THE RELATION OF HYPERHOMOCYSTEINEMIA TO COGNITIVE FUNCTION AND BRAIN ATROPHY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Abstract- Cognitive impairment may be a common even at the onset of multiple sclerosis (MS). In this case-control study, we tried to find out the probable relationship between homocysteine levels and cerebral atrophy or cognitive impairment in patients with multiple sclerosis. One hundred fifty six patients who had MS according to McDonald diagnostic criteria were included in this study. Patients' age, gender, and educational level, MS duration and clinical type, disability, cognitive function state based on minimental state examination (MMSE), presence of hyperhomocysteinemia, and brain atrophy were evaluated. There was no statistically significant relationship between hyperhomocysteinemia and cognitive status. Total homocysteine levels had a significant correlation with MMSE score only in those patients with elementary level of education. Also total homocysteine levels and overall cerebral atrophy did not indicate significant relationship according to those independent variables mentioned above except in the patients with EDSS less than 6. When intercaudate ratio > 0.10 was applied as a criterion for cerebral atrophy, we found that hyperhomocysteinemia related significantly to intercaudate ratio > 0.10 in females, aged between 21 and 30 years, MS duration ≤ 5 years, primary progressive MS and relapsing-remitting MS clinical types, EDSS ≤ 3 and elementary level of education. We suggest applying MMSE only for the first step of cognitive function survey. In the next steps, much more exact test must be used (*e.g.* MSNQ). Also we can not suggest measuring plasma homocysteine level as criterion for monitoring the cognitive function in patients with MS.

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Key words: Multiple sclerosis, hyperhomocysteinemia, cognitive function, brain atrophy

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune inflammatory demyelinating disease of the central nervous system (CNS). The cause of MS remains unknown. The most widely accepted theory is that MS is an inflammatory autoimmune disorder mediated by autoreactive T-cells directed against components of myelin (1, 2).

This disease primarily affects women of Northern European descent who are of child-bearing age. The median and mean age of MS onset are 23.5 and 30 years of age, respectively. It is characterized pathologically by multifocal areas of demyelination with relative preservation of axons, loss of oligodendrocytes, and astroglial scarring. Certain clinical features are typical of MS, but the disease has a highly variable pace and many atypical forms.

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Frank dementia is an uncommon feature of MS, occurring in less than 5 percent of patients. It is usually only encountered in severely affected individuals. However, 34 to 65 percent of patients have cognitive impairment on the basis of neuropsychological testing (3-10). The prevalence of cortical syndromes such as aphasia, apraxia, and agnosia is low. Cognitive impairment may be common even at the onset of MS (2, 3). The most frequent abnormalities are with abstract conceptualization, recent memory, attention, and speed of information processing (11, 12). Different disease courses may have different cognitive profiles. As an example, one study found that patients with relapsing-remitting MS generally had better cognitive performance than patients with progressive types of MS (13).

The degree of cognitive decline in patients with MS correlates with the severity of cerebral pathology on MRI (3, 14-16). In addition, cerebral atrophy on T1-weighted MRI correlates with cognitive impairment, suggesting that gray as well as white matter pathology may contribute to the cognitive decline in patients with MS (17). However, there is a controversy on this issue. Intercaudate ratio (ICR) is one of the most important linear measurement methods of cerebral atrophy, studied as a probable marker of tissue destruction in MS. ICR calculates in MRI as intercaudate distance divided by horizontal width of internal table of the skull in the same level.

There is conflicting evidence about whether homocysteine is an independent risk factor for dementia. Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine. Homocysteine is metabolized by one of two divergent pathways: transsulfuration; and remethylation. The transsulfuration of homocysteine to cysteine is catalyzed by cystathionine-ß-synthase, a process that requires pyridoxal phosphate (vitamin B6) as a cofactor. Remethylation of homocysteine produces methionine. This reaction is catalyzed either by methionine synthase or by betaine homocysteine methyltransferase. Vitamin B12 (cobalamin) is the precursor of methylcobalamin, which is the cofactor for methionine synthase. Homocysteine is an excitatory amino acid and markedly enhances the vulnerability of neuronal cells to excitotoxic and oxidative injury and causes disruption of the blood-brain barrier.

