospital between the period of June 2012 and June 2015. A total of 133 cases of MCI were screened. Among these cases, five cases were excluded. Finally, 128 cases of MCI patients were enrolled; in which 55 cases were male and 73 cases were female. Patient's age ranged from 49 to 77 years old, with an average age of 63±6.21 years old. Educational levels were 5-16 years. All patients underwent the fol-

Table 1. Comparison of MCI group and control group

Groups	Age	Years of education	Male	Hypertension	Diabetes	Hyperlipidemia
MCI	64±7.51	8±3.12	55	31	25	29
Control	63±8.46	9±4.10	43	24	17	21
t/x^2	0.581	0.987	0.518	0.263	0.784	0.553
P	0.56	0.33	>0.05	>0.05	>0.05	>0.05

lowing neuropsychological scale tests: Montreal Cognitive assessment (MoCA) scale, assessment of activity of daily living (ADL) and clinical dementia rating (CDR) scale, Hamilton Rating Scale for depression and anxiety, and Hachinski ischemic index scale.

Diagnostic criteria of patients with aMCI [8] according to the Petersen standard [9] and the diagnostic criteria proposed by the international MCI working group in 2004 are listed as follows: (1) patients who have complained of subjective memory impairment, and (or) loss of memory confirmed by the families of these patients; (2) patients who have objective memory loss in neuropsychological tests, and an Hachinski ischemic index of <4 points; (3) activity of daily living (ADL) of the patients are normal or almost normal, and have an ADL of <22 points and a clinical dementia rating (CDR) = 0.5; (4) patients who do not comply with the diagnosis of mental disorders in the United States and the diagnostic criteria for dementia in DSM-IV; (5) patients with only memory loss, and without other cognitive disorders.

All 112 cases of controls were individuals who underwent physical examinations in our hospital at the same period. These individuals had no cognitive impairments in clinical and cognitive function tests, a CDR = 0.5, an ADL <22 points, and a Hachinski ischemic index <4 points. Among these individuals, 43 cases were male and 69 cases were female; and their age ranged from 48 to 75 years old, with an average age of 62±7.35 years old. Furthermore, their educational levels were 5-19 years.

Exclusion criteria: (1) patients with a history of mental illness, congenital mental retardation and depression; (2) patients with nervous system disease that may cause brain dysfunction such as Parkinson's disease, cerebral vascular disease, and other similar diseases; (3) patients with internal systemic disease that may affect central nervous system functions such as thyroid disease, severe anemia, lack of folic acid, $VitB_{12}$, malnutrition and serious heart, liver, lung, kidney and other organic diseases; (4) patients who are alcohol or drug addicts; (5) patients who could not co-operate to complete the cognitive function examination and the determination of P300 for various of reasons such as old age, low educational level or other factors; (6) patients who took drugs one month prior to the study, which could affect Hcy levels, such as contraceptives, anti-epileptic drugs, dopaminergic drugs and folic acid and/or $VitB_{12}$.

Research methods

Detection method of serum Hcy, folic acid and $VitB_{12}$: In the morning, 2-3 ml of fasting venous blood were collected and submitted for inspection within half an hour. Total serum Hcy concentrations were determined through the enzymatic conversion method using a Hitachi 7180 automatic biochemical analyzer. This kit was provided by Beijing Jiuqiang Biotechnology Co. ltd. Normal concentration of Hcy was within 5-14 umol/L, and the patient was considered with hyperhomocysteinemia if the value is higher than the normal value. At the same time, 3-4 ml of cubital venous blood was collected, and the concentrations of folic acid and $VitB_{12}$ were determined on the right day using the Access automated microparticle chemiluminescent immunoassay system (BECKMAN), and a matched kit was provided by the manufacturer.

Neuropsychological scale test: All subjects underwent strict neuropsychological scale tests, as follows: general situation questionnaire, MoCA, CDR, ADL, Hachinski and HAMD anxiety and depression scale. According to the collected data, medical history and relevant neuropsychological scale test results, diagnoses were independently performed by two neurologists. If two results were inconsistent, a third doctor would diagnose again.

