Homocysteine Serum Level as a Biomarker of Multiple Sclerosis Disability and Cognitive Impairment

Rasha Hatem Ali[1], Ibrahim Abdel-Razik [1], Mohammed Salah ELsayed AlAhmar [1], Hesham Abdel-Shafy Dawood[2]*, Khaled A Elkapany[1]

[1]Department of Neuro Psychiatry, Faculty of Medicine, Menoufia University, Menoufia, Egypt
[2]Department of Neuropsychiatry, Shebin Elkom Teaching Hospital, Menoufia, Egypt

*Corresponding Author**

Hesham Abdel-Shafy Dawood,

Department of Neuropsychiatry,

Shebin Elkom Teaching Hospital, Menoufia,

Egypt

E-mail: heshampsycho@gmail.com

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Abstract

Background: Multiple sclerosis (MS) is considered a progressive irreversible disease that makes neurological dysfunction. Homocysteine is a biomarker that is linked to physical disability and cognitive impairment in patients with MS.

Objective: To study the prevalence of hyperhomocysteinemia in MS patients. To study the possible association between hyperhomocysteinemia (HHcy) and physical disability, and cognitive impairment in MS patients.

Patients and Methods: This was a case-control study that was carried out on 40 MS patients, and 40 controls comparable in age, sex, and socioeconomic status. Patients underwent Cognitive, physical, and laboratory assessments including homocysteine serum level.

Results: Homocysteine serum level was significantly higher in the cases group than in the control group. HHcy is associated with cognitive impairment in MS patients. Physical disability assessed by EDSS score was not associated with HHcy in our research. Homocysteine serum level had a sensitivity of 85% and specificity of 57.5% at best cutoff value ≥13.75, with an accuracy of 96.3% for predicting multiple sclerosis disability and cognitive impairment.

Conclusion: Higher circulating homocysteine levels were present in MS patients compared with controls. Physical disability was associated with HHcy in our research. Hhcy is not associated with cognitive impairment in patients suffering from MS

Keywords: Biomarkers • Cognitive Impairment • Homocysteine Level • Multiple Sclerosis • Physical Disability

Introduction

Multiple Sclerosis (MS) is considered an autoimmune disease in which the inflammatory cells attack the central myelin impairing its function. Myelin regeneration requires normal pathway of Folate &vit. B12 methylation. Vitamin B12 and folate are involved in the synthesis of methionine from homocysteine [1].

Reduced level of vit B12 and folate results in hyperhomocysteinemia which causes hypomethylation of myelin binding protein MBP. Methylation of MBP is important for myelin repair of the affected nerve cells. So hyperhomocysteinemia is considered a risk factor for many neurological diseases such as dementia and MS [2].

Blood levels of homocysteine will help to assess methylation of MBP and subsequently remyelination [3]. Myelin regeneration & remyelination are important for MS remission [4]. Hyperhomocysteinemia is proved to be associated with clinical progression of MS [5].

That hypothesis is supported by some studies and theories which state that homocysteine produces reactive oxygen species that cause neuronal damage and by stimulation of NMDA receptors that damage nerve cells DNA causing apoptosis [6].

Aim of the Work

To study the prevalence of hyperhomocysteinemia in MS patients. To study the possible association between hyperhomocysteinemia and disability, cognitive impairment in MS patients.

Patients and Methods

This is cross-sectional study was carried out on 40 Multiple Sclerosis (MS) patients, and 40 healthy controls comparable in age, sex, and socioeconomic status. MS patients diagnosed according to revised McDonald criteria 2017. Cases were collected in the study from patients who visited the multiple sclerosis outpatient clinics at Menoufia University Hospital and the neuropsychiatric clinic in Shibin Al Kom Teaching Hospital with the inclusion criteria during the period from October 2019 to March 2021. Informed consent was obtained from the controls.

Ethical consideration

The study was approved by the Ethical Committee of the Faculty of Medicine, Menoufia University. A written informed consent was obtained from either patient or his/her legal guardian after simple and clear explanation of the research objectives (IRB number is 7/20/20 NEUR44).

All patients were selected according to the inclusion and exclusion criteria as follows.

