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# Correlation of serum Hcy metabolism with nerve cytokine and apoptosis molecule levels in patients with epilepsy

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ABSTRACT

**Objective:** To study the correlation of serum homocysteine (Hcy) metabolism with nerve cytokine and apoptosis molecule levels in patients with epilepsy. **Methods:** Patients with epileptic seizures and non-epileptic seizures who were treated in our hospital between January 2014 and December 2016 were selected as epileptic seizure group and non-epileptic seizure group respectively, and healthy volunteers during the same period were as selected as control group. The serum was collected to determine the levels of Hcy metabolism indexes, nerve cytokines and apoptosis molecules. **Results:** Serum Hcy, BDNF, NGF, NSE, IGF-I, Bax, Caspase-3, MDA and 8-OHdG levels of epileptic seizure group were significantly higher than those of non-epileptic seizure group and control group while VitB12, Fol and Bcl-2 levels were significantly lower than those of non-epileptic seizure group and control group; serum Hcy, VitB12, Fol, BDNF, NGF, NSE, IGF-I, Bcl-2, Bax, Caspase-3, MDA and 8-OHdG levels of non-epileptic seizure group were not significantly different from those of control group; serum Hcy level in patients with epileptic seizure was positively correlated with BDNF, NGF, NSE, IGF-I, Bax, Caspase-3, MDA and 8-OHdG levels, and negatively correlated with Bcl-2 level while VitB12 and Fol levels were negatively correlated with BDNF, NGF, NSE, IGF-I, Bax, Caspase-3, MDA and 8-OHdG levels, and positively correlated with Bcl-2 level. **Conclusion:** Abnormal serum Hcy metabolism in patients with epileptic seizures can aggravate the neuron injury and apoptosis.

## 1. Introduction

Epilepsy is a common chronic disease of the nervous system, it is characterized by abnormal discharge of central neurons, will cause the brain dysfunction and is manifested as motor, sensory, consciousness and plant nerve dysfunction, and some patients are complicated by learning and memory hypofunction as well as emotional and psychological change[1,2]. The pathogenesis of epilepsy has not been fully elucidated, the abnormal secretion of nerve cytokines and the abnormal apoptosis and damage of neurons is thought to be closely associated with the occurrence of diseases[3], but there is no clear report o the pathway that adjusts nerve cytokine secretion and neuron apoptosis in patients with epilepsy. Homocysteine (Hcy) is the intermediate product in the process of methionine metabolism in the body, it can affect the secretion of cytokines and the apoptosis of cells through a variety of ways, and hyperhomocysteinemia has been proven to be a risk factor for epilepsy attack[4]. In the following studies, the correlation of serum Hcy metabolism with nerve cytokines and apoptosis molecule in patients with epilepsy was analyzed.

## 2. Subject information and research methods

### *2.1 General information of subjects*

Patients with epileptic seizures and non-epileptic seizures who were treated in our hospital between January 2014 and December

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2016 were selected as epileptic seizure group and non-epileptic seizure group respectively, and all patients were in accordance with the diagnostic criteria for the disease, and signed informed consent. There were 32 cases in epileptic seizure group, including 19 men and 13 women that were 21-64 years old; there were 46 cases in non-epileptic seizure group, including 26 men and 20 women that were 23-62 years old. 60 healthy volunteers who received physical examination during the same period were selected as the control group, including 35 men and 25 women that were 25-60 years old. There was no significant difference in the general data of the three groups ($P$>0.05).

### 2.2 Clinical research methods

#### 2.2.1 Serum collection methods

5 mL of peripheral venous blood was collected from epileptic seizure group during epileptic seizure, 5 mL of fasting peripheral venous blood was collected from non-epileptic seizure group in the morning, and 5ml of peripheral venous blood was collected from control group of volunteers during physical examination. The peripheral venous blood was let stand at room temperature for half an hour, naturally coagulated and was then centrifuged in centrifuge for 10 min at a speed of 3 000 r/min, and the upper serum was separated and placed in the -80 ℃ refrigerator.

#### 2.2.2 Serum sample detection methods

Serum samples were taken and thawed, fluorescence polarization immunoassay was used to determine Hcy content, microparticle enzyme immunoassay was used to determine VitB12 and Fol contents, enzyme-linked immunosorbent assay kits were used to determine BDNF, NGF, NSE, IGF-I, Bcl-2, Bax and Caspase-3 contents, and radioimmunoprecipitation kits were used to determine MDA and 8-OHdG.

### 2.3 Statistical processing

SPSS 20.0 software was used for statistical processing, data comparison among three groups was by variance analysis, correlation analysis was by Pearson test and $P<0.05$ indicated statistical significance in differences.

