



## Plasma Homocysteine Levels in Neuromyelitis Optica

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### Abstract

**Background and purpose:** Homocysteine has been implicated in many kinds of neurologic diseases by inducing oxidative injury which is considered one of the pathogenic mechanisms of neuromyelitis optica (NMO). The aim of this study was to investigate whether there were any relationship between plasma homocysteine and clinical features of NMO patients.

**Methods:** We measured plasma homocysteine in 66 patients with NMO and 66 controls.

**Results:** The patients with NMO had significantly higher homocysteine level than that of healthy control ( $P < 0.001$ ). The average homocysteine level of patients with NMO was in normal range. No correlation was found between clinical or magnetic resonance imaging features of patients with NMO and the level of homocysteine. The inactive NMO patients with relapse in follow-up had significantly higher homocysteine level than those without relapse in follow-up ( $P = 0.045$ ), and the entire inactive NMO patient with relapse had at least one relapse within the first year of follow-up.

**Conclusions:** The elevated homocysteine levels in NMO patients might be just a secondary change.

### Keywords

Homocysteine, Neuromyelitis optica, Oxidative injury

### Methods

#### Patients

Our database comprised 66 patients with NMO who were diagnosed and admitted from July 2006 to June 2013 in the Multiple Sclerosis Center of the Third Affiliated Hospital of Sun Yat-sen University. NMO was diagnosed according to the 2006 Wingerchuk criteria [10]. Among them, 35 patients were in inactive stage with no relapse for at least 3 months and 31 patients were in active stage within 7 days after a acute demyelinating event confirmed by enhancing lesions (brain and/or spinal cord and/or optic nerve) in T1-weighted spin-echo images. All of the patients in active stage received high-dose corticosteroids (intravenous methylprednisolone 1 g per day for 5 days) during the acute period, another course of high-dose corticosteroids therapy would be given if no obvious recovery was attained. For steroid-refractory patients, plasma exchange or intravenous immunoglobulin were applied. Prednisone combined with azathioprine was used for prevention of relapse and the dose of prednisone was gradually reduced to complete withdrawal. All of the patients were followed up in the outpatient department once a month after discharge. Age- and sex-matched controls were selected sequentially from healthy volunteers. None of the patients and controls had received vitamin B12 and folate supplementary products, drugs that increase serum Hcy level, and corticosteroids in the past 3 months. Blood collection and laboratory analysis.

After overnight fasting, a total of 3 ml of venous blood was collected and centrifuged immediately at 6 to 7 a.m. in the second day after admission. All samples were transferred to the laboratory in the ice box. Homocysteine was measured using high-performance liquid chromatography ( $\mu\text{mol/L}$ ). The cutoff concentration value for hyperhomocysteinemia was  $13.9\mu\text{mol/L}$ .

Serums from all patients were tested for anti-AQP4 antibody by a commercial sampling kit (Euroimmun, Germany) according to the manufacturer's instructions. Laboratory tests were performed in all patients to exclude infectious diseases, connective tissue diseases, vascular diseases and metabolic disorders.

#### Magnetic Resonance Imaging (MRI) scanning

Brain and spinal cord MRI scans were performed in all patients

### Introduction

Neuromyelitis Optica (NMO) is an inflammatory demyelinating disease that preferentially affects optic nerve and spinal cord. Although the pathogenesis of NMO is not completely clear, some studies have suggested that oxidative injury is involved in NMO [1,2].

Homocysteine (Hcy) is a non-essential sulfur-containing amino acid derived from methionine by demethylation. Recent studies indicate that homocysteine might cause neuronal damage by triggering oxidative injury and DNA damage [3-6]. And homocysteine has been implicated in many kinds of neurologic diseases, such as multiple sclerosis (MS), stroke, and dementia [7-9]. However, little is known about the relationship between Hcy and NMO. In the present study, we investigated whether there were any relationship between plasma Hcy and clinical features of NMO patients.

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**Table 1:** Demographic characteristics and Hcy levels of NMO patients and controls.

	NMO (n = 66)	HC (n = 66)	P
Gender, F:M	54:12	54:12	1
Age, years	47.2 ± 12.4	44.2 ± 14.7	0.213
Disease duration, months	49.1 ± 54.6	-	-
EDSS	3.7 ± 2.2	-	-
NMO-IgG seropositivity	45 (68.2%)		
Hcy (μmol/L)	9.8 ± 3.7	7.8 ± 2.0	<0.001

Abbreviations: Hcy: Homocysteine; NMO: Neuromyelitis Optica; HC: Healthy Control; F: Female; M: Male; EDSS: Expanded Disability Status Scale.