Elevations in the plasma homocysteine concentration can occur due to genetic defects in the enzymes involved in homocysteine metabolism, to nutritional deficiencies in vitamin cofactors, or to other factors including some chronic medical conditions and drugs (4-10). Hyperhomocysteinemia interfere with the synthesis of S-adenosylmethionine and thus with methyl donations for neurotransmitters, which are essential for nerve conduction in MS patients. Clinical evidence suggests a strong relationship between higher total homocysteine level and brain atrophy in healthy elderly subjects as well as in elderly at risk of and with Alzheimer's disease. It is believed that MS relates to the high levels of total homocysteine, even with 60% prevalence (18). Several recent reports have documented vitamin B_{12} deficiency in MS (with unknown etiology) that in turn increases plasma levels of total homocysteine. Vitamin B_{12} is important for myelin synthesis and integrity. Vitamin B_{12} in combination with folic acid and vitamin B_6 have been shown to reduce high plasma levels of homocysteine.

In this study, we tried to find out the probable relationship between homocysteine levels and cerebral atrophy or cognitive impairment in patients with MS.

MATERIALS AND METHODS

This is a case-control study which involved patients with MS in Imam Khomeini hospital, department of neurology, during 2004 to 2005. Patients who had MS according to McDonald diagnostic criteria (2) and had been assessed by brain MRI in the recent one year included in this study. Co-existence of depression, euphoria, malnutrition and the use of special drugs (*e.g.* anticholinergics and benzodiazepines) as the etiologies of cognitive dysfunction and the use of vitamin B12, B6 and folic acid were considered as the exclusion criteria. Informed consent obtained after explaining the plan to each patient.

Patients' age, gender, and educational level, MS duration, and clinical type, disability degree,

cognitive function state based on mini mental state examination (MMSE), presence of hyperhomocysteinemia, and brain atrophy were evaluated. Data were collected from different sources such as medical files, physical examination to determine EDSS and MMSE, brain MRI for atrophy and ICR calculation and chemical lab data for measuring nonfasting plasma total homocysteine concentration. Patients were classified to case and control groups in three different ways: MMSE score (cut off, 26), overall brain atrophy and ICR (cutoff, 0.10). Patients with cognitive impairment, overall brain atrophy or ICR greater than 0.10 formed case group and the others considered as controls.

SPSS 11.5 (statistical package) was used for data analysis consist of squared chi and fisher's exact test. A *P* value less than 0.05 was considered significant.

RESULTS

A total of 156 patients were included in this study that consisted of 49 males (31.4%) and 107 females (68.6%). Patients' characteristics, disability degrees, MMSE scores and imaging results, and MS different clinical types and its duration were abstracted in Table 1.

Comparison of variables in case and control groups is shown in Table 2. Considering MMSE score, significant differences between case and control group were found in educational level, overall brain atrophy and ICR (*P* values were 0.004, 0.018 and 0.006, respectively). Other variables did not show statistically significant difference. Classification of patients according to overall brain atrophy led to find some other significant differences such as age groups, disease duration, EDSS and ICR (*P* values were 0.004 and < 0.01 for the last three variables, respectively) and classification according to ICR discovered significant difference between two groups in age, disease duration, MS clinical types, EDSS, total homocysteine concentration, and MMSE score (*P* values were < 0.01, < 0.01, 0.007, < 0.01, 0.006 and 0.006, respectively).

There was no statistically significant relationship between hyperhomocysteinemia and cognitive status. After classification of case and control groups to more specialized subgroups according to the patients' sex, and age, disease duration, and clinical types, EDSS and educational levels, total homocysteine levels had a significant correlation with MMSE score only in those patients with elementary level of education (r, 0.361, *P*, 0.008). Also total homocysteine levels and overall cerebral atrophy did not indicate significant relationship according to those independent variables mentioned above except in the patients with EDSS less than 6 (χ^2, 4.922, *P*, 0.045). When ICR > 0.10 was applied as a criterion for cerebral atrophy, we found that hyperhomocysteinemia related significantly to ICR > 0.10 in females (*P*, 0.004), aged between 21 and 30 years (*P*, 0.004), MS duration ≤ 5 years, PPMS (0.045) and RRMS (0.001) clinical types, EDSS ≤ 3 (0.005) and elementary level of education (0.005).

