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Investigation of The Effect of Serum Homocysteine Level on Cognitive Functions in Multiple Sclerosis Patients

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Abstract

Objective: In this study, we aimed to investigate whether serum homocysteine level was higher in patients with MS, and evaluate the effect of this situation on cognitive functions. In addition, we aimed to determine whether high blood homocysteine level is associated with socio-demographic and clinical features and determine the affected cognitive functions with neuropsychological tests.

Methods: We looked at changes in the levels of vitamin B12 and folic acid associated with high levels of serum homocysteine levels. Cognitive performance, and laboratory values of 60 cases with Relapsing-remitting MS (RRMS) patient, and 30 healthy volunteers were compared. The frontal lobe activities, information processing speed, flexibility, and the ability of the calculation, as well as cognitive functions of attention and visuospatial perception and construction of complex skills were also evaluated. For these purposes, Paced Auditory Serial Addition Test (PASAT), Line Orientation Test (LOT) and Stroop Test were used.

Results: Test performance for all of the test steps were found to be defective in MS group more than the control group subjects. In addition, all steps of the test performance were found to be impaired in both patients and healthy subjects with high levels of homocysteine($p<0.001$).

Conclusion: In this study visual memory processes, visual-spatial functions, construction skills, planning, programming and executive functions were found to be significantly deteriorated and the reaction time prolonged in RRMS patients with high levels of homocysteine compared with healthy control group

Keywords: Multiple sclerosis, cognitive impairment, serum homocysteine levels

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INTRODUCTION

MS is a chronic inflammatory, demyelinating and neurodegenerative disease accompanied by focal demyelinating plaques and axonal degeneration that may affect cortex and deep gray matter, primarily white matter in the central nervous system (CNS) (1,2). The disease manifests itself with symptoms associated with the involved CNS region.

Impairment of memory and cognitive performance is an important problem affecting functionality in patients. This is thought to be caused not only by MS itself, but also by other underlying damage mechanisms. Studies have shown that the neurotoxic effects of homocysteine may be associated with thrombosis, central nervous system (CNS) developmental disorders and neurodegenerative diseases, psychiatric disorders such as depression and schizophrenia, and some cancers (3).

The main purpose of this study was to investigate the relationship between homocysteine levels and impaired cognitive performance in MS patients. In this context, to investigate whether blood homocysteine levels increase in MS patients and its effect on cognitive functions. Sociodemographic characteristics related to high levels of serum homocysteine, EDSS score, as well as hyper-homocysteinemia, which impaired cognitive functions and which functional brain regions are most affected by neurocognitive tests are our secondary goals. It is also aimed to investigate the changes in vitamin B12 and folic acid levels simultaneously with high blood homocysteine levels. The results obtained will provide important information on the etiology of cognitive impairment in MS patients and whether treatments to reduce homocysteine levels can be effective.

METHODS

Between January 2012 and October 2012, 61 RRMS patients admitted to the MS Outpatient Clinic of Neurology Department of Ondokuz Mayıs University Medical Faculty Hospital were included in the study. The control group consisted of 32 healthy volunteers. 2012/451 was approved by the ethics committee of Ondokuz Mayıs University Medical Faculty Hospital dated 23.02.2012 and numbered B.30.2.ODM.0.20.08 / 949.

Informed consent form was obtained from all MS cases and healthy volunteers included in the study. Patients aged between 18-55 years who were diagnosed with MS according to McDonald's criteria were included in the study. Patients with clinical features compatible with secondary progressive, primary progressive, and other demyelinating diseases, those who use drugs such as B12 and / or folic acid that may affect blood homocysteine levels, those who have a disease that may cause neuroleptic, antipsychotic, antidepressant-like drug use, and those who may use such cognitive functions. Illiteracy, patients with definite diagnosis of MS were not included in the study. During the outpatient controls, detailed neurological examination was performed. Patients who had received an attack treatment were enrolled in the remission period at least six weeks after treatment. Healthy control group was formed; Those who used drugs that could affect blood homocysteine levels such as B12 and / or folic acid, who had any disease that could affect cognitive functions, or those who could use drugs that could

affect cognitive functions, illiterate, and any known chronic diseases were excluded from the study.

Depression affects many cognitive functions such as working memory, information processing speed, learning and memory functions in MS patients (4,5). In addition, antidepressants, anticonvulsants, neuroleptics and glucorticocytoids may negatively affect cognitive performance (6).