**Table 2:** Results of Spearman correlation analysis in patients with NMO.

	Age	Disease duration	EDSS	Relapse number	Number of brain MRI T2 lesions	Length of spinal cord MRI T2 lesions
Hcy						
r	-0.065	-0.159	-0.081	-0.130	-0.058	-0.057
P	0.606	0.201	0.520	0.300	0.644	0.649

Abbreviations: Hcy: Homocysteine; NMO: Neuromyelitis Optica; EDSS: Expanded Disability Status Scale; MRI: Magnetic Resonance Imaging.

**Table 3:** Comparison of Hcy levels in NMO patients with different disease activity, gender, NMO-IgG seropositivity and occurrence of relapse.

NMO	Hcy (μmol/L)	P
Active	9.7 ± 4.1	0.818
Inactive	9.8 ± 3.3	
Female	9.5 ± 3.1	0.385
Male	11.0 ± 5.6	
NMO-IgG positive	9.7 ± 3.6	0.95
NMO-IgG negative	9.8 ± 3.9	
Relapse	9.3 ± 3.3	0.067
No relapse	11.3 ± 4.6	

Abbreviations: Hcy: Homocysteine; NMO: Neuromyelitis Optica.

using a GE 1.5T MR scanner (General Electric, Milwaukee, Wisconsin, USA). The slice thickness of the axial scans was 5 mm. Conventional MRI protocols were used in all patients: T1 with and without gadolinium enhancement (400/15.5 ms, TR/TE) and T2 (2500–3500/100 ms, TR/TE) for spinal cord MRI; and T1 with and without gadolinium enhancement (2128–2300/11.6–12.4 ms, TR/TE), T2 (4600–4640/97.8–102 ms, TR/TE), and fluid-attenuated inversion recovery (FLAIR) (8800/120 ms, TR/TE) for brain MRI [11]. Each patient in acute stage underwent MRI scanning at the time prior to corticosteroid treatment. Brain abnormalities were assessed with T2-FLAIR sequences. All of the MRI scans were analyzed by an experienced neuroradiologist and a neurologist. The final assessments were made by consensus.

### Statistical analysis

The data were expressed as mean ± standard deviation (SD). Chi-square test and t-test were used for qualitative and quantitative data, respectively. Correlations between plasma Hcy and clinical and MRI features of NMO patients were analyzed by Pearson correlation analysis. Statistical analysis was performed by SPSS version 17.0. Values of  $p=0.05$  were considered statistically significant.

### Results

In our study, the patients with NMO had significantly higher Hcy level than that of healthy control (HC) ( $P<0.001$ ) (Table 1). The average Hcy level of patients with NMO was in normal range.

In NMO patients, the Hcy level was not correlated with age, disease duration, Expanded Disability Status Scale (EDSS) score, relapse number, number of brain MRI lesions, or length of spinal cord MRI lesions (Table 2).

Furthermore, we classified the NMO patients into different groups according to disease activity, gender, and occurrence of relapse. There were no differences between NMO patients in active and inactive stage, male and female, NMO-IgG positive and negative,

or patients with and without relapse in follow-up (Table 3).

Finally, further analysis showed that the inactive NMO patients with relapse in follow-up had significantly higher Hcy level than those without relapse in follow-up ( $P=0.045$ ), and no differences were found in Hcy levels between the active NMO patients with and without relapse in follow-up (Table 4). And for the patients in inactive stage with relapse in follow-up, there was at least one relapse that occurred in the first year of follow-up.

### Discussion

Previous studies have demonstrated that Hcy might cause neuronal damage by triggering oxidative injury which is implicated in NMO [3-6]. However, it is unclear whether Hcy play a role in pathogenesis of NMO. To our knowledge, this is the first study investigating the relationship between Hcy and NMO. In the present study, we found the level of Hcy elevated in NMO patients within normal range and the inactive NMO patients with relapse in follow-up had higher Hcy level.