Inclusion criteria

Age above 16 years [7]. Both sexes are included. Patients diagnosed as having MS according to McDonald's criteria 2017 [8]. Any clinical type of MS: clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS).

Exclusion criteria

Presence of any condition associated with elevated homocysteine values, such as renal impairment, hypothyroidism, hemolytic anemia, taking drugs that cause elevated serum Homocysteine levels such as phenytoin, isoniazid, methotrexate, and L-dopa. Pre-existing cognitive impairment or physical disability.

Study grouping

Cases group: Included 40 patients diagnosed with MS.

Control group: Included 40 volunteer's healthy subjects, matched for age, sex and socioeconomic status.

All patients were subjected to:

1. Detailed history and examination (general and neurological) using MS sheet including age, sex, MS duration and types, attacks numbers.
2. Laboratory assessment: Serum levels of homocysteine: determined using human HCY ELIZA kite, complete blood count (CBC), renal, liver, and Thyroid function tests.
3. MRI brain and cervical spine: All MR imaging was performed with Titan Excelart vantage series, 1.5 Tesla. Axial T2-weighted spin-echo, T1-weighted spin-echo, fluid-attenuated inversion recovery (FLAIR), and with contrast to show the new lesions.
4. EDSS scores for assessment of physical disability which was done at the time of sample taking.

Cognitive assessment was done at the time of sample taking through; Montreal cognitive assessment MoCA (Arabic version): for general cognitive screening. Arabic version of Addenbrooke's Cognitive Examination (ACE- III): for assessing five domains of cognition; attention, memory, verbal fluency, language, and visuospatial abilities. Paced Auditory Serial Addition Task (PASAT): for assessing working memory and information processing. Controlled Oral Word Association Test (COWAT): for assessing verbal fluency. Trail making test (part B): for assessing information processing speed and task switching for executive functions.

Human HCY ELIZA kite

1. Principle of the assay: Samples were collected at time of examination and total Homocysteine was quantified by sandwich enzyme immunoassay technique based on antibody binding.
2. Detection range: 0.78nmol/ml-50nmol/ml.
3. Sensitivity: The minimum detectable dose of human HCY is typically less than 0.195 nmol/ml.
4. Specificity: This assay has high sensitivity and excellent specificity for detection of human HCY. No significant cross-reactivity or interference between human HCY and analogues was observed.

Statistical analysis

Results were tabulated and statistically analyzed using a standard computer program using Microsoft Excel 2017 and SPSS V.25 program for MICROSOFT WINDOWS 10. Descriptive statistics: was in the form of the mean (±) SD for quantitative data and frequency and proportion for qualitative data. Non-normally distributed numerical data were presented as median (IQR). Analytical statistics: were Standard student-t test (t), Chi-Squared (χ2), Mann-Whitney test, Student's t-test, Spearman's correlation (r), and The ROC (receiver operating characteristic) curves. P value <0.05 was considered statistically significant.

Results

40 patients were included in this study; age was increased among the cases group (32.08 ± 8.37) than the control group (28.50 ± 3.88). Also, most of the cases (70%) were females and (30%) were males. There was no significant difference between the studied group regarding age and gender (P<0.005).

The majority of cases had Relapsing-remitting multiple sclerosis (95%), and only (5%) of them had Primary Progressive multiple sclerosis. There was no CIS or SPMS in our sample. 82% of patients had less than or equal to 3 attacks vs. (17.5%) who had more than 3 attacks. Also, half of the cases had a duration of illness of more than 5 years, and 52.5% had a duration of treatment of less than 3 years. While 57.5% had EDSS less than 5.5. Cognitive impairment was found in 67.5% of patients, 60% of patients had 3-10 lesions, while, 65% had Brain atrophy, as shown in Table 1.

Table 1. Pattern of MS illness, course, prognosis, and factors affecting (n=40).