## 3. Results

### 3.1 Serum Hcy, VitB12 and Fol levels

Analysis of serum Hcy metabolism indexes Hcy (μmol/L), VitB12 (pg/mL) and Fol (ng/mL) levels among epileptic seizure group, non-epileptic seizure group and control group was as follows: serum Hcy level of epileptic seizure group was significantly higher than that of non-epileptic seizure group and control group ($P$<0.05) while VitB12 and Fol levels were significantly lower than those of non-epileptic seizure group and control group ($P$<0.05); serum Hcy, VitB12 and Fol levels of non-epileptic seizure group were not significantly different from those of control group ($P$>0.05).

**Table 1.**

Serum Hcy, VitB12 and Fol levels in three groups of subjects.

| Groups | $n$ | Hcy | VitB12 | Fol |
|---|---|---|---|---|
| Epileptic seizure group | 32 | 18.49±2.13$^{*a}$ | 424.52±67.15$^{*a}$ | 7.69±0.93$^{*a}$ |
| Non-epileptic seizure group | 46 | 8.92±1.14 | 683.94±86.58 | 10.32±1.53 |
| Control group | 60 | 8.59±1.08 | 691.23±91.35 | 10.93±1.78 |

$^{*}$: compared with control group, $P<0.05$; $^{a}$: compared with non-epileptic seizure group, $P<0.05$.

### 3.2 Serum nerve cytokine levels

Analysis of serum nerve cytokines BDNF (ng/mL), NGF (pg/mL), NSE (ng/mL) and IGF-I (ng/mL) levels among epileptic seizure group, non-epileptic seizure group and control group was as follows: serum BDNF, NGF, NSE and IGF-I levels of epileptic seizure group were significantly higher than those of non-epileptic seizure group and control group ($P$<0.05); serum BDNF, NGF, NSE and IGF-I levels of non-epileptic seizure group were not significantly different from those of control group ($P$>0.05). Pearson correlation analysis showed that serum Hcy level in patients with epileptic seizure was positively correlated with BDNF, NGF, NSE and IGF-I levels while VitB12 and Fol levels were negatively correlated with BDNF, NGF, NSE and IGF-I levels.

### 3.3 Serum apoptosis molecule levels

Analysis of serum apoptosis molecules Bcl-2, Bax, Caspase-3, MDA (μmol/L) and 8-OHdG (pg/mL) levels among epileptic seizure group, non-epileptic seizure group and control group was as follows:

**Table 2.**

Serum nerve cytokine levels in three groups of subjects.

| Groups | $n$ | BDNF | NGF | NSE | IGF-1 |
|---|---|---|---|---|---|
| Epileptic seizure group | 32 | 1.32±0.18$^{*\&}$ | 596.71±77.42$^{*\&}$ | 23.19±3.29$^{*\&}$ | 118.41±15.62$^{*\&}$ |
| Non-epileptic seizure group | 46 | 0.54±0.07 | 324.52±56.38 | 9.48±1.15 | 46.48±6.72 |
| Control group | 60 | 0.57±0.09 | 330.12±59.32 | 9.84±1.04 | 50.21±7.41 |

$^{*}$: compared with control group, $P<0.05$; $^{\&}$: compared with non-epileptic seizure group, $P<0.05$.

**Table 3.**

Serum apoptosis molecule levels in three groups of subjects.

| Groups | *n* | Bcl-2 | Bax | Caspase-3 | MDA | 8-OHdG |
|---|---|---|---|---|---|---|
| Epileptic seizure group | 32 | 4.75±0.62*& | 2.57±0.33*& | 7.49±0.93*& | 11.32±1.58*& | 575.82±71.35*& |
| Non-epileptic seizure group | 46 | 6.95±0.83 | 1.03±0.18 | 3.25±0.56 | 6.42±0.89 | 302.45±44.86 |
| Control group | 60 | 7.02±0.91 | 1.08±0.15 | 3.08±0.51 | 5.98±0.82 | 310.32±41.77 |

*: compared with control group, $P<0.05$; &: compared with non-epileptic seizure group, $P<0.05$.

serum Bcl-2 level of epileptic seizure group was significantly lower than that of non-epileptic seizure group and control group ($P<0.05$) while Bax, Caspase-3, MDA and 8-OHdG levels were significantly higher than those of non-epileptic seizure group and control group ($P<0.05$); serum Bcl-2, Bax, Caspase-3, MDA and 8-OHdG levels of non-epileptic seizure group were not significantly different from those of control group ($P>0.05$). Serum Hcy level in patients with epileptic seizure was negatively correlated with Bcl-2 level and positively correlated with Bax, Caspase-3, MDA and 8-OHdG levels; VitB12 and Fol levels were positively correlated with Bcl-2 level, and negatively correlated with Bax, Caspase-3, MDA and 8-OHdG levels.