Table 1. Patients' demographic characteristics, disease type and duration, physical exams and paraclinical data*

Variable	Count
Gender	
Female	49 (31.4%)
Male	107 (68.6%)
Age groups	
≤ 20 years	16 (10.3%)
21-30 years	50 (32.1%)
31-40 years	55 (35.3%)
41-50 years	25 (16%)
≥ 50 years	10 (6.4%)
Educational level	
Elementary	53 (34%)
High school	59 (37.8%)
Master	35 (22.4%)
Higher	5 (3.2%)
MS clinical types	
Primary progressive	44 (28.2%)
Secondary progressive	34 (21.8%)
Relapsing- remitting	67 (42.9%)
Progressive relapsing	11 (7.1%)
EDSS	
≤ 3	61 (39.1%)
3.5-6	74 (47.4%)
> 6	21 (13.5%)
Disease duration	
≤ 1 year	38 (24.4%)
1-5 years	76 (48.7%)
>5 years	42 (26.9%)
Total homocysteine (μmol/L)†	12.8 (8.6)
MMSE score	146 (93.6%)
< 26	10 (6.4%)
≥ 26	66 (42.3%)
Overall brain atrophy	
Intercaudate ratio	83 (53.2%)
≤ 0.10	73 (46.8%)
> 0.10	

*Data are given as number (percent) unless specified otherwise.
† Mean (SD).

Table 2. Comparison of variables according different classification bases*

Variable	MMSE			Brain atrophy			ICR		
	Case	**Control**	***P***	**Case**	**Control**	***P***	**Case**	**Control**	***P***
Gender									
Female	9 / 90	98/67.1	0.173	45/68.2	62/68.9	1.00	45/61.6	62/74.7	0.087
Male	1 / 10	48/32.9		21/31.8	28/31.1		28/38.4	21/25.3	
Age groups									
≤ 20 years	1 / 10	15/10.3	0.218	2 / 3	14/15.6	0.004	1/1.4	15/18.1	<0.01
21-30 years	1 / 10	49/33.6		15/22.7	35/38.9		17/23.3	33/39.8	
31-40 years	3 / 30	52/35.6		31/47	24/26.7		31/42.5	24/28.9	
41-50 years	4 / 40	21/14.4		14/21.2	11/12.2		18/24.7	7/8.4	
≥ 50 years	1 / 10	9/6.2		4/6.1	6/6.7		6/8.2	4/4.8	
Educational level									
Unlettered	2 / 20	2 / 1.3	0.004	3 / 4.5	1/1.1	0.420	4/5.5	0/0	0.087
Elementary	5 / 50	48/32.8		25/37.9	28/31.1		29/39.7	24/28.9	
High school	2 / 20	57/39.1		22/33.3	37/41.1		22/30.1	37/44.6	
Master	1 / 10	34/23.3		13/19.7	22/24.4		16/21.9	19/22.9	
Higher	0	5 / 3.5		3/4.5	2/2.2		2/2.7	3/3.6	
MS clinical types									
Primary progressive	6 / 60	38/26	0.083	23/34.8	21/23.3	0.108	28/38.4	16/19.3	0.007
Secondary progressive	2 / 20	32/21.9		16/24.2	18/20		18/24.7	16/19.3	
Relapsing- remitting	1 / 10	66/45.2		21/31.8	46/51.1		21/28.8	46/55.4	
Progressive relapsing	1 / 10	10/6.9		6/9.1	5/5.6		6/8.2	5/6	
EDSS									<0.01
≤ 3	1 / 10	60/41.1	0.149	15/22.7	46/51.1	<0.01	12/16.4	49/59	
3.5-6	7 / 70	68/46.6		36/54.5	39/43.3		45/61.6	30/36.1	
> 6	2 / 20	18/12.3		15/22.7	5/5.6		16/21.9	4/4.8	
Disease duration									
≤ 1 year	1 / 10	37/25.4	0.452	8/12.1	30/33.3	<0.01	10/13.7	28/33.7	<0.01
1-5 years	5 / 50	71/48.6		30/45.5	46/51.1		33/45.2	43/51.8	
>5 years	4 / 40	38/26		28/42.4	14/15.6		30/41.1	12/14.5	
Hyperhomocysteinemia	2 / 20	40/27.4	1.00	20/30.3	22/24.4	0.467	30/41.1	12/14.5	<0.01
tHcy concentration (μmol/L) †	13.8/13.3	12.8/8.2	0.727	13.3/8	12.4/9	0.531	14.8/9.9	11.1/6.9	0.006
MMSE score									
< 26	10 / 6.4	-----		8/12.1	2/2.2	0.018	9/12.3	1/1.2	0.006
≥ 26	-----	146/93.6		58/87.9	88/97.8		64/87.7	82/98.8	
Overall brain atrophy	8 / 80	58/39.7	0.018	66/42.3	90/57.7		54/74	12/14.5	<0.01
ICR > 0.10	9 / 90	64/43.8	0.006	54/81.8	19/21.1	<0.01	73/46.8	83/53.2	