Hamilton Depression Scale (HDRS) was applied to all MS cases. Neuropsychometric examination was performed by excluding depression in patients who had a score below seven points. After the antidepressant treatment, neuropsychometric examination was performed for individuals above seven points of HDS when they scored less than seven points of HDS. Patients using antidepressants, anticonvulsants, neuroleptics, and glucorticocytoid for these or other reasons were not included in the study because their cognitive performance could be affected.

Neurological disabilities of the patients were taken into consideration when creating a neuropsychometric battery. Patients with severe visual impairment and impaired speech function were excluded from the study.

MS patients who had normal and high homocysteine levels were compared with; Age, education level, EDSS, disease duration, vitamin B12, folate levels, PASAT, Line direction Determination Test (LDDT) and Stroop test performances. The data obtained were evaluated statistically. All data analyzed for MS patients were also examined in the healthy control group.

Pearson chi-square test and Spearman correlation analysis were applied to the parameters in the MS and healthy control groups, whether they were parametric or not. Each parameter was considered a constant variable and the effects and significance levels of the other variables were examined.

Three neurocognitive tests and EDSS scales lasting approximately one hour in total were administered to the patient and control groups. It basically measures the activities of the frontal lobe; Stroop Test, which demonstrates the ability to change perceptual set-up in line with changing demands and under a “disruptive effect,, the ability to suppress a habitual behavior pattern and to conduct unusual behavior; The Paced Auditory Serial Addition Test (PASAT), a test that measures attention, as well as information processing speed, flexibility, and computational ability, has recently been used frequently to assess cognitive abilities in MS patients; Line Perception Detection Test (BIMC), which assesses the viscospasial perception and construction skills, also called complex perceptual functions.

Stroop test considerations: The time periods during which the test steps are applied and the time differences between them and the number of errors and corrections made. On the other hand, the number of correct answers is important in LDDT and PASAT.

Determination of plasma homocysteine levels; Chromsystems Diagnostics devices and kits were studied. In the isocratic system, the fluorescence detector was measured by HPLC (high performance liquid chromatography) method and the results were obtained in µmol / L. In this method, plasma is separated from whole blood by centrifugation at 4000 rpm for 5 minutes. Precipitant is added to plasma to remove proteins. The resulting supernatant is treated by derivatization and reduction to give homocysteine.

In our study, blood samples were taken at least 12 hours after fasting. The samples were centrifuged as soon as possible after blood collection. In our study, we accepted that the limit at which cognitive influences started, that is, 10 µmol / L and higher, was high homocysteine. Plasma B12 levels below 197 pg / ml and blood folic acid levels below 4.6 ng / ml were considered low.

Statistical Analysis

If the probability of error (p) obtained as a result of statistical analysis is less than 0.05, the result was considered statistically significant, and SPSS (ver. 15.0) program was used in all statistical calculations.

Descriptive statistics of the obtained data are given in tables as mean ± standard deviation (SD), number and% value. In the normality test of numerical variables, Kolmogorov-Smirnov test was used if the number of individuals examined was> 50 and Shapiro-Wilk test was used if the number of individuals examined was <50. In the comparison of patient and control groups in terms of numerical variables showing normal distribution (parametric), hypothesis test and T test of the difference between two independent sample means were used. Mann-Whitney U test was used to compare these two groups in terms of numerical variables that do not show normal distribution (non-parametric).

In addition, Pearson chi-square test was used for parametric data and Spearman correlation analysis was used for non-parametric data in the analysis of the relationship between categorical data and disease and linear relationships between numerical variables. Homocysteine, B12 vitamin, folic acid levels, PASAT, Stroop and Line orientation tests were evaluated separately by using in-group and inter-group data. When evaluating the data, the data that corresponds to the normal distribution are expressed as mean ± standard deviation, and the frequency data are expressed as a percentage.

RESULTS

61 RRMS patients were included in the study. The control group consisted of 32 healthy volunteers. 42 of the patients were female (68.9%), 19 were male (31.1%), 22 of the controls were female (68.8%) and 10 (31.3%) were male. The mean age of the MS patients was 35.2 ± 9.53 years and the mean age of the control group was 36.1 ± 10.59 years. There was no significant difference between the groups in terms of gender and age (p = 0.992, p = 0.690). The mean disease duration was 8.3 ± 5.88 years. The mean EDSS score was 1.7 ± 1.63 (0-6). 38 (62.3%) patients were classified as 0-2, 18 (29.5%) were classified as 2.5-4, 5 (8.2%) were classified as> 4.