Variables	Studied cases (No=40)	
Type of MS	No.	%
RRMS	38	95
Primary Progressive	2	5
No. of attacks/year		
≤3 attack year	33	82.5
>3 attack year	7	17.5
Duration of illness		
< 1 year	6	15
year	140	35
>5 year	20	50
Duration of treatment with DMT		
< 3 years	21	52.5
3-8 years	14	35
> 8 years	5	12.5
EDSS		
< 5.5	23	57.5
> 5.5	17	42.5
Cognitive impairment	27	67.5
Patients with intact cognition	13	32.5
Patients with cognitive impairment		
No of lesions		
<3	7	17.5
03-Oct	24	60
>10	9	22.5
Brain atrophy		
Brain atrophy	14	65
No brain atrophy	26	35

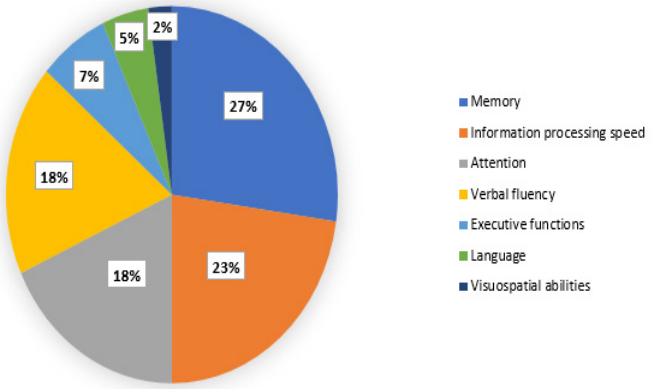

Figure 1. Types and prevalence of cognitive domains affected.

32.5% of patients had cognitive impairment. Types & percentages of cognitive domains affected in our sample are shown in Figure 1. regarding combined affected domains memory and attention found in 38.46%, verbal fluency and executive function found in 38.46%, and visuospatial abilities and language represented by 30.77%.

Homocysteine serum level was significantly higher among the cases group (18.66 mmol/l±3.93 mmol/l) than the control group (9.98 mmol/l±2.71 mmol/l) as in Table 2.

There was a significant relation between homocysteine serum levels with EDSS (P=0.001). While, there were no significant relations between homocysteine serum levels with genders, type of MS, number of attacks, cognitive impairment, duration of illness, duration of treatment, number of lesions, and brain atrophy (P>0.05) as shown in Table 3.

There was no significant relation between homocysteine serum levels with any type of the studied cognitive domains (P>0.05), as shown in Table 4.

Homocysteine serum level had a sensitivity of 85% and specificity of 57.5% at best cutoff value ≥13.75, with an accuracy of 96.3% for predicting multiple sclerosis disability and cognitive impairment (P=0.001) as shown in Table 5 and Figure 2.

Discussion

Multiple sclerosis (MS) is considered a progressive irreversible disease that affects the central nervous system (CNS) causing neurological damage. It affects more than two million young persons around the world [9]. The main feature of MS lesions is inflammation followed by demyelination of nerve cells and oligodendrocytes loss associated with limited remyelination [10]. The pathology of MS is multifactorial. It involves genetic and environmental factors. Markers as vitamin b12 and folate are also included in the process of disease exacerbation [11].

In our study, homocysteine serum level was significantly higher among the cases group (18.66 micromol\L ± 3.93micromol\L) than in the control group (9.98 micromol\L ± 2.71micromol\L). That agreed with Russo et al. (2008), Ashtari et al. (2005), and Li et al. (2020) [12-14]. And against the findings of Fahmy et al. (2018) who used a sample of 45 MS patients and 20 matched healthy controls and found no significant difference [15].

Hyperhomocysteinemia (HHcy) can produce cerebral ischemia in the form of lacunar infarctions increasing the white-matter lesion load in MS patients, which will lead to more cognitive impairment. HHcy is proven to have a role in cortical atrophy and dementia making more cognitive impairment [16]. Homocysteine can also activate macrophages making apoptosis leading to neurodegeneration [17].

Table 2. Comparison between cases and control groups regarding homocysteine level (n=80).

Homocysteine Level (mmol/l)	Studied Groups				
	Cases (No=40)	Control (No=40)	Total (N=80)	t	P Value
Mean ± SD	18.66±3.93	9.98±2.71	14.32±3.32	11.491	<0.001*
Range	10.5-26	6 - 14	6 - 26		
t:Student t test, *: Significant					

Table 3. Homocysteine serum level in relation to studied variables among cases group (n=40).