## 4. Discussion

Epileptic seizures can be divided into generalized seizure, partial seizure, special type of seizure and the seizures difficult to classify, and recurrent epilepsy will cause the brain dysfunction and affect the daily life[5,6]. At present, the pathogenesis of epilepsy is not clear, and hyperhomocysteinemia is considered to be an independent risk factor for epileptic seizures[7]. Hcy is the intermediate product during methionine metabolism, methylenetetrahydrofolate reductase and methionine synthase are the main catalytic enzymes in the process, and the Fol and VitB12 are the coenzymes; when Fol and VitB12 are insufficient, there will be Hcy metabolism disorders and hyperhomocysteinemia[8,9]. Related animal studies have found that Hcy can affect electrical activity of brain, activate the lesions causing epilepsy and cause seizures[10]. In the study, analysis of Hcy metabolism in patients with epilepsy showed that serum Hcy level of epileptic seizure group was significantly higher than that of control group while VitB12 and Fol levels were significantly lower than those of control group; serum levels of above Hcy metabolism indexes in non-epileptic seizure group were not significantly different from those in control group. This suggests that abnormal Hcy metabolism is not involved in the occurrence of epilepsy, but is closely related to epileptic seizures; VitB12 and Fol deficiency can cause the metabolism disorders and constant accumulation of Hcy, and high Hcy levels can cause epileptic seizures.

Abnormal discharge of neurons in the process of epileptic seizures can cause cellular damage, and also cause the secretion of a variety of nerve cytokines from neurons into the blood as well as the increased compensatory expression of nerve cytokines. BDNF is the most abundant neurotrophic factor in brain tissue, NGF is the cytokine that has a promoting effect on nerve growth, BDNF and NGF have protective effect on neurons and they can promote the repair and regeneration of neurons; neuron injury can on the one hand, cause BDNF and NGF to be released into the blood circulation, and on the other hand, also increase the compensatory expression of BDNF and NGF and promote nerve repair[11,12]. NSE is a cytokine with catalytic effect in neurons, IGF-I is a cytokine with hormone effect, they can promote the proliferation of neurons and inhibit the apoptosis of neurons, and the NSE and IGF-I release increase in the process of neuron discharge and damage[13]. In the study, analysis of serum contents of these nerve cytokines in patients with epilepsy showed that serum BDNF, NGF, NSE and IGF-I levels of epileptic seizure group were significantly higher than those of control group, while serum BDNF, NGF, NSE and IGF-I levels of non-epileptic seizure group were not significantly different from those of control group. This suggests that neuron damage is closely associated with epileptic seizures, and neuron injury can cause multiple neurocytokines to be secreted into the bloodstream. In order to further clarify the effect of Hcy elevation on nerve cytokine secretion, the correlation between Hcy metabolism and nerve cytokine contents was analyzed in the study, and the results showed that serum Hcy level in patients with epileptic seizures was positively correlated with BDNF, NGF, NSE and IGF-I levels while VitB12 and Fol levels were negatively correlated with BDNF, NGF, NSE and IGF-I levels. This suggests that abnormal Hcy metabolism can increase the secretion of nerve cytokines and increase the damage of neurons to cause seizures.

Abnormal discharge of neurons in patients with epileptic seizures is not only closely related to the abnormal secretion of nerve cytokines, but also involves the excessive apoptosis of neurons. Bax and Bcl-2 are important regulatory molecules of apoptosis, the former promotes apoptosis, the latter inhibits apoptosis, and they are involved in the regulation of mitochondrial apoptosis together; Caspase-3 is an important downstream effector molecule in the mitochondrial pathway, its activation is regulated by the common regulation of Bax and Bcl-2 and it can mediate cell apoptosis. The increase in Bax and the decrease in Bcl-2 will cause the Caspase-3 overactivity and cause apoptosis[14,15]. Oxidative stress damage is considered as

an important upstream factor that affects the apoptosis mediated by Bax/Bcl-2, excessively produced oxygen free radicals will increase the generation of Bax, decrease the generation of Bcl-2 and lead to cell apoptosis, and meanwhile, they have oxidation reaction with lipid and nucleic acid and generate the corresponding products MDA and 8-OHdG[16,17]. In the study, analysis of serum contents of these apoptosis molecules in patients with epilepsy showed that serum Bcl-2 level of epileptic seizure group was significantly lower than that of control group while Bax, Caspase-3, MDA and 8-OHdG levels were significantly higher than those of control group; serum Bcl-2, Bax, Caspase-3, MDA and 8-OHdG levels of non-epileptic seizure group were not significantly different from those of control group. This suggests that neuron apoptosis is closely associated with seizures. In order to further clarify the effect of Hcy elevation on neuron apoptosis, the correlation between Hcy metabolism and neuron apoptosis was analyzed in the study, and the result showed that serum Hcy level in patients with epileptic seizure was negatively correlated with Bcl-2 level and positively correlated with Bax, Caspase-3, MDA and 8-OHdG levels; VitB12 and Fol levels were positively correlated with Bcl-2 level, and negatively correlated with Bax, Caspase-3, MDA and 8-OHdG levels. This suggests that abnormal Hcy metabolism can promote the apoptosis of neurons to cause seizures.

To sum up, it is believed that abnormal serum Hcy metabolism is closely associated with epileptic seizures; the unusually elevated Hcy can aggravate neuron injury and apoptosis to cause seizures.

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