Homocysteine levels were significantly higher in MS patients than in the control group (p = 0.044). There was no statistically significant difference between the mean values of blood homocysteine levels in MS (50.05) and control groups (41.19) (p = 0.133).

When MS and control groups were compared with B12 vitamin and folic acid levels by logarithmic statistical analysis, no statistically significant difference was found between B12 vitamin levels (p = 0.209) and folic acid levels (p = 0.125).

PASAT test scores were significantly lower (p <0.001) in patients with high homocysteine levels than the normal group.

It was found that the BICT scores were significantly different in patients with high

homocysteine levels compared to those with normal homocysteine levels (p <0.001).

In the evaluation of data between homocysteine levels and Stroop test performances in MS patients; Card reading times and time differences were longer in MS patients with higher homocysteine levels than those with normal homocysteine levels. The number of errors and corrections was higher than the homocysteine normal group. The p values obtained for all stroop test steps were found to be less than 0.05, meaningful. There were no significant differences between the levels of serum homocysteine, age, disease duration, EDSS, educational level, and vitamin B12 levels (p> 0.05).

There was a negative correlation between homocysteine levels and vitamin B12 (r = -0.364, p = 0.004) and folic acid (r = -0.408, p = 0.001) levels. As vitamin B12 and folate levels decrease, homocysteine levels increase.

In both patients and healthy controls, the test performance was found to be impaired for all test steps in individuals with high homocysteine levels (Table 1-2).

Table 1. Comparison of the data of individuals in the healthy control group with high homocysteine levels and normal in terms of different variables and p values obtained

HEALTHY CONTROL GROUP		HOMOCYSTEINE LEVEL (µmol / l)		
		Normal Average ± ss	High Average ± ss	P Value
AGE		35.7 ± 10.83	37.5 ± 10.27	0.725
VITAMIN B12 (pg / ml)		257.2 ± 97.68	275.3 ±182.63	0.735
FOLATE (ng / ml)		9.3 ± 3.27	8.8 ± 5.05	0.247
PASAT		44.0 ± 10.49	27.0 ± 14.95	**0.002**
LDDT		27.2 ± 2.33	19.3 ± 6.25	**0.004**
STROOP TEST	1.Card time / sec	13.7 ± 3.21	24.6 ± 9.87	**0.042**
	3.Card time / sec	18.2 ± 5.53	33.8 ± 13.27	**0.013**
	5.Card time / sec	28.0 ± 11.17	64.1± 31.3	**0.005**
	5-1 Card time / sec	14.3 ± 8.64	39.5 ± 22.42	**0.005**
	5-3 Card time / sec	9.8 ± 6.36	30.3 ± 19.52	**0.005**
	5.Card correction	0.85 ± 1.08	3.0 ± 1.09	**0.001**
	5.Card error	0.15 ± 0.36	2.1 ± 0.98	**<0.001**

Table 2. Comparison of the data of MS cases with high and normal homocysteine levels in terms of different variables and p values obtained

HEALTHY CONTROL GROUP		HOMOCYSTEINE LEVEL (µmol / l)		
		Normal Average ± ss	High Average ± ss	P Value
AGE		35.7 ± 8.50	34.4 ± 11.09	0.596
DISEASE DURATION		8.7 ± 6.32	7.6 ± 5.21	0.568
VITAMIN B12 (pg / ml)		312.4 ±133.63	253.0 ± 99.12	0.098
FOLATE (ng / ml)		8.9 ± 3.61	7.1 ± 2.33	**0.036**
PASAT		36.9 ± 12.7	23.0 ± 10.64	**< 0.001**
LDDT		25.9 ± 3.12	20.4 ± 5.91	**< 0.001**
STROOP TEST	1.Card time / sec	12.7 ± 5.96	19.1 ± 9.60	**0.044**
	3.Card time / sec	19.0 ± 8.55	29.6 ± 15.38	**0.021**
	5.Card time / sec	37.0 ± 21.44	65.0 ± 39.33	**0.007**
	5-1 Card time / sec	24.3 ± 17.48	45.8 ± 30.97	**0.010**
	5-3 Card time / sec	18.0 ± 14.58	35.3 ± 25.16	**0.008**
	5.Card correction	1.7 ± 1.70	2.7 ± 1.53	**0.015**
	5.Card error	0.59 ± 1.14	1.5 ± 1.47	**0.003**

DISCUSSION

In most studies, high homocysteine levels were found to be associated with cognitive impairment in MS patients. Our study was conducted on Turkish population with similar characteristics. Different cognitive functions will be evaluated by different cognitive tests, and folic acid and B12 levels were evaluated simultaneously, which distinguishes our study from other studies.