Variables	No.	Homocysteine serum level Mean ±SD	U	P Value	95% CI
Gender					
Male	12	13.44±5.23	0.921	0.36	-5.05
Female	28	14.6±6.09			
Type of MS					
RRMS	38	17.10±2.16	2.67	0.085	-3.69
Primary progressive MS	2	21.08±1.13			
Number of attacks/years					
≤3	33	18.21±4.05	2.074	0.059	-5.28
>3	7	20.7±2.64			
Cognitive impairment					
Patients with intact cognition	27	18.91±3.48	0.532	0.601	-6.32
Patients with cognitive impairment	13	18.12±4.85			
Duration of illness					
< 1 year	6	17.25±3.76	H=0.459	0.635	13.30-21.20
1-5 year	14	19.07±3.30			17.16-20.98
>5 year	20	18.79±4.45			16.70-20.87
Duration of treatment					
< 3 years	21	17.69±4.47	H=0.616	0.852	15.66-19.72
3-8 years	14	19.34±3.13			17.53-21.14
< 8 years	5	20.80±2.61			17.56-24.04
EDSS					
< 5.5	23	16.75±3.36	4.35	0.001**	15.30-18.20
> 5.5	17	21.24±3.14			19.62-22.85
No of lesions					
<3	7	15.00±3.54	H=2.027	0.146	9.37-20.63
03-Oct	24	19.01±3.64			17.47-20.54
>10	9	19.17±4.29			16.44-21.89
Brain atrophy					
Brain atrophy	14	17.98±3.81	1.481	0.151	
No brain atrophy	26	19.91±3.98			
U: Mann-Whitney test, H: Kruskal Wallis test, CI: Confidence Interval Level, * Significant 95%					

Table 4. Homocysteine serum level in relation to type of cognitive impairment among cases group (n=13).

Type of cognitive impairment	No.	Homocysteine serum level Mean± SD	U	P value
Memory				
Affected	12	17.63±4.72	0.969	0.347
Intact	1	19.10±3.55		
Attention				
Affected	8	17.44±4.44	0.89	0.395
Intact	5	18.96±3.81		
Information processing				
Affected	10	18.65±4.96	0.004	0.997
Intact	3	18.66±3.63		
Verbal fluency				
Affected	8	17.06±3.91	1.288	0.225
Intact	5	19.05±3.90		
Executive functions				
Affected	3	16.50±3.91	0.993	0.412
Intact	10	18.83±3.94		
Visuospatial abilities				
Affected	1	12.00±0.00	1.759	0.087
Intact	12	18.83±3.83		

Language			1.083	0.464
Affected	2	15.25±4.60		
Intact	11	18.83±3.88		
U: Mann-Whitney Test				

Table 5. ROC Curve of homocysteine serum level to predict multiple sclerosis disability and cognitive impairment.

	Cutoff	AUC	Sensitivity	Specificity	Std. Error	Sig.	95% Confidence Interval	Accuracy %
Homocysteine serum level	>13.75	0.96	85%	57.50%	0.018	<0.001*	0.928 - 0.997	96.30%
AUC: Area Under Curve, CI: Confidence Interval, *significant								

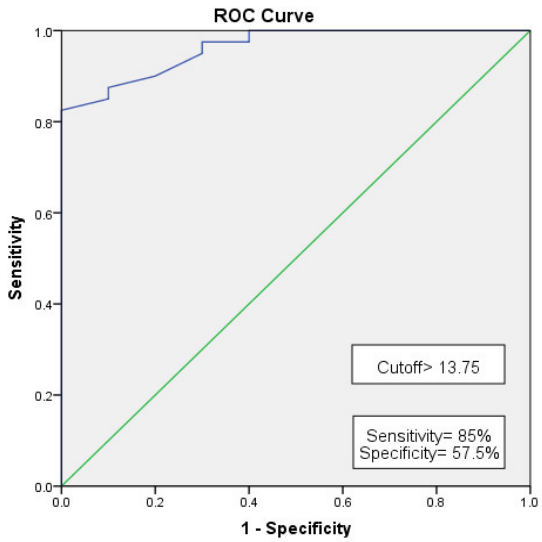

Figure 2. ROC Curve of homocysteine serum level to predict multiple sclerosis disability and cognitive impairment