Abbreviation: ICR, intercaudate ratio; tHcy, total homocysteine.
*Data are given as number /percent unless specified otherwise.
†Mean / SD

DISCUSSION

Similar to other studies, female to male ratio in our study was 2.2:1. Patients in the fourth decade of life were in majority and those in the third decade of life were in the next turn. Relapsing-remitting type of MS is the most common type. A same pattern was observed in our study (42.9%) but primary progressive type was found with a higher rate compared with many of neurological references (28.2 *vs.* 10-19%).

More than one fourth of our patients had hyperhomocysteinemia. In a similar study Russo *et al.* (18) and Reynolds (19) reported increased prevalence rate for hyperhomocysteinemia. Conversely, Rio *et al.* (20) and Goodkin *et al.* (21) found different data. On the other hand, the mean total homocysteine concentration in our study was 12.8 ± 8.6 μmol/L which was more than those reported by Vrethem *et al.* (22) and Rio *et al.* (20).

According to MMSE score (cutoff, 26), there was only 6.4% of patients with some degrees of cognitive impairment while several studies suggested a higher frequency (34-65%). It seems that this difference arose from low sensitivity of MMSE to distinguish mild cases of cognitive impairment. Truelle *et al.* reported that 55% of patients with a history of MS lower than 5 years had obvious memory disorder (23). We only found 5.3% of these patients with a MMSE score less than 26. Several studies suggested a less probability of cognitive impairment in the patients with relapsing-remitting type of MS, but we did not find statistically significant relationship

between them. In addition we did not observe significant relationship between plasma homocysteine concentration and cognitive function perhaps as the reason of small case population. Exact diagnosis of brain atrophy, particularly in the early stage of the disease needs to achieve volumetric MRI. Similar to our study, Losseff *et al.* (24), Simon *et al.* (25) and Bakshi *et al.* (26) proved the concordance of brain atrophy in patients with MS and physical disability degree. The significant relationship between total homocysteine concentration and global brain atrophy has been shown in several disease linked to hyperhomocysteinemia. However, we could not find this relationship but there was another statistically significant relationship between total homocysteine concentration and ICR. In addition, ICR groups had direct relation with age groups, MS duration, overall cerebral atrophy, and EDSS and diverse relation with cognitive status and MMSE mean score.

We suggest applying MMSE only for the first step of cognitive function survey. In the next steps, much more exact tests must be used (*e.g.* MSNQ). Currently we can not recommend measuring plasma homocysteine level as a lab criterion for monitoring the cognitive function in patients with MS. Finally, it seems that ICR is not only a valuable criterion for the diagnosis of cerebral atrophy but also correlates to hyperhomocysteinemia in MS. Thus, it is recommended to prepare another trial to assess the effect of therapeutic intervention diminishing tHcy plasma concentration (*e. g.* vit. B12, B6 and folic acid prescription) on cerebral atrophy in MS (based on ICR).

Conflict of interests

The authors declare that they have no competing interests.

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