Determination of P300 potential: All patients underwent the determination of P300 potential within one week upon enrollment. The British Oxford OXFORD multimedia EMG/evoked potential system was adopted. In a quiet shielded room, the subjects was in the supine position,

Table 2. Comparison of serum Hcy (umol/l), folic acid (ng/ml), $VitB_{12}$ (pg/ml) and P300 ($\bar{x}$±s)

Groups (cases)	HCY	Folic acid	$VitB_{12}$	Latency of P300	Wave amplitude of P300
MCI (128)	16.21±3.98	9.53±3.73	497.15±214.72	377.62±105.61	4.09±1.39
Control (112)	12.97±4.39	11.98±4.81	527.89±151.64	310.87±93.82	3.95±1.06
t	2.256	2.214	0.640	2.588	0.462
P	0.030	0.031	0.525	0.012	0.646

Table 3. Comparison of MoCA, P300 among three subgroups of MCI ($\bar{x}$±s)

Groups (cases)	MoCA	Latency of P300	Wave amplitude of P300
Moderately increased Hcy (6)	21.45±1.47[1]	402.22±80.44[1]	4.14±1.37
Mildly increased Hcy (57)	22.35±1.63	344.96±98.74	3.92±1.25
Normal Hcy (65)	22.85±1.66	323.28±90.87	4.06±1.11
F	3.983	3.231	0.160
P	0.024	0.047	0.852

Note: Superscript 1 means that compared with Normal Hcy, $P<0.05$.

kept awake and concentrated the mind. In accordance with the international brain electro system, the recording electrode was placed over the central midline, the reference electrode was placed on the lobe of the right ear, the forehead electrode was grounded, the impedance among electrodes was <5 KΩ, analysis time was 600 ms, tone pip stimuli was used in the test, the probability of non-target stimuli (frequency was 1,000 Hz) was 80%, and the intensity was 80 dB; which appeared regularly. The probability of the target stimuli (frequency was 4,000 Hz) was 20%, and the intensity was 90 dB; the target stimuli appeared randomly and interspersed in the non-target stimuli. The subjects needed to press the key as a reaction to the target stimuli, and the instrument automatically record the reaction time. The test was repeated twice, and the average value was used.

Statistical methods: All data were analyzed with SPSS 16.0 statistical software. Measurement data were expressed as $\bar{x}$±SD, and were analyzed using variance analysis and *t*-test. Count data were expressed in percentage, and X^2-test was performed. Pearson correlation analysis method was used for correlation analysis. $P<0.05$ was considered statistically significant.

Results

General comparison

In general conditions, there were no significant differences in age, gender, years of education and underlying diseases of patients between the two groups (*t*-test was performed for age and years of education, X^2-test was performed for gender and underlying diseases, $P>0.05$; **Table 1**).

Determination of serum Hcy, folic acid, $VitB_{12}$ and P300

The level of Hcy in the MCI group was higher than in the control group. Folic acid level was lower than that in control group, and there was no significant difference in $VitB_{12}$ level between these two groups. Compared with the control group, the latency of P300 in patients with MCI was prolonged, and the difference was statistically significant ($P<0.01$). There was no significant difference in the wave amplitude of P300 between these two groups (**Table 2**).

Correlations between P300 latency, Hcy and MoCA

A correlation analysis was performed among serum Hcy level, latency and the amplitude of P300, and the MoCA score of patients in MCI group. MoCA scores revealed negative correlations with serum Hcy ($r = -0.476$, $P = 0.002$) and P300 latency ($r = -0.315$, $P = 0.048$); but these did not reveal any significant correlation with P300 amplitude ($r = -0.122$, $P = 0.452$).

The MCI group

According to the level of Hcy, the MCI group was divided into three subgroups: six cases in the moderately increased Hcy group (>30 mmol/L), 57 cases in the mildly increased Hcy group (30>Hcy>14 mmol/L), and 65 cases in the normal Hcy group (<14 mmol/L) [10]. MoCA score and P300 results among these three groups were compared. Results revealed that the MoCA score in the moderately increased Hcy group was lower than in the normal Hcy group; and the latency of P300 was prolonged. There

were no significant differences in MoCA score and P300 latency between the mildly increased Hcy group and normal Hcy group, and there were no significant differences in P300 amplitudes among these three groups (**Table 3**).