Upon studying different cognitive domains affected in our sample, we found that memory was the most affected domain (~92%) of the whole sample followed by information processing (~77%), then attention and verbal fluency came at the third level by (~61%) of each, executive dysfunction (23%), language dysfunction (~15%), Visuospatial disabilities (~8%) came at the end of list. Upon analysis of the combined cognitive domain affection memory dysfunction associated with attention deficit represent (~38%) of cases, also, verbal fluency dysfunction associated with executive dysfunction represents (~38%) of cases, while visuospatial dysfunction associated with language dysfunction represents ~ (31%) of cases.

That was incongruent with Botchorishvili et al. (2021), who found that memory was the most affected domain and Zwecker et al. (2018) who found that 61.5% of their sample was cognitively affected [18,19]. Affection was mainly as follows: verbal fluency (88.6%), short-term memory (70.5%), visual-spatial learning (59.1%), and sustained attention (29.5%).

Cognitive impairment in MS shows variability in types and severity. The most frequently affected domains are memory, information processing, attention, executive functions, and verbal fluency and the least affected is visuospatial ability [20]. Multiple sclerosis patients with frontal-subcortical affection mostly are presented with impaired memory, decision-making, and goal-directed behavior. This pattern is associated with loss of tissue integrity and organization in fronto subcortical tracts especially frontolateral areas as measured by MRI brain with diffusion. Frontolateral areas are associated mainly with executive functions [21]. Memory and executive functions are closely related to the prefrontal cortex. Executive functions are the cognitive abilities required for complex goal-directed behavior and that includes several functions including memory, that may explain why memory is the most affected cognitive domain [22]. Regarding memory, the most frequently involved domain in MS. Deficits in episodic memory tests are consistently found in patients with MS. Benedict et al. (2009) said that memory impairment in patients with MS is linked to the frontal/subcortical axi dysfunction which may lead to deficient encoding; mesial temporal lobe dysfunction could also lead to deficient consolidation [23].

The present study demonstrated that there were no significant relations between homocysteine serum levels with gender, number of attacks, cognitive impairment, number of lesions, duration of illness, duration of treatment, and brain atrophy (P>0.05). While there was a significant relation between homocysteine serum and physical disability assessed with EDSS (P=0.001). This finding was matching with Guzel et al. (2016), Fahmy et al. (2018), and Mititelu et al. (2020) [11,15,24].

In the current study, there was no significant relation between homocysteine serum levels with impairment of memory, attention, information processing, verbal fluency, executive functions, visuospatial abilities, and language. According to Fahmy et al. (2018) & in contrast to us, high homocysteine levels are associated with impairments in attention, memory, language, verbal fluency, information processing speed, and executive functions [15].

Differences with our study may be due to a small number of cognitively affected patients. According to Russo et al. (2008), nonverbal reasoning, visual attention, visuospatial memory, and visuospatial ability were the cognitive abilities in patients with hyperhomocysteinemia (HHcy) that were most significantly impacted [12].

The present study demonstrated that homocysteine serum level had a sensitivity of 85% and specificity of 57.5% at best cutoff value ≥13.75, with an accuracy of 96.3% for predicting multiple sclerosis disability and cognitive impairment. Mititelu et al. (2021) used homocysteine cobalamin ratio as a predictor toll for MS disease progression and it was a very good index of disease severity with an AUC of 1.00 ((95% CI of 1.00) according to ROC analysis [11]. Fahmy et al. (2018) assessed homocysteine, folic acid &Vitamin b12 and correlates them with cognitive impairment in MS patients and found that only homocysteine could be a marker for cognitive impairment in patients with MS [15].

Finally, we concluded that homocysteine serum levels increased in MS patients. HHcy is associated with increased physical disability in MS patients and is not related to other factors.

Conclusion

Higher circulating homocysteine levels were noted in MS patients compared with controls. Physical disability was associated with HHcy in our research. No association was noticed between HHcy and cognitive impairment in MS patients.

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