In a study, the relationship between cognitive functions and homocysteine, vitamin B12 and folic acid levels was investigated using multiple neuropsychiatric tests. 93 MS and 53 healthy controls were taken, and there was no difference between B12 and folic acid levels between control and MS groups. It was shown that homocysteine levels were higher in MS patients compared to the control group and cognitive performance was significantly impaired in those with high homocysteine levels (Russo et al., 2008). Similarly, in our study, homocysteine levels were found to be significantly higher in MS patients compared to the control group, and test performance for all PASAT, LDDT scores, and Stroop test steps was found to be poor in individuals with high homocysteine levels.

Durga et al. and Brattstorm and Wilcken, in their critical review of studies, have stated that there is sufficient evidence supporting a relationship between white matter and homocysteine (7,8). Increased homocysteine levels disrupt the endothelial structure, causing spasm and ischemia (9,10). In another study, homocysteine has been shown to induce apoptosis in neuronal cell cultures with copper and beta amyloid-peptide-mediated toxic effects (11). It is known that the earliest and lasting disorder in the pathogenesis of MS is the structural alteration of blood brain barrier and perivenular lymphocyte accumulation (12). The role of homocysteine on neurodegenerative processes in MS can be explained by these mechanisms.

In one study, healthy elderly individuals, patients with Alzheimer's Dementia and Parkinson's, and other patients with cognitive impairment and MS patients were evaluated to investigate the effect of homocysteine in various conditions. After all; It was found that high homocysteine concentration was significantly associated with impaired cognitive performance at 6 years of follow-up in normal aging population and was higher in Parkinson's patients than in normal population. It was found that there was no significant elevation in dementia patients and higher homocysteine levels in MS patients than in the normal population. This was found to be significantly associated with impaired cognitive and motor performance. This shows us that homocysteine has a neurotoxic effect under all conditions and this effect is obvious enough to be clinically detectable (13).

In a study by Aksungar et al., It was stated that the blood homocysteine levels of MS patients were significantly higher than those of healthy individuals and serum B12 and folate levels were not correlated with homocysteine (14). Despite this study, in another study, Vrethem et al. reported that vitamin B12, folate and serum methylmalonic acid levels did not decrease in MS patients (15). There are other studies with low correlation between B12 and homocysteine ratio (16).

Despite normal B12 levels measured in serum, intracellular levels of vitamin B12 decrease as age progresses. As in this study, the normal B12 and folate levels despite homocysteine elevation may be due to a polymorphism in the MTHFR gene coding in

MS patients or due to any enzyme defect in the transsulfurization pathway. MTHFR and other regulatory enzymes in the transsulfurization pathway are required for methylenetetrahydrofolate regeneration and normal homocysteine metabolism. In our study, the absence of a correlation between homocysteine levels and vitamin B12 levels may be due to such an underlying pathology. however, the fact that we could not perform MTHFR gene analysis, and we could not determine it precisely may be a shortcoming of our study.

In a study conducted with 35 MS patients and 30 healthy controls, Kocer et al. Found that homocysteine levels were normal in the control group and high in 20% of the MS group. Vitamin B12 levels were found to be lower in MS patients with prolongation of VEP, posterior tibial SEP P1 and P2 latencies compared to MS patients with normal latency. It was stated that there was a close relationship between MS and vitamin B12 deficiency, and homocysteine elevation was due to vitamin B12 deficiency.

Last studies about reduction of plasma homocysteine levels and increase of B12 levels in impaired cognition patients, taking vitamins B6, B12, and/or folic acid supplements, at least 1 month of supplementation, findings support that reduce homocysteine levels and elevated plasma B12 levels. Thus, the cognitive impairment caused by high homocysteine levels can treated like this way as possible as (17).

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