Discussion

MCI is a clinical condition between AD and normal aging, which has become a research hot topic. Searching for related factors on the incidence of MCI and early intervention can delay the occurrence of AD, and this has important clinical significance. This study aims to investigate the correlation between cognitive function and Hcy in patients with MCI, and provide evidence for clinical intervention.

Hcy is a kind of amino acid containing sulfhydryl group that is produced in the metabolism of methionine *in vivo*. The metabolism of Hcy is mainly converted into methionine and tetrahydrofolate, which was catalyzed by methionine synthase and methylenetetrahydrofolate reductase. The methylation process needs the participation of $VitB_{12}$ and folic acid. The deficiency of folic acid and $VitB_{12}$ may affect enzyme activity and Hcy metabolism conversion, which result in hyperhomocysteinemia [11]. In this study, we found that the level of Hcy in patients with MCI was higher than in the control group, and the level of folic acid was lower than in the control group. However, there was no significant difference in $VitB_{12}$ levels between these two groups. These indicate that folic acid deficiency may be the cause of the increase in Hcy levels in patients with MCI.

Event related potential P300 is the endogenous component that reflects the advanced processing of feeling. Its latency reveals the evaluation time of stimulus in cognitive activities, and it is the index of information processing speed. In addition, its amplitude indicates the extent of effective resource mobilization in brain information processing, and it is an indicator of the excitability of brain [12]. P300 is not affected by culture and educational levels, and it can reflect cognitive process objectively [13]. The present study revealed that P300 latency reflects the states of brain function such as perception, information coding, and cognitive integration speed up to a certain extent [14]. A prolong P300 latency can be considered as evidence for clinical prediction of early cognitive decline [15]. The results of this study revealed that latency of P300 in the MCI group were significantly prolonged, compared with that in control group; and there was no significant difference in the amplitudes. This was considered to be related to stable P300 latency and high sensitivity. Meanwhile, correlation analysis revealed that P300 latency was negatively correlated to MoCA score. This further confirmed that prolonged P300 latency can objectively reflect mild cognitive dysfunction, which has important significance in early diagnosis of MCI patients.

At present, research results on the relationship between Hcy and cognitive function have been inconsistent. Some studies suggest that there are correlations between these [16], in which the level of cognitive function can be improved by reducing the level of Hcy [17, 18]. Some studies have indicated that these are unrelated [7], in which cognitive function cannot be improved by reducing the level of Hcy [19]. These differences may be related to the different ethnic groups, regions, individuals in groups, and research methods. This study revealed that the level of Hcy in patients with MCI was higher than in the control group. In the MCI group, MoCA score of patients in the Hcy moderately increased subgroup was lower than in the normal Hcy subgroup. Furthermore, P300 latency was longer than that in the normal Hcy subgroup. In addition, the correlation analysis further indicated that the level of Hcy was significantly and negatively correlated to MoCA and P300 latency, which suggest that there are certain correlations between Hcy level and cognitive impairment in MCI patients. This may be related to the increase in the glutamate excitability toxicity induced by Hcy, the reduction in DNA repairing capacity of neurons, the acceleration of the oxidative stress, and the formation of Aβ and damage to hippocampal neurons [20-22].

To conclude, there may be some relevance between Hcy increase and cognitive function impairment in MCI patients. Attention should be given in the detection of Hcy levels in MCI patients, in order to improve the prognosis by intervention.

Since this study was a single center study, the main study objects were elderly people; and the number of enrolled cases was too few to repre-

sent all MCI patients. Furthermore, and the effects of Hcy reduction in patients with MCI on cognitive function was not observed; therefore, improvement is needed in future research works.

Disclosure of conflict of interest

None.

Address correspondence to: Bo Jiang, Department of Neurology, The First Hospital Affiliated To The Chinese PLA General Hospital, 51 Fucheng Avenue, Haidian District, Beijing 100048, China. Tel: +86-10-66848043; Fax: +86-10-66848043; E-mail: bojiangcn@126.com

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