Several researches have reported higher level of Hcy in patients with MS which has some common pathogenic mechanisms with NMO [7,12-15]. Although other studies did not found increased level of Hcy in patients with MS [16-18], a meta-analysis still suggested a significant association between MS and serum Hcy [19]. But only a few associations were found between clinical features of MS and Hcy in these studies: two of them suggested an association between cognitive impairment in MS and Hcy [13,16], one found Hcy levels in patients with multiple sclerosis were associated with male gender while the other did not [12,17], and four studies investigated the relationship between EDSS score of MS and Hcy and found negative results [7,14,15,17]. We found similar results when analyzing the association between clinical features of NMO and Hcy. It seems that the level of Hcy is not related to the severity, prognosis, and lesion extent of NMO. So the elevated Hcy levels in NMO patients might be just a secondary change. There might be other explanations. One could argue that Hcy does cause oxidative injury but its impact is not large enough to affect the severity, prognosis, and lesion extent of NMO. Besides, other factors that play more important role in oxidative injury in NMO may cover the effect of Hcy. But it is unlikely that Hcy within normal range can cause substantial oxidative injury. Besides, C677T polymorphism of MTHFR gene which is the main genetic factor of Hcy was not tested, so we could not exclude its effect on our results.

Further analysis indicated that the inactive NMO patients with relapse within one year had higher Hcy level, while the same results were not found in active NMO patients. It is possible that the Hcy levels reflect the severity of oxidative injury only in inactive NMO patients but not in active NMO patients, since different mechanisms may be involved in different stages of disease. The elevated Hcy might indicate more severe oxidative injury, so the inactive NMO patients with higher Hcy levels might be more prone to relapse. However, the follow-up period varied from several months to several years in our study, so we still can not draw a conclusion that the level of Hcy in patients with NMO in inactive stage might be helpful to predict relapse.

Folate and vitamin B12 have fundamental roles in CNS function especially methionine synthase-mediated conversion of Hcy to methionine, which is essential for synthesis of DNA and RNA [20,21]. Therefore, vitamin B12 and folate deficiency can lead to an increased level of Hcy [21]. Some studies showed the relation between high level of serum Hcy and low levels of serum B12 and folate in patients with MS [15,22,23], while others did not [24,25]. The levels of vitamin B12 and folate were tested in only thirteen patients in our study, among whom no deficiency in vitamin B12 or folate were detected. After searching our database of NMO, we found the levels of vitamin B12 and folate were tested in another nine patients that were not included in the present study because the level of Hcy was not tested. None of these nine patients had deficiency in vitamin B12 and only one of them had a level of folate below normal range.

**Table 4:** Comparison of clinical features and Hcy levels in active/inactive NMO patients with and without relapse in follow-up.

	NMO in active stage		NMO in inactive stage		P1	P2
	Relapse	No relapse	Relapse	No relapse		
Gender, F:M	6:1	19:5	7:1	22:5		
Age, years	43.1 ± 12.0	47.0 ± 14.3	47.4 ± 13.1	41.1 ± 16.2	0.527	0.327
Disease duration, months	69.6 ± 84.6	44.4 ± 55.9	59.1 ± 35.1	45.0 ± 50.2	0.359	0.465
Follow-up, months	25.9 ± 11.6	23.0 ± 19.6	22.0 ± 25.6	17.8 ± 18.3	0.718	0.607
EDSS	2.8 ± 0.9	4.4 ± 2.4	4.0 ± 1.5	3.2 ± 2.3	0.093	0.368
Hcy (μmol/L)	11.0 ± 4.1	9.0 ± 3.2	11.9 ± 3.7	9.2 ± 3.0	0.181	0.045

Abbreviations: Hcy: Homocysteine; NMO: Neuromyelitis Optica; F: Female; M: Male; EDSS: Expanded Disability Status Scale; P1: NMO in active stage with relapse in follow-up vs NMO in active stage without relapse in follow-up; P2: NMO in inactive stage with relapse in follow-up vs NMO in inactive stage without relapse in follow-up.

So vitamin B12 and folate deficiency is uncommon in patients with NMO. Besides, if vitamin B12 and folate deficiency played a role in the elevated level of Hcy in NMO, the level of Hcy should not be in normal range. Therefore, it is unlikely that the elevated level of Hcy in NMO is caused by vitamin B12 or folate deficiency.

There are some limitations in this study: (a) Oxidative stress biomarkers and the cerebrospinal fluid Hcy levels were not measured; (b) sequential change of Hcy levels was unavailable; (c) the follow-up period varied from several months to several years; (d) bias is inevitable in retrospective studies.

In conclusion, the elevated Hcy levels in NMO patients might be just a secondary change.

### Ethics Statement

This research was approved by the ethics committee of the Third Affiliated Hospital of Sun Yat-sen University. All participants involved in this study provided written informed consent.

### Author Contribution

Lei Zhang, Yaqing Shu, and Shaoyang Sun contributed equally to the manuscript.

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